**1.Introduction:**

The skin, one of the body’s largest organs, undergoes aging-related degeneration like all other tissues. It is also the most visibly affected by aging **(Zouboulis and Boschnakow, 2001)**. As the skin ages, the risk of skin conditions, including tumors, increases, often accompanied by psychological stress due to changes in appearance. While public health has traditionally focused on age-related conditions like arthritis, cardiovascular disease, and cancer **(Kligman and Koblenzer, 1997)**, the aging of the skin and related disorders are gaining more attention.

In industrialized countries, many women spend over a third of their lives in the postmenopausal phase **(Kligman and Koblenzer, 1997)**, during which visible aging signs become particularly significant. Skin aging is influenced by several factors including genetics, hormonal changes, UV exposure, lifestyle choices (such as diet, smoking, alcohol, and drug use), infections, tumors, and other environmental influences. Many women experience a noticeable acceleration of skin aging symptoms during menopause, such as increased dryness, reduced firmness and elasticity, and sagging. These symptoms are linked to biological changes like reduced collagen and elastin production, altered collagen type ratios, changes in the extracellular matrix, and decreased blood supply **(Brincat, 2000)**. These visible signs are also supported by histological changes seen in the skin **(Broniarczyk-Dyla and Joss-Wichman, 2001).**

Acne is primarily influenced by genetic and hormonal factors. An increase in androgens during puberty stimulates the sebaceous glands to produce more sebum, contributing to acne development. In women, hormonal fluctuations—especially those occurring before menstruation—can also trigger breakouts.

Another key factor is the bacterial colonization of the skin by *Cutibacterium acnes* (formerly *Propionibacterium acnes*), which plays a significant role in acne pathogenesis.

In addition, several other elements can contribute to the onset of acne. These include environmental factors such as climate changes; the use of certain medications like anabolic steroids, corticosteroids, and progestin-only contraceptives; dietary habits such as high intake of dairy products or foods with a high glycemic index; the prolonged application of oil-based cosmetics; and psychological influences, including stress, anger, and anxiety **(Acne treatment 2)**

Acne Vulgaris is a persistent inflammatory skin condition that primarily affects the pilosebaceous units on the face, particularly during adolescence. It presents with various lesions such as comedones, papules, pustules, nodules, and cysts. Azelaic acid (AZA) is a proven anti-acne agent that is effective against both inflammatory and non-inflammatory types of acne, with a stronger impact on the inflammatory form. It is commonly formulated in 15% and 20% concentrations. **(Article 2)**

AZA is now widely recommended by clinicians as a second-line treatment option. Research indicates that it is also beneficial for maintenance therapy, helping to prolong acne-free periods with results similar to those of adapalene. Given its well-established efficacy and strong safety profile, European evidence-based guidelines endorse AZA with moderate recommendation strength for acne management. The use of AZA in treating acne continues to rise in clinical practice. **(Article 2)**

Melasma is a long-term, acquired pigmentation disorder that affects facial skin, typically appearing as light to dark brown patches with indistinct borders, symmetrically located on areas such as the cheeks, forehead, and jawline. Although hydroquinone (HQ) is considered the gold standard for treating melasma, prolonged use can result in adverse effects like permanent skin discoloration and ochronosis. As a result, there is a growing need for safer topical alternatives. Azelaic acid (AZA), used in concentrations ranging from 15% to 25%, is regarded as a safer and effective substitute for HQ in managing melasma. **(Article 2)**

Psoriasis is a chronic inflammatory skin disease with an immune-related origin, typically characterized by red, scaly patches. Emerging research suggests that azelaic acid (AZA) may help alleviate psoriasis symptoms. In a single-blind randomized clinical trial, patients applied either 15% AZA gel or a placebo to symmetrical lesions on opposite sides of their bodies twice daily for one month. Compared to the placebo, AZA significantly reduced symptoms such as itching, scaling, and plaque thickening (hyperkeratosis), demonstrating notable clinical improvement. **(Article 2)**

