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# Evaluation of the Effects of Age, Sex, and Dexpanthenol-Containing Skin Care on the Facial and Body Skin Microbiome

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**Abstract:** Given the pivotal role played by the microbiome in skin health, it is important to understand how its composition varies with age, sex, and body site and regular use of topical products. Four studies were carried out to determine the effects of long-term (4-week) use of different dexpanthenol-containing topical products on the skin microbiome of a varied population with cosmetically dry skin. The skin microbiome composition was assessed before and after product usage. No significant changes in microbiome richness or diversity were found for the individual test products; however, a meta-analysis of the combined dataset did show changes in microbiome composition as a function of the subject's sex, age, and body site. The work presented here demonstrates how the use of carefully formulated topical products on skin, when used in a way that is representative of real-life usage conditions, can respect the microbial diversity present on skin across a widely varied study population.

**Keywords:** microbiome; topical products; skin health; dexpanthenol



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## 1. Introduction

The skin microbiome, a diverse community of microorganisms including bacteria, fungi, viruses, and mites, plays a crucial role in maintaining skin health by contributing to the skin's barrier function, modulating the immune response, and protecting against pathogens [1,2]. Far from being a static and unchanging part of the skin, the microbiome varies over time as a result of key life stages such as puberty and menopause [3]. It is important that the use of topical skin care products does not negatively impact the microbiome composition and respects the natural balance present at these different life stages as microbiome dysbiosis is linked with a range of skin conditions such as xerosis, atopic dermatitis, and psoriasis [4–8].

Dexpanthenol, also known as provitamin B5, is widely used in skin care products for its moisturizing and healing properties. It is converted to pantothenic acid in the skin, which is essential for cellular metabolism. Dexpanthenol-containing emollients have been previously studied for their role in enhancing skin hydration, promoting barrier regeneration and accelerating wound healing while also demonstrating an anti-inflammatory effect, yet the impact on the skin microbiome was not assessed at that time [9–12].

To close this gap, four human studies were recently carried out to examine the skin microbiome composition and the effects of using topical cosmetic products containing dexpanthenol, along with ingredients such as niacinamide and lipids, which were included with the aim of improving xerotic skin [13]. Three of these studies examined topical moisturizing products, and one looked at products designed to cleanse the skin. While

some preliminary results have been shared with an emphasis on the behavior of the wash products [14], a meta-analysis has now been carried out on the results of the studies to enable a deep dive into the microbiome of the study population and the effects of the products on it. This article discusses the results of these studies and this meta-analysis and how the skin microbiome varies with sex, age, and body location and the usage of a range of topical cosmetic products, as well as the roles that these types of products have in helping to protect the skin.

## 2. Materials and Methods

Four studies to examine the effects of topical moisturizing products on skin microbiome composition were conducted between January and June 2023 in Hamburg, Germany. The studies were conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of proderm GmbH (Hamburg, Germany) (IRB code 2022/028, 15 January 2023, IRB code 2022/029 15 January 2023, IRB code 2023/001 11 January 2023, IRB code 2023/002 11 January 2023). The subjects gave consent to take part in writing in each of the four studies. They also consented that their (pseudo-anonymized) data could be used for further scientific analyses. The key inclusion criteria were as follows: female or male, between 18 to 65 years of age and having cosmetically dry skin (according to Corneometer® CM825 (Courage & Khazaka, Cologne, Germany) values < 37 a.u. (arbitrary units) on one volar forearm at screening). The population demographics across the four studies are given in Table 1.

**Table 1.** Test population demographics.

| Variable | Male | | | Female | | |
|---|---|---|---|---|---|---|
| | N | % | Mean (SD) | N | % | Mean (SD) |
| **Age (Range 22–65)** | | | **52.1 (12.4)** | | | **51.3 (13.1)** |
| Fitzpatrick skin type | | | | | | |
| I | 0 | 0 | | 8 | 12.6 | |
| II | 15 | 53.6 | | 34 | 54.0 | |
| III | 10 | 35.7 | | 19 | 30.2 | |
| IV | 2 | 7.1 | | 1 | 1.6 | |
| V | 1 | 3.6 | | 1 | 1.6 | |
| Ethnicity | | | | | | |
| White | 23 | 82.1 | | 61 | 96.8 | |
| Black | 3 | 10.7 | | 2 | 3.2 | |
| Asian | 2 | 7.2 | | 0 | 0 | |

Each study was based on microbiome samples collected from the participants to test four different treatments (2 test products in a split design each vs. untreated). The product identifying codes are given in Table 2. All products contained dexpanthenol and a range of other ingredients, the compositions of which are listed in Supplementary Materials, Section S1. Two sites were on the face, and two were on the volar forearm. Prior to the start of the study the subjects were instructed not to apply any leave-on cosmetics (e.g., creams or lotions) to the volar forearms within the last 3 days or bring the test area in contact with water (e.g., no bathing and swimming, only careful bathing excluding the test area) within the last 2 h. The products were applied once daily to each site for 28 days by the subjects after being provided with training on how much product to apply and where. The subjects were instructed to apply 0.5 g of the relevant test product to half their face and one volar forearm once daily throughout the study. The subjects were instructed to not apply any leave-on cosmetics (e.g., creams or lotions) other than the provided test products to the test areas throughout the entire course of the study. The last application of the test products was performed 24 ± 2 h prior to the scheduled visits. Only subjects who completed the product usage as outlined in the protocol (the per protocol population) were included in the data analysis.

**Table 2.** Product codes and descriptions.

| Product Code | Product Name |
|---|---|
| DFCD | Dexpanthenol repair complex (DRC) face cream day |
| DFCN | DRC face cream night |
| DFC-SPF | DRC face cream (SPF 25) |
| DFW | Dexpanthenol face wash |
| DBL | DRC body lotion (low lipid level) |
| DBH | DRC body balm (high lipid level) |
| DHC | DRC hand cream |
| DBW | Dexpanthenol body wash |

For microbiome assessment, the participants were swabbed at day 0 (D0) before product usage commenced and day 29 (D29) after 4 weeks of regular product usage, with comparisons made between treated and untreated samples per test area (face and forearm) per time point (D0 and D29). DNA was extracted from the swab samples using the NucleoSpin 96 Soil (Macherey-Nagel, Düren, Germany) kit. Bead beating was performed on a Vortex-Genie 2 horizontally at 2700 rpm for 2 × 5 min. Negative controls (sterile water treated as a sample and no-template PCR controls) were included per batch of samples from the DNA extraction and throughout the laboratory process (including sequencing). A ZymoBIOMICS Microbial Community DNA Standard (Zymo Research, Irvine, CA, USA) was also included in the analysis as a positive (mock) control. Full details on the microbiome assessment are given in Supplementary Materials, Section S2.

The microbiome alpha diversity was calculated as the number of entities detected ("richness") or as the Shannon index based on natural logarithm. The Shannon diversity index accounted for both the number of different species and abundance evenness of the species. To reduce bias from differences in sequencing depth, alpha diversity analyses were based on values calculated from rarefied abundance data (rarefied to a sequencing depth of 4620 reads). A sequencing depth-adjusted richness measure was also calculated as rarefaction may not remove all the sequencing depth-related bias. The paired Wilcoxon signed rank test was used as the statistical test used to detect significant differences in alpha diversity measures between groups.

