Chapter-3

SKIN MICROBIOME AND ACNE: PATHOGENESIS AND MANAGEMENT

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LEARNING OBJECTIVES

1. Describe the composition and ecological organization of the healthy skin microbiome, including the major bacterial, fungal, and viral communities and their roles in maintaining cutaneous homeostasis.
2. Explain the microbial alterations associated with acne vulgaris, with particular emphasis on dysbiosis, Cutibacterium acnes phylotypic diversity, changes in Staphylococcus epidermidis, fungal contributions, and biofilm formation.
3. Discuss the pathogenic mechanisms underlying acne development, including sebum dysregulation, follicular hyperkeratinization, activation of innate and adaptive immune pathways, and inflammatory cytokine responses.
4. Compare the major laboratory and molecular approaches used to investigate the skin microbiome, including 16S rRNA gene sequencing, shotgun metagenomics, culturomics, single-cell sequencing, and bioinformatics-based analyses.
5. Evaluate the impact of microbial dysbiosis on acne pathogenesis and understand how alterations in microbial diversity contribute to disease progression and antibiotic resistance.
6. Assess current and emerging microbiome-targeted therapeutic strategies for acne vulgaris, including conventional antibiotics, retinoids, probiotics, postbiotics, bacteriophage therapy, and skin microbiome transplantation.

3.1 INTRODUCTION

The skin is the largest organ of the human body and serves far more than a simple protective barrier. It functions as a highly dynamic biological ecosystem that supports a diverse community of microorganisms collectively known as the skin microbiome. This microbial community consists of bacteria, fungi, viruses, and microscopic mites that colonize different regions of the skin according to local environmental conditions. [1,2] Distinct microbial populations inhabit sebaceous (oil-rich), moist, dry, and foot-associated skin sites, each providing unique ecological niches. Among bacteria, the dominant phyla include Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes, while Cutibacterium, Staphylococcus, and Corynebacterium represent the major bacterial genera responsible for maintaining microbial balance. The fungal community is largely dominated by Malassezia species, particularly in sebum-rich areas such as the face, scalp, chest, and upper back. Together, these microorganisms contribute to skin health by preventing colonization by pathogenic microbes, regulating local immune responses, maintaining the skin's acidic pH, and preserving the integrity of the epidermal barrier. [3,4] Under normal conditions, the skin microbiome exists in a stable and mutually beneficial relationship with the host. However, disruption of this balanced ecosystem, a condition known as microbial dysbiosis, has emerged as a key factor in the development of several inflammatory skin disorders, including acne vulgaris. Rather than being caused simply by an increase in the number of a single microorganism, acne-associated dysbiosis is characterized by reduced microbial diversity and alterations in the composition of resident microbial communities. In particular, specific

inflammatory phylotypes of Cutibacterium acnes, especially phylotype IA1, become more dominant, while beneficial microbial populations decline. Simultaneously, changes in Staphylococcus epidermidis populations and the fungal microbiota further disturb microbial homeostasis. These alterations activate innate immune pathways, promote persistent follicular inflammation, and create an environment that favors disease progression. Although researchers continue to investigate whether dysbiosis initiates acne or develops as a consequence of inflammation, increasing evidence from longitudinal and metagenomic studies suggests that microbial imbalance plays an active role in triggering the disease rather than merely reflecting its presence. [5,6] Acne vulgaris is one of the most common chronic inflammatory disorders of the pilosebaceous unit, affecting approximately 9.4% of the global population, or nearly 650 million individuals, making it one of the most prevalent skin diseases worldwide. [1] The disease primarily affects adolescents and young adults because hormonal changes during puberty stimulate sebaceous gland activity and increase sebum production. Clinical manifestations are often more severe in adolescent males, whereas persistent and adult-onset acne is increasingly recognized among women. Beyond its visible skin lesions, acne has substantial psychological and social consequences. Many patients experience reduced self-esteem, anxiety, depression, social withdrawal, and impaired quality of life, contributing significantly to disability-adjusted life years (DALYs) and the overall global burden of disease. [1,2] For decades, the pathogenesis of acne was explained by four principal mechanisms: excessive sebum production, abnormal follicular keratinization, colonization of the pilosebaceous follicle by Cutibacterium acnes (formerly Propionibacterium acnes), and the resulting inflammatory response. [5] Although these factors remain fundamental, advances in microbiome research have considerably expanded this traditional concept. Current evidence indicates that acne is not simply the result of an increased abundance of C. acnes. Instead, the composition and diversity of the skin microbiome, particularly the predominance of specific pro-inflammatory C. acnes phylotypes, appear to play a much more important role in determining whether the skin remains healthy or develops inflammation. This shift in understanding has transformed acne research from focusing on a single pathogenic bacterium to viewing the skin microbiome as an interconnected ecological system whose balance is essential for maintaining cutaneous health.