Alopecia areata is an autoimmune condition that causes non-scarring, temporary hair loss. In a study evaluating the effectiveness of 20% AZA cream, patients were compared to a control group using 0.05% topical clobetasol propionate cream, applied nightly over a 12-week period with monthly follow-ups. The findings showed that 20% AZA cream had acceptable efficacy in comparison, indicating its potential as a viable topical treatment for alopecia areata. Another study comparing 20% AZA to 0.5% anthralin also supported its therapeutic benefits. **(Article 2)**

Folliculitis is a frequently recurring skin disorder characterized by inflamed papules and pustules on various parts of the body. According to research, applying 15% AZA foam twice daily for four weeks led to a 78% reduction in folliculitis symptoms. These results suggest that AZA foam may be a promising treatment option or complementary therapy for managing folliculitis. **(Article 2)**

Skin diseases, or dermatoses, encompass a variety of conditions with diverse causes that are sometimes not completely known. Their symptoms can affect all layers of the skin. Most skin lesions are accompanied by localized inflammation. As a result, topical treatments applied directly to the skin are often the primary approach to managing these conditions. In recent years, azelaic acid (AZA), a naturally occurring acid used topically, has gained growing popularity. **(ARTICLE 3)**

Azelaic acid (AZA), sometimes mistakenly called azalea acid due to a confusion with the flower’s name, is actually unrelated to azaleas. AZA naturally occurs in various grains like rye, wheat, and barley. In the human body, small amounts of AZA are found in the urine of healthy people, produced through the ω-oxidation of fatty acids. Moreover, the yeast Pityrosporum ovale on human skin also produces and releases AZA.

The dermatological relevance of AZA was first noted in the 1970s when a dermatologist in Rome observed significant depigmentation in pityriasis versicolor lesions. Further studies showed that the skin yeast Malassezia furfur can break down unsaturated fatty acids into dicarboxylic acids ranging from C8 to C12, including AZA, which can inhibit melanocytes. AZA was first used alongside surgery to treat malignant melanoma in 1980 with positive outcomes.

Subsequent research has highlighted AZA’s impressive effects beyond melanogenesis inhibition, demonstrating antibacterial, anti-keratinization, antioxidant, anti-inflammatory, and anti-melanogenic properties. It is widely employed to treat skin conditions such as rosacea, acne vulgaris, and melasma. Recently, its use in cosmetics has surged, reflecting its broad potential applications.

Therefore, this review comprehensively examines AZA’s mechanisms and clinical uses, aiming to support its application in both medical and cosmetic fields and to encourage further investigation. **( Article 2)**

Azelaic acid (AZA) is a straight-chain saturated dicarboxylic acid with the molecular formula C9H16O4 and a molecular weight of 188.22. It contains two

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carboxyl groups, which cause it to act as a weak acid in water, exhibiting two dissociation constants: pKa1 at 4.5 and pKa2 at 5.3. The absorption of AZA through the skin is highly influenced by the pH and its level of dissociation in topical formulations. Unlike many other compounds, a higher degree of dissociation actually increases AZA’s absorption rate in the skin. This effect is likely due to the improved solubility of AZA when more dissociated, which enhances its overall skin permeability. **(Article 2)**

**1.2Mechanism of Action of AZA**

We have thoroughly examined and clarified the potential ways AZA works, focusing on its antibacterial properties, inhibition of keratinization, suppression of melanogenesis, and its antioxidant and anti-inflammatory effects, **as summarized in Figure 1.**

**1.2.1Bacteriostatic Effect**

AZA demonstrates bacteriostatic activity against various bacteria including *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, as shown in laboratory studies. Its effectiveness depends on concentration and pH, with stronger activity observed at lower pH levels and higher concentrations. Unlike traditional antibiotics, AZA does not promote bacterial resistance. It is effective even against antibiotic-resistant strains of *Propionibacterium acnes* and *Staphylococcus aureus*, linked to its unique mechanism.

AZA crosses bacterial cell membranes via ion transporters in a non-specific manner, lowering the intracellular pH and disrupting the pH balance within the bacteria. To restore this balance, bacteria consume more energy, which ultimately weakens or kills them. Moreover, AZA inhibits bacterial thioredoxin reductase, interfering with protein and DNA synthesis. This broad antibacterial mechanism reduces the likelihood of resistance development.