The beta diversity was calculated as weighted UniFrac distances and Bray–Curtis dissimilarity for the visualization of samples and air swabs. To enable visual interpretation of distances between the samples in a two-dimensional plot, the samples could be projected onto the first two dimensions of a principal co-ordinates analysis (PCoA) of the distances. Each point of the PCoA plot represented a sample. The longer the distance between two samples, the farther apart were the corresponding sample points in the PCoA plot, i.e., points near each other represented samples with similar microbiome compositions. Ellipses were added to the PCoA plots that were centered on the group centroids and covered one standard deviation of the mean of the centroid position, i.e., the ellipses indicated how certain the positions of the group centroids were.

When comparing the beta diversity, a PERMANOVA test was performed using the adonis2 function from the vegan R package with 1000 permutations. While PCoA plots reduced the beta diversity information into two dimensions, PERMANOVA significance tests directly regressed the complete beta diversity information (i.e., the distances) to the groups to be compared.

The meta-analysis merged data from the cosmetic studies focusing on the merged datasets for the phylum and species of the bacteria present. Data were merged using the PANDAS library (Python version 3.10.12). Exploratory analyses were conducted using R libraries (SparkR, sparklyr, dyplr, tidyr, ggplot2, ggpubr, and vegan) (R version 4.3.1). To

assess for pre- and post-treatment changes in the microbiome, Wilcoxon signed rank tests were used (R library stats).

## 3. Results

Alpha Diversity: Changes in the Shannon diversity index for the individual test products are given in Table 3. No significant differences were found for any of the products between baseline and post-treatment.

**Table 3.** Individual product data for median Shannon diversity index (with Q1 and Q3, the 1st and 3rd quartile) at baseline (D0) and post-treatment (D29). NS—not significant at the 95% confidence level.

| Product | Body Site | D0 (Shannon Index) | D29 (Shannon Index) | Difference |
|---|---|---|---|---|
| DFCD | Face | 1.297 (1.120, 1.598) | 1.263 (1.083, 1.559) | −0.034 (NS) |
| DFCN | Face | 2.352 (1.525, 2.224) | 2.149 (1.529, 2.135) | −0.203 (NS) |
| DFC-SPF | Face | 1.601 (1.293, 1.792) | 1.658 (1.326, 1.872) | +0.057 (NS) |
| DFW | Face | 1.831 (1.476, 2.027) | 1.741 (1.287, 1.902) | −0.090 (NS) |
| DBL | Forearm | 2.808 (2.250, 2.637) | 2.900 (2.439, 2.864) | +0.092 (NS) |
| DBH | Forearm | 2.738 (2.442, 2.714) | 3.130 (2.853, 2.986) | +0.392 (NS) |
| DHC | Forearm | 2.444 (2.194, 2.551) | 2.940 (2.449, 2.846) | +0.496 (NS) |
| DBW | Forearm | 2.735 (2.544, 2.859) | 2.894 (2.495, 2.784) | +0.159 (NS) |

Beta diversity: Beta diversity analysis showed no differences between D0 and D29 or between the products and the relevant untreated comparisons at the 95% confidence level.

Abundance: Paired Wilcoxon signed rank tests were used to compare abundances at the level of individual ASVs, species, genera, families, orders, and phyla to test for differences in abundance between treatment time points along with whether the levels increased or decreased (Table 4).

**Table 4.** Taxonomic microbiome changes after 4 weeks of product usage on the face and forearm. NSD—no significant differences (at the 95% confidence level); ASV—amplicon sequence variant.

| Product | Body Site | Taxonomic Changes (D29 vs. D0) | Category | *p* Value |
|---|---|---|---|---|
| DFCD | Face | NSD | - | - |
| DFCN | Face | NSD | - | - |
| DFC-SPF | Face | NSD | - | - |
| WPF | Face | *Corynebacterium tuberculostearicum* ↓ | Species | 0.003 |
| | | *Frankia nodulisporulans* ↑ | Species | 0.002 |
| DBL | Forearm | ASV_0009—*Xanthomonas campestris* ↑ | ASV | <0.001 |
| | | *Xanthomonas campestris* ↑ | Species | <0.001 |
| | | Veillonella ↑ | Genus | <0.001 |
| | | Xanthomonas ↑ | Genus | <0.001 |
| | | Veillonellaceae ↑ | Family | <0.001 |
| | | Xanthomonadaceae ↑ | Family | <0.001 |
| | | Firmicutes_C ↑ | Phylum | <0.001 |
| DBH | Forearm | *Haemophilus_D parainfluenzae* ↑ | Species | 0.001 |
| DHC | Forearm | Epilithonimonas ↑ | Genus | 0.010 |
| | | Rothia ↑ | Genus | 0.007 |
| | | Xanthomonas ↑ | Genus | 0.008 |
| WPB | Forearm | NSD | - | - |

Meta-Analysis: Following the assessment of data from the individual products, a meta-analysis was carried out to determine the effects of topical moisturizer usage on the

overall study population, as well as to further understand the impacts of age, sex, and body site on microbiome composition. Microbial composition for the entire study population, including the top three phyla, at the beginning of the study and after product usage on the face and forearm is shown in Figure 1. Once-daily product usage for 4 weeks did not result in any significant changes to the gross overall microbial population composition.

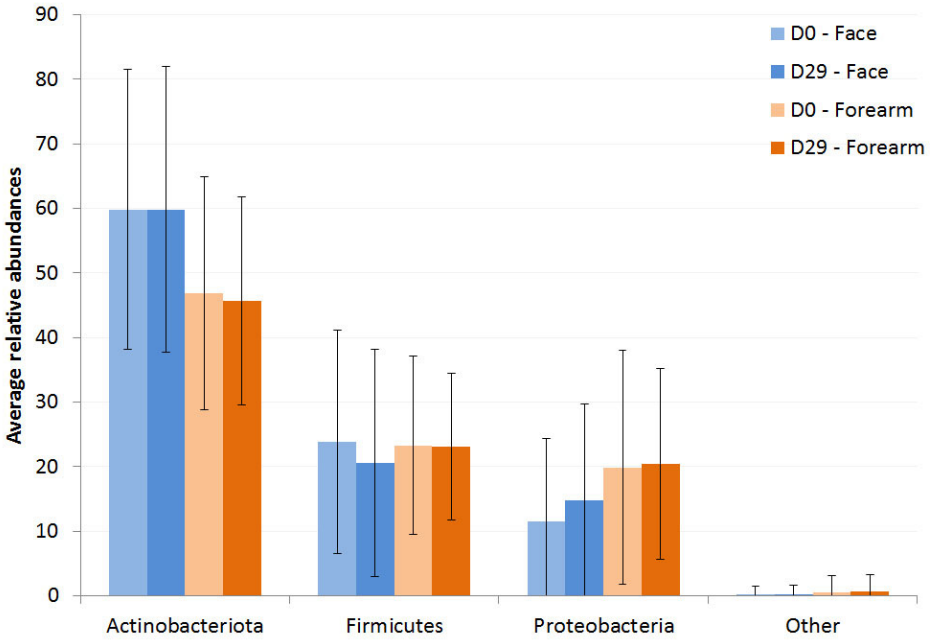


**Figure 1.** Baseline (D0) and post-product usage (D29) average relative abundance of the top three phyla (Actinobacteriota, Firmicutes, and Proteobacteria) and other species by body site (face and forearm).

Microbiome "richness" scores and the Shannon diversity index at baseline (D0) as a function of age group, sex, and body site (face and forearm) are shown in Figure 2. Variation in the richness of the face microbiome by age group was observed but with no clear trend; however, richness was greatest in the oldest age group (60+ years). A non-parametric Kruskal–Wallis test indicated that there were significant differences in both the "richness" ($p = 0.001$) and the Shannon diversity index ($p < 0.001$) of the face by age and sex. There were no significant differences in the "richness" ($p = 0.063$) of the forearm by age and sex; however, there was a significant difference in Shannon diversity index on the forearm ($p < 0.001$).