3.2 THE HEALTHY SKIN MICROBIOME

The skin microbiome is a highly diverse and dynamic community of microorganisms that colonizes the human skin throughout life. Rather than existing as passive inhabitants, these microorganisms establish a mutually beneficial relationship with the host and play a fundamental role in maintaining cutaneous health. The composition of the skin microbiome is influenced by the unique physical and chemical characteristics of different skin sites, including moisture, sebum production, temperature, pH, and oxygen availability. [7-10] In healthy individuals, the microbiome remains in a state of ecological balance, where resident microorganisms interact with one another and with the host immune system to prevent colonization by pathogens, maintain the skin barrier, regulate inflammatory responses, and support tissue repair. Understanding the composition and organization of the healthy skin microbiome provides an essential foundation for explaining how microbial dysbiosis contributes to inflammatory skin disorders such as acne vulgaris.

3.2.1 Composition and Dominant Commensal Microorganisms

The healthy skin microbiome is composed of a wide variety of bacteria, fungi, viruses, and microscopic mites that collectively contribute to skin homeostasis. Among these microorganisms, bacteria constitute the largest and most extensively studied component. At the phylum level, the skin microbiome is predominantly composed of Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes, while the genera Cutibacterium, Staphylococcus, and Corynebacterium represent the dominant bacterial populations found across most skin sites. Cutibacterium acnes is a Gram-positive, anaerobic, lipophilic bacterium that is highly adapted to the lipid-rich environment of the pilosebaceous follicle. In sebaceous regions such as the face, scalp, chest, and upper back, it may account for up to 90% of the total bacterial population. Despite its well-known association with acne, C. acnes is an important commensal organism in healthy skin. [11-14] It contributes to

microbial balance by metabolizing sebum-derived lipids into short-chain fatty acids that help maintain the skin's acidic pH and inhibit the growth of harmful microorganisms. Another key commensal species is Staphylococcus epidermidis, which plays a central role in preserving skin homeostasis. Through the fermentation of glycerol and the production of antimicrobial compounds such as succinic acid and phenol-soluble modulins, S. epidermidis suppresses the growth of pathogenic bacteria, including Staphylococcus aureus, and can also regulate the proliferation of C. acnes under normal physiological conditions. In addition, this organism interacts closely with keratinocytes and immune cells to promote immune tolerance and reduce excessive inflammatory responses. Species belonging to the genus Corynebacterium are particularly abundant in moist areas of the body, including the axillae, groin, and skin folds. Although historically less studied than other commensals, they are now recognized as important contributors to microbial diversity, epithelial homeostasis, and host immune regulation. Alongside bacteria, fungi primarily Malassezia species constitute the dominant fungal community, particularly in sebaceous regions, while bacteriophages and other skin viruses help regulate bacterial populations and maintain microbial equilibrium. [15-18]

3.2.2 Site-Specific Variations in the Skin Microbiome

The composition of the skin microbiome varies considerably across different anatomical locations because each region provides a distinct ecological niche. Variations in sebum secretion, moisture content, temperature, pH, oxygen availability, and exposure to the external environment create selective conditions that favor specific microbial communities. Sebaceous areas, including the face, forehead, scalp, upper chest, and upper back, are enriched with lipophilic microorganisms, particularly Cutibacterium species, which thrive on sebum as their primary energy source. [19-24] Staphylococcus species are also commonly found in these oil-rich regions. Moist regions such as the axillae, groin, antecubital fossae, popliteal fossae, and interdigital spaces are predominantly colonized by Staphylococcus and Corynebacterium species, which are well adapted to humid environments. In contrast, dry skin sites, including the forearms, palms, and legs, generally harbor the greatest microbial diversity, supporting a broader range of bacterial taxa due to the absence of a single dominant ecological factor. Landmark studies have demonstrated that the ecological characteristics of a skin site exert a greater influence on microbial composition than individual genetic differences. Consequently, sebaceous, moist, and dry skin regions each possess distinct microbial communities with specialized functional roles that contribute to the maintenance of overall skin health. [25-29]

3.2.3 Host Factors Influencing Skin Microbiome Composition

The composition and stability of the skin microbiome are regulated by numerous host-related factors that continuously shape the cutaneous environment. Among these, sebum production is one of the most influential determinants.