One study investigated the impact of AZA on the skin microbiota of acne patients. After 28 days of daily application of 15% AZA gel on 55 individuals, microbial diversity improved at acne-affected sites, with modest decreases in bacterial and staphylococcal counts and a notable rise in lactobacilli. Long-term treatment normalized *Propionibacterium* and *Staphylococcus* levels closer to those found in healthy skin. Another study evaluated AZA micro-nanocrystals in an *Propionibacterium acnes* model, revealing that these micro-nanocrystals not only inhibited the bacteria but also showed better anti-acne effects in vivo compared to standard AZA formulations.

**1.2.2 Anti-Keratinizing Effect**

Azelaic acid (AZA) acts as a mild anti-keratinizing agent that reversibly inhibits the growth of keratinocytes in a manner dependent on both the dose and duration of exposure. Research shows that AZA’s inhibitory effect mainly occurs by causing mitochondrial swelling and dilation of the rough endoplasmic reticulum in keratinocytes, which interferes with their differentiation process, especially the final stages of differentiation.

Keratohyalin granules and filaggrin are important markers of terminal keratinocyte differentiation. Mature filaggrin plays a crucial role in bundling keratin filaments into tonofilaments, which provide the main structural framework of keratinocytes. Studies have found that AZA can delay filaggrin production and reduce both the size and number of keratohyalin granules and tonofilament bundles. Additionally, AZA temporarily inhibits the synthesis of DNA, RNA, and proteins in keratinocytes, which further affects their proliferation.

One study demonstrated that applying 20% AZA cream twice daily for 8 to 12 weeks on acne-affected skin significantly reduced or normalized intra- and interfollicular hyperkeratosis. It also notably decreased the number and size of keratohyalin granules in both follicular and epidermal keratinocytes. Another clinical trial compared the effects of 20% AZA cream, 0.05% retinoic acid (RA) cream, and placebo on acne. Thirty participants were divided into three groups, receiving either twice-daily AZA, once-daily RA, or placebo cream. Both AZA and RA significantly decreased the number of comedones compared to placebo, with AZA’s reduction of follicular hyperkeratinization comparable to that of RA.

**1.2.3 Antimelanogenic Effect**

Azelaic acid (AZA) selectively targets overactive and malignant melanocytes without harming normal ones, likely due to the increased membrane permeability of abnormal melanocytes to AZA. Laboratory studies show that AZA can enter these cells, disrupt mitochondrial respiration, cause expansion of the rough endoplasmic reticulum, and inhibit DNA synthesis, which suppresses melanocyte growth and differentiation. Additionally, AZA competitively inhibits tyrosinase, the key enzyme responsible for converting tyrosine into melanin precursors, thus effectively reducing melanin production. These multiple mechanisms explain AZA’s success in treating pigmentation disorders such as melasma and post-inflammatory hyperpigmentation, with its selective targeting contributing to a safer profile.

**1.2.4 Antioxidant and Anti-Inflammatory Activities**

In vitro, AZA acts as a competitive inhibitor of several redox enzymes, including tyrosinase and thioredoxin reductase involved in DNA synthesis. It also exhibits strong anti-inflammatory properties. The antioxidant and anti-inflammatory actions of AZA are interconnected and influence each other.

Neutrophils generate reactive oxygen species (ROS) early in inflammation. Studies indicate that AZA can dose-dependently inhibit the release of ROS, including hydroxyl radicals and superoxide anions, possibly by suppressing the activity of NADPH oxidases (NOXs) on neutrophil membranes. Since ROS are key drivers of inflammation by activating signaling pathways, AZA’s inhibition helps reduce inflammatory responses.

AZA interferes with the NF-κB and MAPK signaling pathways by inhibiting phosphorylation of MAPK p38 and blocking NF-κB’s movement into the nucleus. It also modulates inflammation through activation of PPARγ, which suppresses NF-κB transactivation and lowers pro-inflammatory cytokine production. AZA reduces mRNA expression of inflammatory cytokines such as IL-1β, IL-6, and TNF-α induced by UVB exposure.