Wilcoxon–Mann–Whitney U tests were used to compare the richness of the treated versus untreated groups after product usage (D29). Separate analyses were run for the face and forearm. The results for the face showed no mean difference in the "richness" ($p = 0.545$) or Shannon index ($p = 0.661$). The forearm results also showed no difference in the "richness" ($p = 0.240$) or Shannon index ($p = 0.193$).

Using a meta-analysis, the effects of age and sex on microbiome diversity were examined. The relative abundance of the main microbial phyla on the forearm and face as a function of subject age is given in Figure 3.

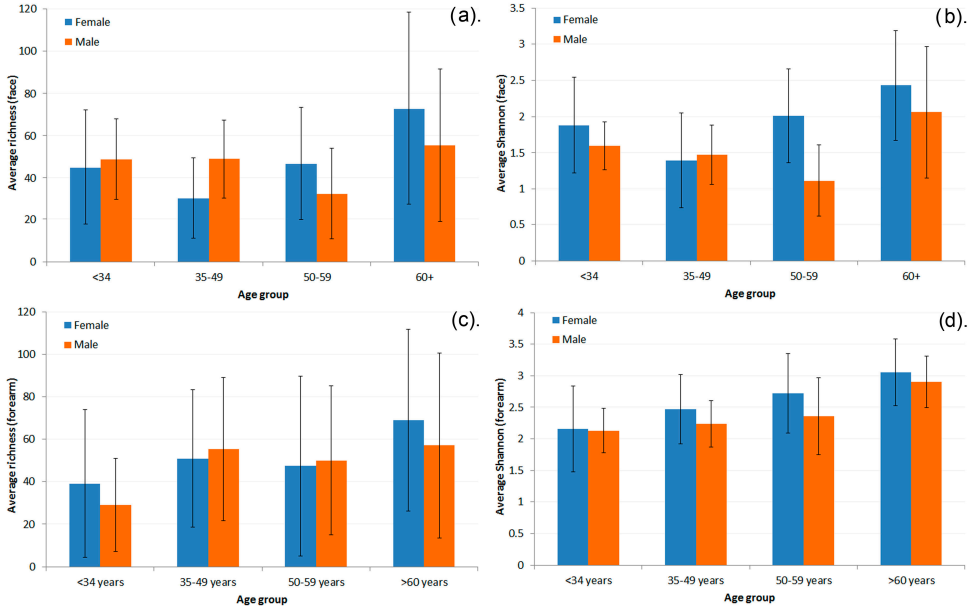


**Figure 2.** Alpha diversity at baseline (D0) by age group and sex shown as mean and standard deviation. (**a**) Variation in the richness on the face. (**b**) Shannon diversity of the facial microbiome. (**c**) Variation in the richness of the forearm. (**d**) Shannon diversity of the forearm microbiome.

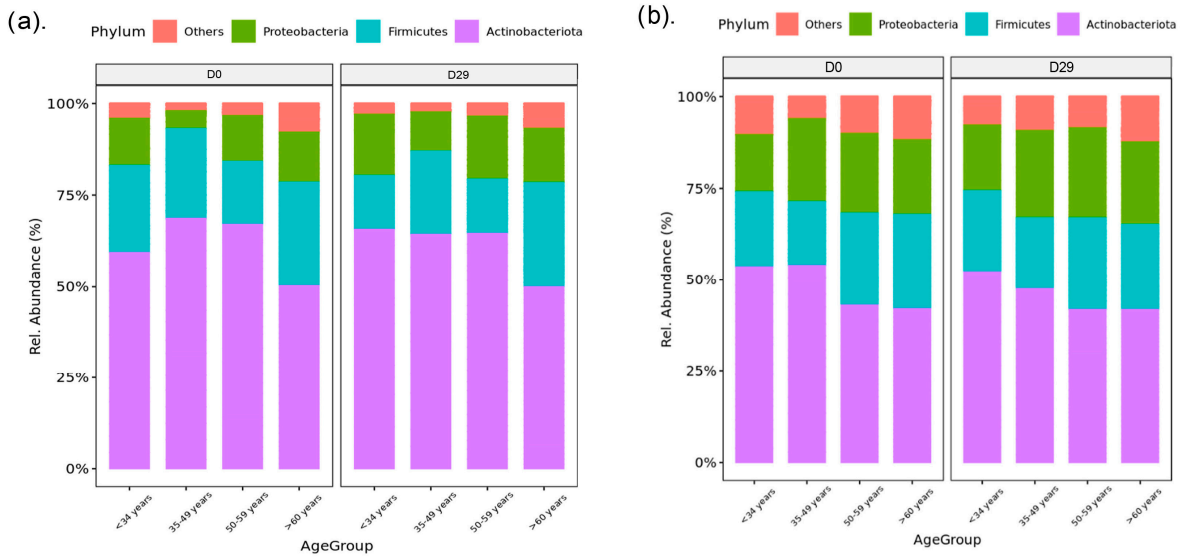


**Figure 3.** Relative abundance of the top three phyla (Actinobacteriota, Firmicutes, and Proteobacteria) for (**a**) the face and (**b**) the forearm, at baseline (D0) and post-treatment (D29), split by age group.

The relative abundance of the main microbial phyla on the forearm and the face as a function of subject sex is given in Figure 4.

Individual Wilcoxon-signed rank tests were used to compare the pre- and post-treatment counts and relative abundance for the face and forearm for the three phyla. There was no significant difference in the pre- and post-treatment for the treatment groups for Actinobacteriota and Firmicutes (both counts and relative abundance, face and forearm, $p > 0.05$). There were significant differences in the pre- and post-treatment groups for Proteobacteria facial relative abundances ($p = 0.045$) and forearm counts ($p = 0.015$) between the pre- and post-treatment groups.

Adverse events: A total of 34 adverse events (AEs) were reported across the four studies. Of the reported AEs, 17 were attributed to the product usage, and 17 occurred in

the untreated groups. All product-related AEs were minor, and all AEs were monitored until resolved.