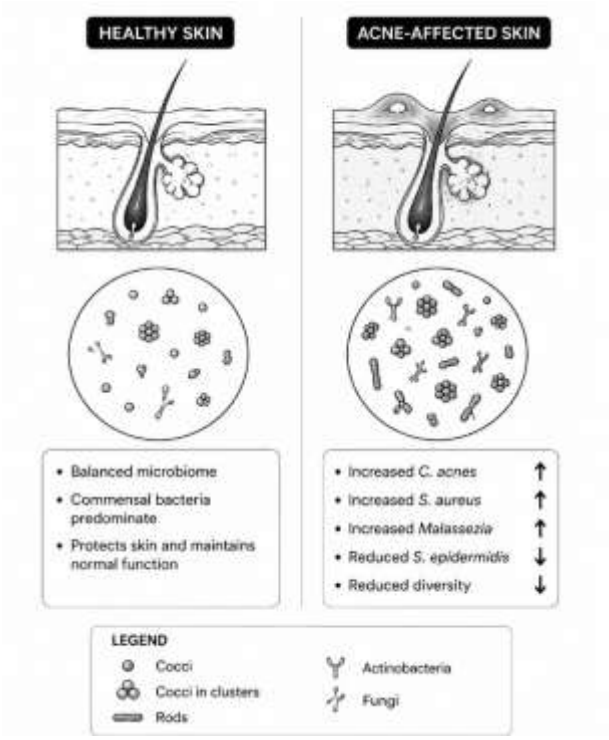


Figure: 3.1 Microbial Changes in Acne

During puberty, increased androgen levels stimulate sebaceous gland activity, creating lipid-rich conditions that favor the growth of lipophilic microorganisms such as Cutibacterium acnes and Malassezia species. This hormonal transition is one of the primary reasons for the marked changes in the skin microbiome observed during

adolescence. The naturally acidic pH of healthy skin, typically ranging from 4.5 to 5.5, provides another important mechanism for maintaining microbial balance. [30] This acidic environment promotes the growth of beneficial commensal microorganisms while restricting the colonization of many pathogenic species. Environmental factors such as local humidity, temperature, ultraviolet radiation, and oxygen availability further influence microbial survival and distribution across different skin sites. Host immune function also plays a critical role in shaping microbial communities. The innate and adaptive immune systems continuously monitor resident microorganisms, allowing beneficial microbes to coexist while limiting excessive microbial proliferation and preventing pathogen invasion. Genetic factors, including variations in genes involved in epidermal barrier function, such as filaggrin, and polymorphisms in innate immune receptors, can further influence microbial colonization patterns and susceptibility to skin disorders. Together, these physiological, immunological, genetic, and environmental factors maintain a balanced skin microbiome that is essential for preserving skin barrier integrity and protecting against inflammatory diseases such as acne vulgaris.

3.3 MICROBIAL CHANGES IN ACNE

Acne vulgaris is increasingly recognized as a disease associated with disturbances in the skin microbiome rather than simply an overgrowth of a single microorganism. Recent advances in genomic sequencing and microbiome research have demonstrated that acne is characterized by alterations in microbial diversity, shifts in the balance of commensal and pathogenic microorganisms, and changes in microbial interactions within the pilosebaceous unit. These microbial changes disrupt skin homeostasis, activate inflammatory pathways, promote biofilm formation, and contribute to disease persistence. Understanding these alterations provides important insights into acne pathogenesis and offers new opportunities for targeted microbiome-based therapies.