Furthermore, AZA inhibits lipid peroxidation of arachidonic acid, potentially decreasing pro-inflammatory products like prostaglandin E2, thromboxane, and leukotrienes.

AZA also reduces expression of toll-like receptor 2 (TLR2), a molecule involved in recognizing pathogens and triggering inflammatory diseases. In rosacea, elevated TLR2 increases keratinocyte production of serine proteases like KLK5, which leads to accumulation of the inflammatory peptide LL37. LL37 further promotes inflammation by inducing pro-inflammatory cytokines and activating NF-κB, creating a feedback loop that sustains inflammation. AZA inhibits TLR2, KLK5, and LL37, providing a rationale for its effectiveness in rosacea treatment. This action led to its FDA approval for papulopustular rosacea in 2002.

Similarly, overactive TLR2 also plays a key role in acne pathogenesis, where *Propionibacterium acnes* stimulates TLR2 and triggers inflammation. AZA’s inhibition of TLR2 helps explain its benefit in treating acne vulgaris. **(Article 2)**

**1.3 Concentration and Dosage Forms:**

In clinical practice, azelaic acid (AZA) is most commonly used in concentrations of 15% and 20%. In certain cases, such as acne treatment, higher concentrations—up to 30%—may be employed. Cosmetic products typically contain lower concentrations of AZA and are often combined with other active ingredients, although 15% and 20% formulations are also commercially available in skincare.

AZA’s percutaneous (skin) absorption is relatively limited and depends on both the concentration and the formulation used. While higher concentrations may improve absorption, they also increase the likelihood of local side effects. To enhance skin penetration and efficacy, a variety of advanced delivery systems have been developed, such as gels, foams, microemulsions, liposomes, ethosomes, and liquid crystal formulations. These innovative forms aim to boost AZA’s solubility and permeability, potentially allowing for lower doses while maintaining therapeutic effects.

Studies show that about 3–5% of AZA from a single application of cream is retained in the stratum corneum (outermost skin layer), whereas gel formulations can increase absorption up to 8%. These findings offer important guidance for optimizing topical AZA delivery. **(Article 2)**

**1.4 Safety:**

Topical azelaic acid is classified as a pregnancy category B drug, making it suitable for use during pregnancy and in individuals aged 12 and older. The 15% and 20% formulations are generally well tolerated, with mild and temporary side effects such as stinging, burning, or itching being the most common. There are no significant systemic side effects or reports of photosensitivity. Overall, AZA is considered a safe and well-tolerated treatment, with high levels of patient satisfaction. **(Article 2)**

The aim of this study is to investigate differences in the skin microbiota between acne-affected and unaffected areas in individuals with acne vulgaris (AV). In addition, the study evaluates the impact of 15% azelaic acid gel on the composition of the skin microbiota

### **2. Materials and Methods**

#### **2.1 Study Subjects and Sample Collection**

A total of 55 individuals diagnosed with acne vulgaris were enrolled from Jiangnan University, China, following an initial screening process. Prior to participation, each volunteer provided written informed consent. The study protocol was conducted in accordance with the ethical principles set forth in the Declaration of Helsinki.

Participants met the following inclusion criteria: aged between 18 and 35 years, inclusive of both male and female subjects, with a confirmed diagnosis of acne vulgaris. Additionally, their acne severity corresponded to grades 1 to 3 as defined in the 2016 European S3 Guidelines for Acne Management.

Exclusion criteria included the presence of severe systemic illnesses, immune system disorders, or active allergic conditions. Furthermore, individuals were excluded if they had received antibiotics or acne-related treatments within one month prior to the study and were required to avoid such treatments throughout the duration of the trial. Only unrelated participants were included to prevent genetic or familial bias.