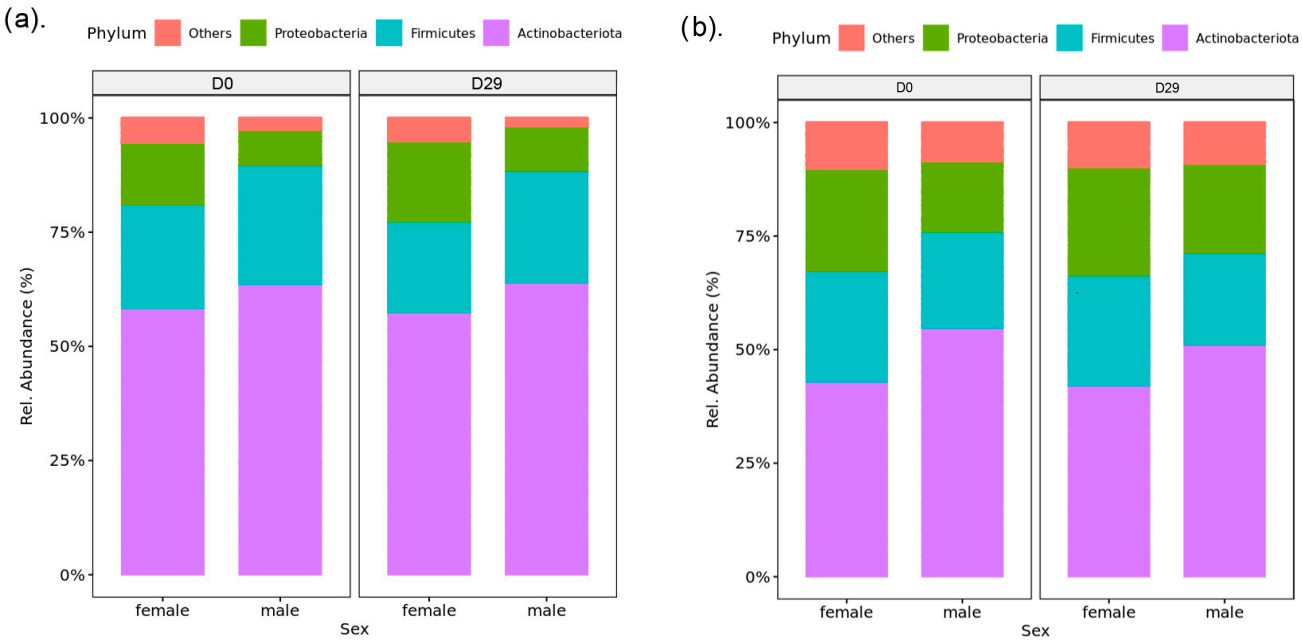


**Figure 4.** Relative abundance of the three top phyla (Actinobacteriota, Firmicutes, and Proteobacteria) for (**a**) the face and (**b**) the forearm, at baseline (D0) and post-treatment (D29), split by sex.

## 4. Discussion

The stratum corneum hosts a diverse ecosystem, with different microbial species colonizing various body sites [2,15–19]. These microbial communities have been shown to actively contribute to skin homeostasis and health [1], and microbiome dysbiosis has been linked with a variety of skin disorders, such as atopic dermatitis, psoriasis, and acne [2,20]. In addition to substances such as sebum, which are produced by the body, the skin is also exposed to a wide variety of external chemical agents including topical products and even pollution, all of which can be expected to have an impact on its properties and therefore the microbiome that is present [21–23]. It is therefore important to understand microbial population variations and the impacts of topical products. In this study, topical product usage had a relatively minor impact on the skin microbiome composition after 4 weeks of use. While no significant changes in overall diversity were found, some changes at the species/genus/phylum levels were observed, including for untreated sites (Table 4). Ingredients like xanthan gum, commonly used in topical products [24–26], may benefit dry skin as previously reported [4]. Xanthan gum was present in a number of the products tested here, and the level present on the skin was found to have increased after usage of products DBL and DHC. This has potential skin benefits when used on dry skin as the Xanthomonas genus has a specific enzymatic system for hydrolyzing keratin [26]. This emphasizes the need to fully understand the composition of the products being used on skin when interpreting microbiome assessments.

How do the results presented here compare with previous studies on the effects of topical products on the skin microbiome? Numerous studies have examined cosmetic effects, including those involving pre-, pro-, or post-biotic ingredients, with claims of enhancing or preserving bacterial diversity [27–30]. Makeup usage has been reported to increase microbiome diversity more than moisturizers and cleansers [31]. While many studies assert improvements in microbiome structure and composition, it is crucial to consider the influence of the study design. For example, some studies applied products twice daily [32–35], while others combined them with the participants' existing routines [36]. Conversely, some research indicates no significant impact on diversity [37], and certain product ingredients

may even harm microbiome diversity [38], highlighting the need for caution in product development. Although research regarding what constitutes "microbiome-friendly" topical products is still in its infancy, it is logical for such products to avoid drastically impacting an already healthy skin microbiome [39]. Additionally, ensuring that study designs accurately reflect real-life product usage is essential to avoid skewed results.

Skin microbiome composition and diversity, while typically stable in the short term, can vary significantly with age, sex, and life events such as puberty, pregnancy, and menopause [16,40–43]. In our data, variations in the richness of the facial microbiome by age group were observed, with the oldest age group (60+ years) showing the greatest richness. Significant differences in both "richness" ($p = 0.001$) and Shannon diversity index ($p < 0.001$) of the face were noted by age and sex. Although no significant differences in "richness" ($p = 0.063$) of the forearm by age and sex were found, a significant difference in Shannon diversity index was observed on the forearm ($p < 0.001$). Generally, microbiome diversity is reported to increase with age, which aligns with our findings [44–46]. While skin microbial composition in healthy subjects is typically stable during adulthood [40,45], physiological changes associated with aging—such as variations in sebum secretion and immune function, as well as decreased sweat production—may impact the skin microbiome of older individuals. Notably, significant modifications in the structure and composition of the skin microbiome have been observed with increased age [47], particularly in females [48]. The skin's ability to retain moisture and its sebum production capabilities are influenced by both aging and sex [49]. Male skin microbiome composition varies dramatically across the first 25 years of life, with an increase in diversity being seen with increasing age [50]. An increase in skin alpha diversity has been reported for post-menopausal females following a decrease in sebaceous gland activity and the subsequent reduction in skin sebum levels [48].

It is often assumed by the consumer that increased microbial diversity on skin is better; this notion requires further exploration. Existing research suggests that drier skin sites tend to be more diverse than those with moist or sebaceous skin [51]. At other sites the question of diversity is further complicated. For example, a healthy vaginal microbiome typically has low microbiome diversity, while a healthy gut microbiome has high diversity [52,53]. The potential for cross talk between these different microbiomes should also be considered [54,55]. Given the variability of the microbiome across the body and during life, rather than simply aiming for increased diversity with the use of topical moisturizing products, there is a growing emphasis on creating products that respect the microbiome present on the skin, especially on skin that is classified as "healthy" as opposed to being in a disease state, as the composition of skin flora is reflective of the changes occurring in the body with age [27,39,56,57].

Specific bacterial species have been linked with a range of skin conditions such as xerosis, atopic dermatitis (AD), and psoriasis [4–8]. However, it is not always clear whether these relationships are causal or correlative [58]. Average bacterial counts of the three most prevalent species (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Cutibacterium acnes*, and others) at baseline (D0) for both females and males, grouped by body site, are shown in Table 5.

Although the overall levels of *S. aureus* are relatively low compared with other bacterial species, females appear to have more of it present than males. Elevated *S. aureus* levels are a potential issue for skin as this can result in increased α-toxin and proteases that can damage the skin barrier and lead to inflammation [59]. *S. aureus* in particular has been widely reported to be elevated in subjects with AD but also to be an initial colonizer of dry skin regions [5,60–63] and is a strong driver of the "itch–scratch cycle" by its ability to directly activate pruriceptor sensory neurons to drive itch [64]. The available study data of differences in skin hydration between males and females widely vary [65]. However, despite strong clinical or objective data on the subject, females do report experiencing dry skin more frequently than males [66]. Elevated baseline levels of *S. aureus* may indicate a higher propensity of females to develop issues with dry, sensitive skin, which would be in line with existing data on sex-related differences in skin sensitivity [67,68]. This emphasizes

the important ongoing need to adequately hydrate and moisturize the skin, especially for females.