A. Cutibacterium acnes Phylotypic Diversity: *Cutibacterium acnes* is the dominant bacterial species inhabiting sebaceous regions of healthy skin and is generally considered a beneficial commensal organism. However, advances in molecular biology have revealed that not all *C. acnes* strains exhibit the same biological behavior. Based on genomic, biochemical, and morphological characteristics, *C. acnes* has been classified into three principal phylotypes Types I, II, and III. Among these, Type I demonstrates the greatest genetic diversity and has been further subdivided into several subgroups, including IA1, IA2, IB, and IC, using whole-genome sequencing techniques. These genetic differences have important clinical implications. Among the various subtypes, phylotype IA1 has consistently been associated with inflammatory acne lesions. Compared with other phylotypes, IA1 strains exhibit enhanced biofilm-forming ability, produce a greater number of virulence factors, and stimulate stronger activation of innate immune pathways. Phylotype II, particularly ribotype RT6, is generally regarded as a beneficial commensal that contributes to maintaining follicular homeostasis and microbial balance. Modern microbiome research has fundamentally changed the traditional understanding of acne. Rather than being caused simply by an increased number of *C. acnes*, acne appears to result from alterations in the relative abundance of different phylotypes. Long-read 16S rRNA sequencing has demonstrated that inflammatory acne lesions are enriched with ribotypes RT4 and RT5, both belonging to the IA1 lineage, whereas healthy skin contains higher proportions of the commensal RT6 ribotype. This reduction in phylotypic diversity promotes immune activation and supports the concept that microbial dysbiosis is an active contributor to acne pathogenesis rather than merely a consequence of inflammation.

B. Loss of Microbial Diversity: Another hallmark of acne-associated dysbiosis is a reduction in the overall diversity of the skin microbiome. Healthy skin supports a balanced microbial ecosystem in which numerous bacterial species coexist and interact through cooperative mechanisms that maintain ecological stability. During acne development, however, this diversity declines significantly, allowing a limited number of pro-inflammatory microorganisms to dominate the follicular environment. High-throughput 16S rRNA sequencing studies have consistently shown that inflammatory acne lesions exhibit lower alpha diversity than healthy skin or non-inflammatory lesions. The loss of microbial diversity weakens several protective ecological mechanisms, including competition for nutrients,

production of antimicrobial compounds, metabolic cross-feeding, and competitive exclusion of pathogenic organisms. As these beneficial interactions diminish, the follicular microenvironment becomes increasingly favorable for the expansion of virulent *C. acnes* phylotypes and other opportunistic microorganisms, thereby sustaining chronic inflammation and lesion formation.

C. The Dual Role of Staphylococcus epidermidis: *Staphylococcus epidermidis* is one of the most abundant commensal bacteria inhabiting healthy human skin and plays an essential role in maintaining microbial homeostasis. Under normal physiological conditions, this organism protects the skin through several beneficial mechanisms. It metabolizes glycerol present in sebum to produce succinic acid and other short-chain fatty acids that lower the local pH, creating conditions that inhibit excessive growth of *C. acnes*. Additionally, *S. epidermidis* produces antimicrobial peptides and lipoteichoic acid, which help regulate innate immune responses and suppress excessive TLR2-mediated inflammation in keratinocytes. Despite these protective functions, not all strains of *S. epidermidis* behave identically. Certain pathogenic strains can produce virulence factors such as delta toxins, which damage keratinocytes, promote inflammation, and facilitate mixed-species biofilm formation together with *C. acnes*. Consequently, the relationship between these two microorganisms is highly dynamic. A balanced interaction supports skin health, whereas disruption of this equilibrium may amplify inflammatory signaling and contribute to acne progression. This dual role highlights the importance of preserving beneficial microbial communities rather than indiscriminately eliminating bacteria through broad-spectrum antimicrobial therapies.

D. Fungal Contributions: The Role of Malassezia: Although bacterial dysbiosis has traditionally received the greatest attention in acne research, increasing evidence indicates that fungal communities also contribute to disease development. Members of the genus *Malassezia* are lipid-dependent yeasts that normally colonize sebaceous areas of healthy skin and represent the dominant component of the cutaneous fungal microbiome. Historically, *Malassezia* species have been primarily associated with disorders such as seborrheic dermatitis and pityriasis versicolor. However, recent studies suggest that these fungi may also influence acne pathogenesis. Species including *Malassezia furfur* and *Malassezia globosa* can cause Malassezia folliculitis, a condition that closely resembles acne vulgaris and may coexist with bacterial acne, making clinical diagnosis more challenging. These fungi metabolize triglycerides present in sebum, releasing free fatty acids capable of activating Toll-like receptor 2 (TLR2) on keratinocytes and stimulating local inflammatory responses. Their interaction with *C. acnes* may further amplify follicular inflammation. Moreover, shotgun metagenomic analyses have reported increased abundance of *Malassezia* species in certain acne cohorts, suggesting that fungal dysbiosis may complement bacterial alterations in promoting disease progression.