All enrolled subjects were instructed to use a 15% azelaic acid topical gel (ZHIRUN Azelaic Acid Acne Clear Aqua Cream-in-gel), provided by Sinomune Pharmaceutical Co., Ltd. (Wuxi, China). Facial skin samples were obtained using sterile cotton swabs from each participant at three time points: prior to the initiation of treatment (baseline), 14 days after treatment onset, and at the 28-day mark. For each sampling, two types of facial areas were targeted: acne-involved regions (on the cheeks) and healthy, non-acne-affected cheek skin.

Sample collection was conducted by trained specialists from Jiangnan University under standardized conditions. Subjects were instructed to refrain from using facial cleansers, skincare products, or cosmetics for at least 24 hours prior to each sampling visit. A sterile buffer solution was prepared, consisting of 0.9% sodium chloride and 0.1% Tween-20 (Sigma-Aldrich Co., Ltd., Darmstadt, Germany).

During the sampling process, technicians wore sterile gloves and masks and used a consistent wiping technique. A 3 × 3 mm² area from both acne-affected and unaffected skin was gently but thoroughly swabbed 30 times using moistened sterile cotton swabs, rotating the swab continuously and applying moderate pressure. Sampling was conducted in a controlled environment with stable humidity and temperature. After collection, each swab was immediately transferred to a sterile tube containing buffer and stored at −80 °C until DNA extraction. All follow-up samples were obtained from the same fixed facial region as the initial baseline collection.

#### **2.2 Sample Microbiome DNA Extraction and 16S rRNA Sequencing**

Genomic DNA was extracted from the collected facial swabs using the Animal Tissue DNA Quick Extraction Kit (OMEGA Bio-Tek, Norcross, GA, USA). The concentration of extracted DNA was quantified using a Qubit 4 Fluorometer.

Samples that met quality control standards underwent polymerase chain reaction (PCR) amplification using universal primers designed for the V3–V4 hypervariable regions of the 16S rRNA gene. Specifically, the primers used were 341F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), as previously validated in the literature.

Subsequent sequencing was conducted using the Illumina HiSeq 2500 high-throughput sequencing platform, operated by MGI Tech Co., Ltd., located in Shenzhen, China.

#### **2.3 Meta-Amplicon Analysis Method**

Initial data processing involved the filtering of sequencing reads to remove low-quality sequences and artifacts, thereby ensuring high-quality data for downstream analysis. In cases where paired-end reads exhibited overlap, they were merged into a single consensus sequence using the software FLASH (Fast Length Adjustment of Short reads), version 1.2.11.

Operational Taxonomic Units (OTUs) were generated by clustering sequence tags, and representative sequences from each OTU were then taxonomically annotated by comparing them against a reference database using the RDP Classifier software (version 2.2), with a minimum sequence identity threshold set at 60%.

To evaluate within-sample microbial diversity (alpha diversity), several indices were calculated, including the Chao index, ACE index, Shannon index, and Simpson index. Between-sample diversity (beta diversity) was assessed using the QIIME software package (version 1.80), which allowed for the comparison of microbial community structure across samples.

The UniFrac distance metric was employed to assess phylogenetic differences between microbial communities. Two types of UniFrac analysis were used: unweighted UniFrac, which considers only the presence or absence of taxa, and weighted UniFrac, which incorporates both phylogenetic distances and species abundance. This allowed for a more comprehensive analysis of shifts in microbial community structure over time and between sample types.

### **3. Results**

#### **3.1 Study Subjects**

Between February and March 2023, a total of 55 participants (27 males and 28 females) with an average age of 25, all diagnosed with mild to moderate acne vulgaris (AV), took part in the study. Over 91% of participants reported positive experiences with the 15% azelaic acid gel, noting no adverse effects such as stinging or redness during use. A total of 212 valid data samples from 53 volunteers were included in the final analysis.

Operational taxonomic units (OTUs) were clustered and annotated using the RDP Classifier (version 1.9.1), resulting in 3684 distinct OTUs identified across all samples for further analysis.

#### **3.2 Comparison of the Taxonomic Composition Between Acne-Affected (A) and Non-Acne-Affected (N) Areas**

Alpha diversity indices (Sobs, Chao, ACE, Shannon, Simpson, and Coverage) were compared using the Wilcoxon rank-sum test. As shown in **Fig. 1**, there were no significant differences between acne-affected and non-affected areas, indicating similar microbial richness and diversity across both skin sites.