**Table 5.** Average bacterial counts of the three most prevalent species (*S. epidermidis*, *S. aureus*, *C. acnes*, and others) for females and males, grouped by body site. All subjects at D0 are included. SD—standard deviation; CI—confidence interval; N—number of subjects.

| Species | Body Site | Sex | Average Count (SD) | Lower CI | Upper CI | N |
|---|---|---|---|---|---|---|
| *S. epidermidis* | Face | Female | 4250.56 (5083.19) | 3352.22 | 5148.90 | 62 |
| | | Male | 7150.76 (9486.93) | 4643.50 | 9658.03 | 28 |
| | Forearm | Female | 1607.17 (2122.55) | 1232.06 | 1982.28 | 63 |
| | | Male | 2441.20 (2658.55) | 1744.85 | 3137.54 | 28 |
| *S. aureus* | Face | Female | 87.57 (317.14) | 14.31 | 160.83 | 36 |
| | | Male | 9.38 (38.85) | −6.81 | 25.57 | 11 |
| | Forearm | Female | 91.43 (370.03) | 4.12 | 178.75 | 36 |
| | | Male | 0.00 (0.00) | 0.00 | 0.00 | 11 |
| *C. acnes* | Face | Female | 21,448.19 (18,560.41) | 18,168.06 | 24,728.32 | 62 |
| | | Male | 31,771.31 (19,974.18) | 26,492.41 | 37,050.21 | 28 |
| | Forearm | Female | 6461.51 (9054.49) | 4861.34 | 8061.69 | 63 |
| | | Male | 8494.68 (9603.19) | 5979.45 | 11,009.91 | 28 |
| Other | Face | Female | 32.28 (391.06) | 29.51 | 35.06 | 62 |
| | | Male | 36.86 (535.64) | 31.08 | 42.63 | 28 |
| | Forearm | Female | 39.48 (440.08) | 36.34 | 42.62 | 63 |
| | | Male | 46.26 (480.83) | 41.13 | 51.39 | 28 |

## 5. Future Opportunities

Future research should focus on elucidating the complexities of the skin microbiome and its interaction with topical products. Investigating the specific mechanisms by which various ingredients influence microbial diversity and composition will be essential. Additionally, longitudinal studies that monitor changes in the skin microbiome over time, particularly in response to different life stages and environmental factors, could provide valuable insights.

Understanding the implications of microbial diversity in relation to skin health will be crucial. This includes determining whether increased diversity is genuinely beneficial or if it varies based on the specific context of skin conditions. More targeted studies should explore the relationship between specific bacterial species and skin disorders, aiming to clarify the causative factors involved. Furthermore, the development of "microbiome-friendly" products should be prioritized, ensuring they do not disrupt the natural balance of the skin microbiome, especially on healthy skin. Research should also assess the real-world application of these products, considering consumer usage patterns and preferences.

Finally, interdisciplinary approaches that integrate dermatology, microbiology, and consumer science may yield comprehensive strategies for enhancing skin health while respecting the delicate balance of the microbiome. As we advance our understanding of the skin microbiome, it will be essential to communicate findings effectively to both the scientific community and consumers, fostering informed decisions regarding skin care products.

## 6. Limitations

It is important to discuss the limitations of retrospective analysis. There was a sample size discrepancy between sexes, with a reduced representation of males ($n$ = 28) compared

with females ($n$ = 63). Additionally, there was an uneven distribution of subjects across various age groups, and the overall base size was low. Although attempts were made to address this imbalance through age stratification, it resulted in the creation of relatively small groups with unequal age ranges, deviating from standard age stratification. Further work is required to focus on specific age groups to further explore the initial results presented here. These clinical studies and the retrospective analysis solely focus on assessing the evolution of the skin microbiome, without considering other skin biomarkers such as hydration levels and housing habits (urban vs. rural), which have been reported to impact microbial diversity [69,70]. The study population was predominantly Caucasian (described as White in Table 1), so it was not possible to examine the effects of ethnicity on microbiome composition. Among the genetic factors that shape the skin microbiome, ethnicity appears to be a secondary, although not insignificant, one [71]. The microbiome is assumed here to be bacterial in nature; however, bacteria form only part of the living ecosystem present on the stratum corneum. For example, fungi can also be considered as part of the overall microbiome of the skin, and they can have a dramatic impact on its health and function [72–75]. Incorporating multiple skin biomarkers could lead to a more comprehensive understanding of the dynamics of the skin microbiome. In addition to parameters such as bacterial diversity, more research is required to assess the metabolite pathways at the molecular level and its impact on the microbiome. Given their potential to act as a food source for bacteria, the interaction of ingredients in the topical formulation with the microbiome will be the subject of future work.

Finally, while the sample size was sufficient for detecting significant changes, a larger cohort would enhance the generalizability of the findings. The study was conducted within a European population, and results may not be applicable to other geographic regions with differing climates and lifestyles

## 7. Conclusions

Microbiome composition is important to skin health, form, and function. However, the skin is a complex structure and subject to changes as a function of age, sex, and body site and the use of topical products. It is therefore important to understand how these intrinsic and extrinsic factors impact microbiome composition. Four studies were carried out to determine the effects of long-term (4-week) use of different dexpanthenol-containing topical skin care products on the skin microbiome of a varied population with cosmetically dry skin. The skin microbiome composition was assessed before and after product usage. No significant changes in microbiome richness or diversity were found for the individual test products; however, a meta-analysis of the combined dataset did show changes in microbiome composition as a function of subject's sex, age, and body site. While low microbial diversity is linked with certain skin conditions such as acne vulgaris, it is not sufficient to state that increased diversity is representative of better quality skin. It is important to demonstrate that the use of carefully formulated topical products on skin, when used in a way that is representative of real-life usage conditions, can respect the microbial diversity present on skin across a widely varied study population without significantly impacting the natural diversity that is present.

**Supplementary Materials:** The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/cosmetics11060213/s1, Section S1: Test product ingredients. Section S2: Microbiome assessment.