E. Biofilm Formation and Antibiotic Resistance: One of the major survival strategies employed by pathogenic *C. acnes* strains is the formation of biofilms within the pilosebaceous follicle. Biofilms are highly organized microbial communities embedded within a self-produced extracellular matrix that protects bacteria from host immune defenses, environmental stress, and antimicrobial agents. This protective environment allows bacteria to persist for prolonged periods and contributes to the chronic nature of acne. Among the different phylotypes, IA1 strains exhibit significantly greater abilities to adhere to follicular surfaces, produce dense biofilms, and resist antimicrobial treatment compared with commensal strains. Biofilm-associated *C. acnes* also activates inflammatory signaling pathways in keratinocytes, including TLR2-mediated responses and the expression of regulatory molecules such as miR-146a, thereby maintaining localized inflammation within the follicular epithelium. Antibiotic resistance has become an increasingly important challenge in acne management. Extensive and prolonged use of topical and systemic antibiotics has led to a substantial rise in resistance against commonly prescribed agents, including erythromycin, clindamycin, and tetracyclines. Resistance rates have increased considerably over the past two decades, particularly in Europe and Asia, reducing treatment effectiveness and highlighting the urgent need for alternative therapeutic approaches. These concerns have accelerated research into microbiome-preserving strategies,

including probiotics, bacteriophage therapy, antimicrobial peptides, and other targeted interventions that aim to restore microbial balance while minimizing the development of antibiotic resistance.

3.4 PATHOGENIC MECHANISMS

The development of acne vulgaris is driven by a complex interaction between microbial dysbiosis, excessive sebum production, abnormal keratinization, and dysregulated immune responses. Rather than functioning independently, these mechanisms influence one another to create a self-perpetuating cycle of follicular obstruction, microbial imbalance, and chronic inflammation. Recent molecular studies have demonstrated that alterations in the skin microbiome particularly the predominance of pro-inflammatory *Cutibacterium acnes* phylotypes activate multiple signaling pathways in keratinocytes, sebocytes, and immune cells, ultimately leading to the formation and progression of acne lesions.

A. *Cutibacterium acnes*-Mediated Activation of Toll-Like Receptors (TLR2 and TLR4): One of the earliest events in acne pathogenesis is the activation of the innate immune system by *Cutibacterium acnes*. Components of the bacterial cell wall, particularly peptidoglycan, lipoteichoic acid, and several secreted virulence factors, are recognized by pattern-recognition receptors (PRRs) expressed on keratinocytes, sebocytes, macrophages, and dendritic cells. Among these receptors, Toll-like receptor 2 (TLR2) and, to a lesser extent, Toll-like receptor 4 (TLR4) play central roles in initiating inflammatory responses. Following recognition of *C. acnes*, TLR2 activation triggers intracellular signaling pathways that culminate in the activation of nuclear factor-kappa B (NF-κB) and other transcription factors. This signaling cascade stimulates the production of numerous pro-inflammatory cytokines and chemokines, including interleukin-1β (IL-1β), IL-6, IL-8, and tumor necrosis factor-alpha (TNF-α). These inflammatory mediators recruit neutrophils and other immune cells into the pilosebaceous unit, creating the characteristic inflammatory environment observed in acne lesions. Notably, not all *C. acnes* strains stimulate the immune system equally. Phylotype IA1, which is strongly associated with inflammatory acne, induces significantly greater TLR2 activation than commensal phylotypes, resulting in more intense cytokine production and tissue inflammation. In addition, *C. acnes* secretes several virulence proteins, including Christie–Atkins–Munch-Petersen (CAMP) factors 1 and 2, which further amplify inflammatory signaling and tissue injury. Because of their important role in disease progression, these virulence factors are being investigated as potential therapeutic targets for future acne treatments.

B. Sebum Dysregulation and Short-Chain Fatty Acid Production: Sebum production is essential for maintaining normal skin hydration and barrier function; however, excessive sebum secretion is one of the major contributors to acne development. During puberty, rising androgen levels stimulate sebaceous gland enlargement and increase lipid production, creating a nutrient-rich environment that favors the proliferation of lipophilic microorganisms, particularly *Cutibacterium acnes*. *C. acnes* produces lipase enzymes that hydrolyze sebum triglycerides into free fatty acids (FFAs). These metabolites exert both beneficial and harmful effects. Under physiological conditions, they contribute to maintaining the acidic pH of the skin, which supports commensal microbial communities and limits colonization by many pathogenic microorganisms. However, excessive accumulation of free fatty acids within the pilosebaceous follicle promotes inflammation by activating inflammatory signaling pathways, including peroxisome proliferator-activated receptor gamma (PPAR-γ) in keratinocytes and sebocytes. In addition to free fatty acids, *C. acnes* produces short-chain fatty acids (SCFAs), particularly propionic acid, during lipid metabolism. These microbial metabolites regulate keratinocyte differentiation, influence sebocyte activity, and modulate the expression of genes involved in inflammation. Although SCFAs contribute to maintaining microbial homeostasis under normal conditions, excessive production in dysbiotic follicles may enhance inflammatory responses and support acne progression.