To evaluate compositional similarity and dissimilarity among microbial communities, cluster analysis and unweighted UniFrac analysis were conducted. As illustrated in **Fig. 2**, there was a statistically significant difference in the overall microbiome composition between acne-affected (A) and non-acne (N) areas (p < 0.05).

#### **3.3 Microbial Community Composition in Acne-Affected (A) vs. Non-Acne-Affected (N) Areas**

According to **Fig. 3A**, the dominant bacterial genera in both acne-affected and unaffected regions were *Propionibacterium*, *Corynebacterium*, and *Prevotella*. While the general structure of microbial communities was similar, certain genera showed notable differences in abundance.

As shown in **Fig. 3B**, *Propionibacterium* levels were slightly higher in acne-affected areas compared to non-acne skin (53.18% vs. 50.54%, p = 0.58). *Staphylococcus* (5.24% vs. 4.44%, p = 0.47) and *Veillonella* (1.40% vs. 0.83%, \***p < 0.001**) were also more abundant in acne regions. On the other hand, *Prevotella* (4.44% vs. 4.50%, *p = 0.02*) and *Corynebacterium* (5.39% vs. 6.37%, p = 0.09) showed a slightly reduced presence in acne-affected areas.

### **3.4 Effect of 15% Azelaic Acid Gel on Microbiome Composition**

Analysis at the genus level before and after treatment with 15% azelaic acid gel revealed a gradual decrease in the relative abundance of *Propionibacterium* (52.18% → 51.84% → 50.62%) and *Staphylococcus* (5.24% → 3.52% → 3.26%) over time. However, these changes were not statistically significant (**Fig. 4B**). After 28 days of daily application (A28), the microbial profile of acne-affected areas, particularly the levels of *Propionibacterium* and *Staphylococcus*, closely resembled that of non-acne-affected skin.

### **3.5 15% Azelaic Acid Gel Increases Microbial Diversity in Acne-Affected Skin**

As illustrated in **Fig. 5**, treatment with 15% azelaic acid gel led to an improvement in the overall microbial diversity of acne-affected skin. At baseline (A0), the affected areas exhibited higher values for Sobs, Chao, ACE, and a lower Simpson diversity index, indicating a less even microbial distribution. After 28 days of treatment, these indices reflected a significant enhancement in both microbial richness and evenness, aligning more closely with the diversity levels observed in healthy, non-acne-affected skin.

After 28 days of treatment with 15% azelaic acid gel (A28), there was a notable shift in the microbial community structure compared to baseline (A0), as shown in **Fig. 6**. This indicates that the treatment led to substantial alterations in the composition and diversity of microorganisms in acne-affected skin. Furthermore, when comparing the microbiota at A28 with that of non-acne-affected areas (N), unweighted UniFrac analysis revealed a statistically significant difference (**p < 0.05**), confirming that despite improvement, the microbial community in treated areas remained distinct from healthy skin (**Fig. 6B**)

### **4. Discussion**

Azelaic acid (AZA) has been extensively studied and is recognized as an effective treatment for acne vulgaris (AV). According to the 2016 European evidence-based guidelines, AZA is recommended for managing mild to moderate papulopustular AV. Its therapeutic effects are attributed to its ability to inhibit *C. acnes* protein synthesis and its bacteriostatic, anti-inflammatory, antioxidant, and anti-keratinizing properties. Both 15% AZA gel and 20% AZA cream have shown effectiveness against inflammatory and non-inflammatory acne. Moreover, 15% AZA gel demonstrates similar efficacy to 0.1% adapalene gel, without causing side effects, and may also help prevent recurrence by reducing sebum production.