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## References

1. Sfriso, R.; Egert, M.; Gempeler, M.; Voegeli, R.; Campiche, R. Revealing the secret life of skin—With the microbiome you never walk alone. *Int. J. Cosmet. Sci.* **2020**, *42*, 116–126. [CrossRef] [PubMed]
2. Byrd, A.L.; Belkaid, Y.; Segre, J.A. The human skin microbiome. *Nat. Rev. Microbiol.* **2020**, *16*, 143–155. [CrossRef] [PubMed]
3. Townsend, E.C.; Kalan, L.R. The dynamic balance of the skin microbiome across the lifespan. *Biochem. Soc. Trans.* **2023**, *51*, 71–86. [CrossRef] [PubMed] [PubMed Central]
4. Murphy, B.; Grimshaw, S.; Hoptroff, M.; Paterson, S.; Arnold, D.; Cawley, A.; Adams, S.E.; Falciani, F.; Dadd, T.; Eccles, R.; et al. Alteration of barrier properties, stratum corneum ceramides and microbiome composition in response to lotion application on cosmetic dry skin. *Sci. Rep.* **2022**, *12*, 5223. [CrossRef] [PubMed]
5. Ogonowska, P.; Szymczak, K.; Empel, J.; Urbaś, M.; Woźniak-Pawlikowska, A.; Barańska-Rybak, W.; Świetlik, D.; Nakonieczna, J. *Staphylococcus aureus* from Atopic Dermatitis Patients: Its Genetic Structure and Susceptibility to Phototreatment. *Microbiol. Spectr.* **2023**, *11*, e04598-22. [CrossRef]
6. Wrześniewska, M.; Wołoszczak, J.; Świrkosz, G.; Szyller, H.; Gomułka, K. The Role of the Microbiota in the Pathogenesis and Treatment of Atopic Dermatitis-A Literature Review. *Int. J. Mol. Sci.* **2024**, *25*, 6539. [CrossRef] [PubMed] [PubMed Central]
7. Korneev, A.; Peshkova, M.; Koteneva, P.; Gundogdu, A.; Timashev, P. Modulation of the skin and gut microbiome by psoriasis treatment: A comprehensive systematic review. *Arch. Dermatol. Res.* **2024**, *316*, 374. [CrossRef] [PubMed]
8. Thio, H.B. The Microbiome in Psoriasis and Psoriatic Arthritis: The Skin Perspective. *J. Rheumatol. Suppl.* **2018**, *94*, 30–31. [CrossRef] [PubMed]
9. Stettler, H.; de Salvo, R.; Olsavszky, R.; Nanu, E.A.; Dumitru, V.; Trapp, S. Performance and Tolerability of a New Topical Dexpanthenol-Containing Emollient Line in Subjects with Dry Skin: Results from Three Randomized Studies. *Cosmetics* **2021**, *8*, 18. [CrossRef]
10. Barrionuevo-Gonzalez, A.; Trapp, S.; de Salvo, R.; Reitmann, M.; Cassar, E.; Rharbaoui, S.; Reber, F.; Stettler, H. Three New Dexpanthenol-Containing Face Creams: Performance and Acceptability after Single and Repeated Applications in Subjects of Different Ethnicities with Dry and Sensitive Skin. *Cosmetics* **2021**, *8*, 93. [CrossRef]
11. Barrionuevo-Gonzalez, A.; Trapp, S.; de Salvo, R.; Olsavszky, R.; Nanu, E.A.; Dopcea, I.; Dumitru, V.; Stettler, H. Dexpanthenol-Containing Wash Gels: Skin Hydration, Barrier Function, and Cosmetic Performance Upon Single and Repeated Usage in Subjects with Dry Skin. *Cosmetics* **2021**, *8*, 44. [CrossRef]
12. Proksch, E.; de Bony, R.; Trapp, S.; Boudon, S. Topical use of dexpanthenol: A 70th anniversary article. *J. Dermatolog. Treat.* **2017**, *28*, 766–773. [CrossRef] [PubMed]
13. Stettler, H.; Crowther, J.M.; Brandt, M.; Boxshall, A.; Lu, B.; de Salvo, R.; Laing, S.; Hennighausen, N.; Bielfeldt, S.; Blenkiron, P. Multi parametric biophysical assessment of treatment effects on xerotic skin. *Skin Health Dis.* **2021**, *1*, e21. [CrossRef] [PubMed] [PubMed Central]
14. Qaizar, Z.; Sun, C.; Unbereit, K.; Stettler, H. Skin microbiome effects of long term dexpanthenol based gentle wash and emollient products formulated for dry skin. In Proceedings of the European Academy of Dermatology and Venereology Conference, Berlin, Germany, 11–14 October 2023.
15. Kligman, A.M. A brief history of how the dead stratum corneum became alive. In *Skin Barrier*; Elias, P.M., Feingold, K.R., Eds.; Informa Healthcare Inc.: New York, NY, USA, 2010; pp. 15–24.
16. Pagac, M.P.; Stalder, M.; Campiche, R. Menopause and facial skin microbiomes: A pilot study revealing novel insights into their relationship. *Front. Aging* **2024**, *5*, 1353082. [CrossRef] [PubMed] [PubMed Central]
17. Crowther, J.M. Stratum corneum or stratum ecologica? *Int. J. Cosmet. Sci.* **2019**, *41*, 200–201. [CrossRef] [PubMed]
18. Pérez-Losada, M.; Crandall, K.A. Spatial diversity of the skin bacteriome. *Front. Microbiol.* **2023**, *14*, 1257276. [CrossRef] [PubMed] [PubMed Central]