C. Follicular Hyper-Keratinization and Comedone Formation: Abnormal follicular keratinization represents one of the earliest pathological events in acne development. Under normal conditions, keratinocytes lining the follicular canal undergo controlled proliferation and desquamation, allowing sebum to flow freely

onto the skin surface. In acne, this process becomes dysregulated, resulting in excessive keratinocyte proliferation and impaired shedding of dead epithelial cells. Exposure to *C. acnes* and its inflammatory products stimulates keratinocytes to release interleukin-1 alpha (IL-1α), a cytokine that promotes hyperproliferation and abnormal differentiation of follicular epithelial cells. The resulting accumulation of keratinocytes and sebum leads to the formation of a microcomedone, the earliest clinically inapparent acne lesion. As the follicular opening becomes obstructed, an anaerobic and lipid-rich microenvironment develops within the pilosebaceous unit. These conditions provide an ideal ecological niche for the expansion of pathogenic *C. acnes* phylotypes and facilitate biofilm formation. Simultaneously, activation of intracellular signaling pathways, particularly NF-κB and the NLRP3 inflammasome, further amplifies inflammatory responses and perpetuates follicular obstruction. Consequently, follicular hyperkeratinization not only initiates comedone formation but also establishes the conditions necessary for chronic microbial colonization and persistent inflammation.

D. Innate and Adaptive Immune Responses: The inflammatory response in acne involves coordinated activation of both the innate and adaptive immune systems. Innate immunity provides the first line of defense following microbial dysbiosis. Activation of keratinocytes, sebocytes, macrophages, and dendritic cells by *C. acnes* results in the secretion of chemokines such as CXCL1, CXCL2, and IL-8, which recruit neutrophils to the affected follicle. Neutrophil infiltration contributes to the formation of inflammatory papules and pustules through the release of reactive oxygen species, proteolytic enzymes, and additional inflammatory mediators. Macrophages also play a critical role by phagocytosing *C. acnes* and producing cytokines including IL-1β, IL-6, and TNF-α, thereby amplifying local inflammation and promoting communication between innate and adaptive immune pathways. Adaptive immunity further sustains chronic inflammation through activation of antigen-specific T lymphocytes. Among these, T helper 17 (Th17) cells have emerged as key mediators of acne pathogenesis. Pro-inflammatory *C. acnes* phylotype IA1 preferentially stimulates Th17 differentiation and the production of interleukin-17 (IL-17), a cytokine that promotes keratinocyte proliferation, disrupts epidermal barrier function, and recruits additional neutrophils to inflammatory sites. At the same time, elevated TNF-α produced by activated macrophages and T cells increases vascular permeability, enhances sebaceous gland dysfunction, and further intensifies inflammation. Chronic acne lesions are also associated with impaired immune regulation. Reduced activity of regulatory T (Treg) cells and decreased production of the anti-inflammatory cytokine IL-10 limit the resolution of inflammation, allowing inflammatory pathways to remain persistently activated. This imbalance between pro-inflammatory and regulatory immune responses contributes to the chronic and recurrent nature of acne vulgaris.