The skin microbiota plays an essential role in the pathogenesis of AV. Beyond *C. acnes*, other microorganisms contribute to acne development. For instance, *Staphylococcus epidermidis* has been associated with inflammation and follicular dilation in acne, as observed by M. Bek Thomsen et al., since it was detected only in affected follicles. These inflamed follicles tended to host more *Staphylococcus* species and showed a simpler but more heterogeneous microbiota structure. Additionally, *Malassezia* species can promote AV by hydrolyzing triglycerides into free fatty acids, triggering follicular hyperkeratinization and comedone formation.

Although AZA is known for its clinical effectiveness, its specific impact on the skin microbiome remains underexplored. In this study, we compared the bacterial diversity and community structure between acne-affected and unaffected skin in AV patients, and investigated how the application of 15% AZA gel influences microbial dynamics using 16S rRNA gene sequencing.

We found that the general structure and composition of the bacterial community were similar in acne-affected and unaffected regions, with *Propionibacterium*, *Corynebacterium*, *Staphylococcus*, and *Prevotella* as the dominant genera (**Fig. 3B**). While *Propionibacterium* showed higher relative abundance in acne-affected skin (53.18% vs. 50.54%, p = 0.58), and *Staphylococcus* also showed a similar trend (5.24% vs. 4.44%, p = 0.47), these differences were not statistically significant. Prior studies have shown that a dynamic equilibrium between *Propionibacterium* and *Staphylococcus* is essential for maintaining skin homeostasis. Shao et al. highlighted that the abundance of these bacteria is positively correlated with acne severity. Ruan et al. also observed significant associations between increased *Propionibacterium*, *Staphylococcus*, and *Corynebacterium* levels and clinical acne parameters. In contrast, a study by Sorel Fitz-Gibbon revealed that while *C. acnes* abundance was similar in both acne and non-acne groups, strain-level differences were evident. Strains from clades IA1 and IC were more associated with acne, while others (e.g., IA2, IB, II, III) were linked to healthy skin.

Although our study did not reveal major differences in microbial composition between acne-affected and unaffected skin, the resolution was limited by the inability to identify specific strains. Despite this, our results provide insight into general microbial shifts.

Following 28 days of daily application of 15% AZA gel, alpha and beta diversity indices significantly improved in acne-affected areas (**Fig. 5**, **Fig. 6**), suggesting enhanced microbial richness and structure. In terms of genus abundance, the levels of *Propionibacterium* (52.18% → 51.84% → 50.62%) and *Staphylococcus* (5.24% → 3.52% → 3.26%) gradually declined with continued treatment (**Fig. 4B**).

Interestingly, *Lactobacillus* levels increased significantly in treated areas compared to both untreated and normal skin (**Fig. 4B**). Certain strains like *Lactobacillus paracasei* CNCM-I 2116 have been shown to support skin barrier function and reduce inflammation. Similarly, *Lactobacillus reuteri* can inhibit *C. acnes* and *S. epidermidis*, controlling acne in both human and animal studies.

We also observed a marked increase in *Streptococcus* and *Enhydrobacter* levels after treatment (**Fig. 4B**). *Streptococcus* species may aid acne resolution by enhancing ceramide production, producing antimicrobial peptides, and downregulating pro-inflammatory signals like IL-8 through NF-κB pathway inhibition. A recent study by O'Neill et al. identified a strain of *Staphylococcus capitis* (S. capitis E12) capable of selectively suppressing *C. acnes* more effectively than conventional antibiotics. While our study did not determine strain-level effects of *S. capitis*, the increase in this genus presents an intriguing possibility for future research.

Overall, this study suggests that 15% AZA gel helps regulate core skin bacteria—including *Propionibacterium*, *Staphylococcus*, *Lactobacillus*, *Prevotella*, and *Enhydrobacter*—enhancing microbial diversity and reducing dysbiosis in acne-prone areas. This microbial modulation may contribute to its clinical effectiveness against AV.

However, the study has some limitations. Swab samples were taken only from the surface of skin in mild to moderate AV cases. As a result, deeper follicular microbiota and more severe acne types were not investigated. Furthermore, our analysis could not resolve microbial differences at the strain level. Future studies should examine the impact of AZA across varying acne severities and utilize higher-resolution sequencing to explore strain-specific responses.