19. Brandwein, M.; Fuks, G.; Israel, A.; Hodak, E.; Sabbah, F.; Steinberg, D.; Bentwich, Z.; Shental, N.; Meshner, S. Biogeographical Landscape of the Human Face Skin Microbiome Viewed in High Definition. *Acta Derm. Venereol.* **2021**, *101*, adv00603. [CrossRef] [PubMed] [PubMed Central]
20. Kim, J.; Park, T.; Kim, H.J.; An, S.; Sul, W.J. Inferences in microbial structural signatures of acne microbiome and mycobiome. *J. Microbiol.* **2021**, *59*, 369–375. [CrossRef] [PubMed]
21. McCall, L.I.; Callewaert, C.; Zhu, Q.; Song, S.J.; Bouslimani, A.; Minich, J.J.; Ernst, M.; Ruiz-Calderon, J.F.; Cavallin, H.; Pereira, H.S.; et al. Home chemical and microbial transitions across urbanization. *Nat. Microbiol.* **2020**, *5*, 108–115. [CrossRef] [PubMed] [PubMed Central]
22. Gu, X.; Li, Z.; Su, J. Air pollution and skin diseases: A comprehensive evaluation of the associated mechanism. *Ecotoxicol. Environ. Saf.* **2024**, *278*, 116429. [CrossRef] [PubMed]
23. Wang, D.Q.; Li, X.; Zhang, R.Y.; Yuan, C.; Yan, B.; Humbert, P.; Quan, Z.X. Effects of Investigational Moisturizers on the Skin Barrier and Microbiome following Exposure to Environmental Aggressors: A Randomized Clinical Trial and Ex Vivo Analysis. *J. Clin. Med.* **2023**, *12*, 6078. [CrossRef] [PubMed] [PubMed Central]
24. Palaniraj, A.; Jayaraman, V. Production, recovery and applications of xanthan gum by *Xanthomonas campestris*. *J. Food Eng.* **2011**, *106*, 1–12. [CrossRef]
25. Dai, X.; Gao, G.; Wu, M.; Wei, W.; Qu, J.; Li, G.; Ma, T. Construction and application of a *Xanthomonas campestris* CGMCC15155 strain that produces white xanthan gum. *MicrobiologyOpen* **2019**, *8*, e631. [CrossRef] [PubMed]
26. De Toni, C.H.; Richter, M.F.; Chagas, J.R.; Henriques, J.A.; Termignoni, C. Purification and characterization of an alkaline serine endopeptidase from a feather-degrading *Xanthomonas maltophilia* strain. *Can. J. Microbiol.* **2002**, *48*, 342–348. [CrossRef] [PubMed]
27. Han, J.H.; Kim, H.S. Skin Deep: The Potential of Microbiome Cosmetics. *J. Microbiol.* **2024**, *62*, 181–199. [CrossRef] [PubMed]
28. Woolery-Lloyd, H.; Andriessen, A.; Day, D.; Gonzalez, N.; Green, L.; Grice, E.; Henry, M. Review of the microbiome in skin aging and the effect of a topical prebiotic containing thermal spring water. *J. Cosmet. Dermatol.* **2023**, *22*, 96–102. [CrossRef] [PubMed]
29. Li, M.; Mao, J.; Diaz, I.; Kopylova, E.; Melnik, A.V.; Aksenov, A.A.; Tipton, C.D.; Soliman, N.; Morgan, A.M.; Boyd, T. Multi-omic approach to decipher the impact of skincare products with pre/postbiotics on skin microbiome and metabolome. *Front. Med.* **2023**, *10*, 1165980. [CrossRef] [PubMed] [PubMed Central]
30. De Almeida, C.V.; Antiga, E.; Lulli, M. Oral and Topical Probiotics and Postbiotics in Skincare and Dermatological Therapy: A Concise Review. *Microorganisms* **2023**, *11*, 1420. [CrossRef] [PubMed] [PubMed Central]
31. Staudinger, T.; Pipal, A.; Redl, B. Molecular analysis of the prevalent microbiota of human male and female forehead skin compared to forearm skin and the influence of make-up. *J. Appl. Microbiol.* **2011**, *110*, 1381–1389. [CrossRef] [PubMed]
32. Santamaria, E.; Åkerström, U.; Berger-Picard, N.; Lataste, S.; Gillbro, J.M. Randomized comparative double-blind study assessing the difference between topically applied microbiome supporting skincare versus conventional skincare on the facial microbiome in correlation to biophysical skin parameters. *Int. J. Cosmet. Sci.* **2023**, *45*, 83–94. [CrossRef] [PubMed]
33. Lee, H.J.; Jeong, S.E.; Lee, S.; Kim, S.; Han, H.; Jeon, C.O. Effects of cosmetics on the skin microbiome of facial cheeks with different hydration levels. *MicrobiologyOpen* **2018**, *7*, e00557. [CrossRef] [PubMed] [PubMed Central]
34. Cui, S.; Pan, M.; Tang, X.; Liu, G.; Mao, B.; Zhao, J.; Yang, K. Metagenomic insights into the effects of cosmetics containing complex polysaccharides on the composition of skin microbiota in females. *Front. Cell. Infect. Microbiol.* **2023**, *13*, 1210724. [CrossRef] [PubMed] [PubMed Central]
35. Iglesia, S.; Kononov, T.; Zahr, A.S. A multi-functional anti-aging moisturizer maintains a diverse and balanced facial skin microbiome. *J. Appl. Microbiol.* **2022**, *133*, 1791–1799. [CrossRef] [PubMed]
36. Hwang, B.K.; Lee, S.; Myoung, J.; Hwang, S.J.; Lim, J.M.; Jeong, E.T.; Park, S.G.; Youn, S.H. Effect of the skincare product on facial skin microbial structure and biophysical parameters: A pilot study. *MicrobiologyOpen* **2021**, *10*, e1236. [CrossRef] [PubMed] [PubMed Central]
37. Callejon, S.; Giraud, F.; Larue, F.; Buisson, A.; Mateos, L.; Grare, L.; Guyoux, A.; Perrier, E.; Ardiet, N.; Trompezinski, S. Impact of Leave-on Skin Care Products on the Preservation of Skin Microbiome: An Exploration of Ecobiological Approach. *Clin. Cosmet. Investig. Dermatol.* **2023**, *16*, 2727–2735. [CrossRef] [PubMed] [PubMed Central]
38. Zhao, H.; Yu, F.; Wang, C.; Han, Z.; Liu, S.; Chen, D.; Liu, D.; Meng, X.; He, X.; Huang, Z. The impacts of sodium lauroyl sarcosinate in facial cleanser on facial skin microbiome and lipidome. *J. Cosmet. Dermatol.* **2024**, *23*, 1351–1359. [CrossRef] [PubMed]
39. van Belkum, A.; Lisotto, P.; Pirovano, W.; Mongiat, S.; Zorgani, A.; Gempeler, M.; Bongoni, R.; Klaassens, E. Being friendly to the skin microbiome: Experimental assessment. *Front. Microbiomes* **2023**, *1*, 1077151. [CrossRef]
40. Oh, J.; Byrd, A.L.; Park, M.; NISC Comparative Sequencing Program; Kong, H.H.; Segre, J.A. Temporal Stability of the Human Skin Microbiome. *Cell* **2016**, *165*, 854–866. [CrossRef] [PubMed] [PubMed Central]
41. Park, J.; Schwardt, N.H.; Jo, J.H.; Zhang, Z.; Pillai, V.; Phang, S.; Brady, S.M.; Portillo, J.A.; MacGibeny, M.A.; Liang, H.; et al. Shifts in the Skin Bacterial and Fungal Communities of Healthy Children Transitioning through Puberty. *J. Investig. Dermatol.* **2022**, *142*, 212–219. [CrossRef] [PubMed] [PubMed Central]
42. Leem, S.; Keum, H.L.; Song, H.J.; Gu, K.N.; Kim, Y.; Seo, J.Y.; Shin, J.G.; Lee, S.G.; Lee, S.M.; Sul, W.J.; et al. Skin aging-related microbial types separated by Cutibacterium and α-diversity. *J. Cosmet. Dermatol.* **2024**, *23*, 1066–1074. [CrossRef] [PubMed]
43. Radocchia, G.; Brunetti, F.; Marazzato, M.; Totino, V.; Neroni, B.; Bonfiglio, G.; Conte, A.L.; Pantanella, F.; Ciolli, P.; Schippa, S. Women Skin Microbiota Modifications during Pregnancy. *Microorganisms* **2024**, *12*, 808. [CrossRef] [PubMed] [PubMed Central]