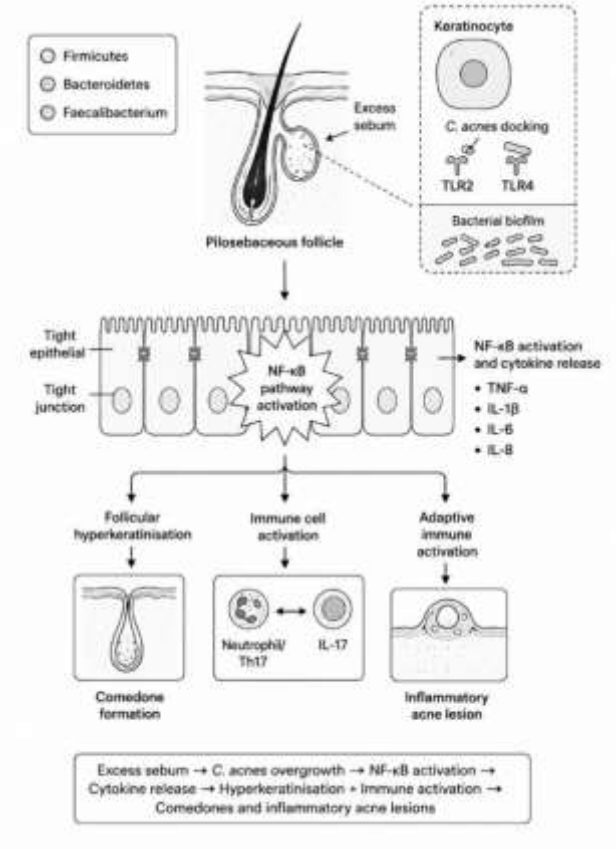


Figure: 3.2 Pathogenesis of Acne

Collectively, these interconnected pathogenic mechanisms demonstrate that acne is not solely a disorder of bacterial colonization or excessive sebum production but a multifactorial inflammatory disease involving complex interactions between the skin microbiome, sebaceous gland physiology, follicular biology, and both innate and adaptive immune responses. Understanding these molecular pathways has laid the foundation for the development of targeted

therapies aimed at restoring microbial balance while selectively modulating inflammatory signaling.

3.5 DIAGNOSTIC AND ANALYTICAL APPROACHES

Understanding the role of the skin microbiome in acne vulgaris has become possible because of remarkable advances in molecular biology, sequencing technologies, and computational analysis. Traditional microbiological methods relied heavily on culture-based techniques, which could identify only a limited proportion of microorganisms capable of growing under laboratory conditions. However, many members of the skin microbiome are difficult or impossible to culture using conventional methods. Modern high-throughput sequencing and multi-omics technologies now provide a comprehensive view of microbial composition, genetic diversity, metabolic activity, and host–microbe interactions. These approaches have significantly improved our understanding of microbial dysbiosis, identified acne-associated microbial signatures, and opened new avenues for precision diagnostics and microbiome-targeted therapies.

3.5.1 16S rRNA Gene Profiling

Among the available molecular techniques, 16S ribosomal RNA (16S rRNA) gene sequencing remains the most widely used method for characterizing bacterial communities in the skin microbiome. The technique targets conserved regions of the bacterial 16S rRNA gene while amplifying hypervariable regions, such as V1–V9 or V3–V4, allowing accurate taxonomic identification of bacteria without the need for culture. Because of its cost-effectiveness and high sensitivity, 16S rRNA sequencing has become the standard approach for investigating microbial diversity in acne research. Studies comparing acne lesions, adjacent non-lesional skin, and healthy controls have consistently demonstrated that acne is associated with reduced bacterial diversity and significant alterations in microbial composition. Rather than simply showing an increase in the total abundance of *Cutibacterium acnes*, these investigations reveal an enrichment of specific pro-inflammatory ribotypes, particularly phylotype IA1, accompanied by shifts in the relative abundance of other commensal organisms such as *Staphylococcus* and *Corynebacterium*. These findings support the concept that microbial imbalance, rather than bacterial overgrowth alone, contributes to acne pathogenesis. Recent advances in long-read sequencing platforms, including Oxford Nanopore Technologies, have further improved the resolution of 16S rRNA analysis. Unlike conventional short-read sequencing, long-read approaches can sequence the full-length 16S rRNA gene, enabling discrimination between closely related *C. acnes* ribotypes such as RT4, RT5, and RT6. This higher taxonomic resolution provides deeper insights into strain-specific pathogenicity and enhances the ability to identify clinically relevant microbial signatures associated with acne severity and treatment response.