44. Somerville, D.A. The normal flora of the skin in different age groups. *Br. J. Dermatol.* **1969**, *81*, 248–258. [CrossRef] [PubMed]
45. Shibagaki, N.; Suda, W.; Clavaud, C.; Bastien, P.; Takayasu, L.; Iioka, E.; Kurokawa, R.; Yamashita, N.; Hattori, Y.; Shindo, C.; et al. Aging-related changes in the diversity of women's skin microbiomes associated with oral bacteria. *Sci. Rep.* **2017**, *7*, 10567. [CrossRef] [PubMed] [PubMed Central]
46. Murphy, B.; Hoptroff, M.; Arnold, D.; Cawley, A.; Smith, E.; Adams, S.E.; Mitchell, A.; Horsburgh, M.J.; Hunt, J.; Dasgupta, B.; et al. Compositional Variations between Adult and Infant Skin Microbiome: An Update. *Microorganisms* **2023**, *11*, 1484. [CrossRef] [PubMed] [PubMed Central]
47. Leyden, J.J.; McGinley, K.J.; Mills, O.H.; Kligman, A.M. Age-related changes in the resident bacterial flora of the human face. *J. Investig. Dermatol.* **1975**, *65*, 379–381. [CrossRef] [PubMed]
48. Kim, J.H.; Son, S.M.; Park, H.; Kim, B.K.; Choi, I.S.; Kim, H.; Huh, C.S. Taxonomic profiling of skin microbiome and correlation with clinical skin parameters in healthy Koreans. *Sci. Rep.* **2021**, *11*, 16269. [CrossRef] [PubMed] [PubMed Central]
49. Luebberding, S.; Krueger, N.; Kerscher, M. Skin physiology in men and women: In vivo evaluation of 300 people including TEWL, SC hydration, sebum content and skin surface pH. *Int. J. Cosmet. Sci.* **2013**, *35*, 477–483. [CrossRef] [PubMed]
50. Hu, X.; Tang, M.; Dong, K.; Zhou, J.; Wang, D.; Song, L. Changes in the skin microbiome during male maturation from 0 to 25 years of age. *Skin Res. Technol.* **2023**, *29*, e13432. [CrossRef] [PubMed] [PubMed Central]
51. Russo, E.; Di Gloria, L.; Cerboneschi, M.; Smeazzetto, S.; Baruzzi, G.P.; Romano, F.; Ramazzotti, M.; Amedei, A. Facial Skin Microbiome: Aging-Related Changes and Exploratory Functional Associations with Host Genetic Factors, a Pilot Study. *Biomedicines* **2023**, *11*, 684. [CrossRef] [PubMed] [PubMed Central]
52. Ottinger, S.; Robertson, C.M.; Branthoover, H.; Patras, K.A. The human vaginal microbiota: From clinical medicine to models to mechanisms. *Curr. Opin. Microbiol.* **2024**, *77*, 102422. [CrossRef] [PubMed] [PubMed Central]
53. Lozupone, C.A.; Stombaugh, J.I.; Gordon, J.I.; Jansson, J.K.; Knight, R. Diversity, stability and resilience of the human gut microbiota. *Nature* **2012**, *489*, 220–230. [CrossRef] [PubMed] [PubMed Central]
54. Chaudhary, P.P.; Kaur, M.; Myles, I.A. Does "all disease begin in the gut"? The gut-organ cross talk in the microbiome. *Appl. Microbiol. Biotechnol.* **2024**, *108*, 339. [CrossRef] [PubMed] [PubMed Central]
55. Zhou, Z.; Feng, Y.; Xie, L.; Ma, S.; Cai, Z.; Ma, Y. Alterations in gut and genital microbiota associated with gynecological diseases: A systematic review and meta-analysis. *Reprod. Biol. Endocrinol.* **2024**, *22*, 13. [CrossRef] [PubMed] [PubMed Central]
56. Wallen-Russell, C.; Wallen-Russell, S. Meta Analysis of Skin Microbiome: New Link between Skin Microbiota Diversity and Skin Health with Proposal to Use This as a Future Mechanism to Determine Whether Cosmetic Products Damage the Skin. *Cosmetics* **2017**, *4*, 14. [CrossRef]
57. Carvalho, M.J.; Oliveira, A.L.S.; Santos Pedrosa, S.; Pintado, M.; Pinto-Ribeiro, I.; Madureira, A.R. Skin Microbiota and the Cosmetic Industry. *Microb. Ecol.* **2023**, *86*, 86–96. [CrossRef] [PubMed]
58. Hwang, J.; Thompson, A.; Jaros, J.; Blackcloud, P.; Hsiao, J.; Shi, V.Y. Updated understanding of Staphylococcus aureus in atopic dermatitis: From virulence factors to commensals and clonal complexes. *Exp. Dermatol.* **2021**, *30*, 1532–1545. [CrossRef] [PubMed]
59. Blicharz, L.; Rudnicka, L.; Samochocki, Z. *Staphylococcus aureus*: An underestimated factor in the pathogenesis of atopic dermatitis? *Postepy. Dermatol. Alergol.* **2019**, *36*, 11–17. [CrossRef] [PubMed] [PubMed Central]
60. Tauber, M.; Balica, S.; Hsu, C.Y.; Jean-Decoster, C.; Lauze, C.; Redoules, D.; Viodé, C.; Schmitt, A.M.; Serre, G.; Simon, M.; et al. *Staphylococcus aureus* density on lesional and nonlesional skin is strongly associated with disease severity in atopic dermatitis. *J. Allergy. Clin. Immunol.* **2016**, *137*, 1272–1274.e3. [CrossRef] [PubMed]
61. Cleary, J.M.; Lipsky, Z.W.; Kim, M.; Marques, C.N.H.; German, G.K. Heterogeneous ceramide distributions alter spatially resolved growth of *Staphylococcus aureus* on human stratum corneum. *J. R. Soc. Interface* **2018**, *15*, 20170848. [CrossRef] [PubMed] [PubMed Central]
62. Imokawa, G.; Abe, A.; Jin, K.; Higaki, Y.; Kawashima, M.; Hidano, A. Decreased level of ceramides in stratum corneum of atopic dermatitis: An etiologic factor in atopic dry skin? *J. Investig. Dermatol.* **1991**, *96*, 523–526. [CrossRef]
63. Watanabe, M.; Tagami, H.; Horii, I.; Takahashi, M.; Kligman, A.M. Functional analyses of the superficial stratum corneum in atopic xerosis. *Arch. Dermatol.* **1991**, *127*, 1689–1692. [CrossRef] [PubMed]
64. Deng, L.; Costa, F.; Blake, K.J.; Choi, S.; Chandrabalan, A.; Yousuf, M.S.; Shiers, S.; Dubreuil, D.; Vega-Mendoza, D.; Rolland, C.; et al. *S. aureus* drives itch and scratch-induced skin damage through a V8 protease-PAR1 axis. *Cell* **2023**, *186*, 5375–5393.e25. [CrossRef] [PubMed]
65. Rahrovan, S.; Fanian, F.; Mehryan, P.; Humbert, P.; Firooz, A. Male versus female skin: What dermatologists and cosmeticians should know. *Int. J. Womens Dermatol.* **2018**, *4*, 122–130. [CrossRef] [PubMed] [PubMed Central]
66. Jemec, G.B.; Serup, J. Scaling, dry skin and gender. A bioengineering study of dry skin. *Acta Derm. Venereol. Suppl.* **1992**, *177*, 26–28. [CrossRef] [PubMed]
67. Wang, X.; Su, Y.; Zheng, B.; Wen, S.; Liu, D.; Ye, L.; Yan, Y.; Elias, P.M.; Yang, B.; Man, M.Q. Gender-related characterization of sensitive skin in normal young Chinese. *J. Cosmet. Dermatol.* **2020**, *19*, 1137–1142. [CrossRef] [PubMed] [PubMed Central]
68. Farage, M.A. The Prevalence of Sensitive Skin. *Front. Med.* **2019**, *6*, 98. [CrossRef]
69. Ying, S.; Zeng, D.N.; Chi, L.; Tan, Y.; Galzote, C.; Cardona, C.; Lax, S.; Gilbert, J.; Quan, Z.X. The Influence of Age and Gender on Skin-Associated Microbial Communities in Urban and Rural Human Populations. *PLoS ONE* **2015**, *10*, e0141842. [CrossRef] [PubMed] [PubMed Central]
70. Callewaert, C.; Ravard Helffer, K.; Lebaron, P. Skin Microbiome and its Interplay with the Environment. *Am. J. Clin. Dermatol.* **2020**, *21* (Suppl. S1), 4–11. [CrossRef] [PubMed] [PubMed Central]

71. Skowron, K.; Bauza-Kaszewska, J.; Kraszewska, Z.; Wiktorczyk-Kapischke, N.; Grudlewska-Buda, K.; Kwiecińska-Piróg, J.; Wałecka-Zacharska, E.; Radtke, L.; Gospodarek-Komkowska, E. Human Skin Microbiome: Impact of Intrinsic and Extrinsic Factors on Skin Microbiota. *Microorganisms* **2021**, *9*, 543. [CrossRef] [PubMed] [PubMed Central]
72. Nguyen, U.T.; Kalan, L.R. Forgotten fungi: The importance of the skin mycobiome. *Curr. Opin. Microbiol.* **2022**, *70*, 102235. [CrossRef] [PubMed] [PubMed Central]
73. Jo, J.H.; Kennedy, E.A.; Kong, H.H. Topographical and physiological differences of the skin mycobiome in health and disease. *Virulence* **2017**, *8*, 324–333. [CrossRef] [PubMed] [PubMed Central]
74. Meray, Y.; Gençalp, D.; Güran, M. Putting It All Together to Understand the Role of *Malassezia* spp. in Dandruff Etiology. *Mycopathologia* **2018**, *183*, 893–903. [CrossRef] [PubMed]
75. Leyden, J.J.; Kligman, A.M. Interdigital athlete's foot: New concepts in pathogenesis. *Postgrad. Med.* **1977**, *61*, 113–116. [CrossRef] [PubMed]