3.5.2 Shotgun Metagenomics and Resistome Analysis

While 16S rRNA sequencing primarily identifies bacterial taxa, shotgun metagenomic sequencing provides a much broader and more comprehensive analysis of the skin microbiome. Instead of sequencing a single marker gene, this technique sequences all microbial DNA present within a sample, allowing researchers to identify bacteria, fungi, viruses, and other microorganisms simultaneously while also characterizing their functional genetic potential. Shotgun metagenomics enables the reconstruction of complete microbial genomes and facilitates the identification of genes involved in virulence, metabolic pathways, biofilm formation, host adaptation, and antimicrobial resistance. In acne research, this technology has revealed that pathogenic *C. acnes* strains possess unique genetic determinants associated with enhanced inflammatory potential and persistence within pilosebaceous follicles. One of the major advantages of shotgun sequencing is its ability to investigate the microbial resistome, which represents the complete collection of antimicrobial resistance genes present within a microbial community. Studies of acne-associated microbiomes have identified resistance determinants such as tetM, tetW, ermB, and ermC, explaining the increasing prevalence of resistance to tetracyclines, macrolides, and clindamycin observed in clinical practice. Furthermore, metagenomic analyses have demonstrated that mobile genetic elements facilitate horizontal transfer of resistance genes between bacterial populations, highlighting the impact of prolonged antibiotic exposure on microbial evolution. These

findings are particularly valuable for guiding antibiotic stewardship and developing microbiome-preserving therapeutic strategies.

3.5.3 Culturomics and Single-Cell Sequencing

Although culture-independent methods dominate microbiome research, culturomics has re-emerged as an important complementary approach for studying viable microorganisms. Culturomics combines high-throughput cultivation under diverse environmental conditions with rapid microbial identification using techniques such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and confirmatory 16S rRNA sequencing. By employing numerous specialized culture media and incubation conditions, culturomics has enabled the isolation of previously uncultivable or rarely detected skin microorganisms, substantially expanding our knowledge of microbial diversity and functional capabilities. Unlike sequencing-based approaches, culturomics allows direct phenotypic characterization of microorganisms, including antimicrobial susceptibility testing, metabolic profiling, and investigation of microbial interactions. This makes it particularly valuable for validating genomic findings and exploring the biological behavior of clinically important organisms. Another rapidly emerging technology is single-cell sequencing, which enables genomic or transcriptomic analysis of individual microbial cells rather than entire mixed populations. This approach overcomes the limitations of bulk sequencing by revealing cellular heterogeneity within microbial communities. In acne research, single-cell technologies have demonstrated that different *C. acnes* cells within the same follicle may exhibit distinct gene expression patterns, virulence characteristics, and metabolic activities, highlighting the complexity of microbial behavior during disease progression. The integration of single-cell sequencing with host transcriptomics, proteomics, and metabolomics has given rise to advanced multi-omics approaches that simultaneously evaluate microbial composition, host immune responses, protein expression, and metabolic activity. These integrated analytical strategies are providing unprecedented insights into host–microbe interactions, identifying biomarkers of disease susceptibility, and supporting the development of personalized microbiome-based therapeutic interventions. As sequencing technologies continue to evolve, combining culturomics, single-cell genomics, and multi-omics analyses is expected to significantly advance precision dermatology and improve the diagnosis and management of acne vulgaris.

3.6 THERAPEUTIC IMPLICATIONS

Growing knowledge of the skin microbiome has transformed the way acne vulgaris is understood and managed. Traditional acne therapies primarily focused on reducing bacterial load, controlling sebum production, and suppressing inflammation. However, recent research suggests that successful treatment should also preserve or restore the natural balance of the skin microbiome rather than simply eliminating microorganisms. This shift has led to the development of microbiome-friendly therapeutic strategies that aim to reduce inflammation, limit antimicrobial resistance, and promote long-term skin health. Emerging treatments, including probiotics, postbiotics, bacteriophage therapy, and microbiome transplantation, are increasingly being investigated as potential alternatives or adjuncts to conventional therapies.

3.6.1 Antibiotics and the Skin Microbiome

Topical and systemic antibiotics have been a cornerstone in the management of moderate-to-severe acne for several decades. Commonly prescribed agents include tetracyclines such as doxycycline and minocycline, as well as topical antibiotics like clindamycin and erythromycin. These medications reduce the burden of Cutibacterium acnes and suppress inflammatory responses, leading to a noticeable reduction in acne lesions. Despite their clinical effectiveness, antibiotics are not selective for pathogenic bacteria and therefore disrupt the overall balance of the skin microbiome. Prolonged antibiotic exposure can also affect the gut microbiome, altering microbial diversity and potentially influencing systemic immune responses. One of the major concerns associated with long-term antibiotic therapy is the emergence of antimicrobial resistance. Resistant strains of C. acnes have become increasingly common worldwide, particularly against macrolides and tetracyclines, while resistance genes may also spread among other commensal skin bacteria. This growing problem has prompted international acne management guidelines to recommend

limiting the duration of antibiotic therapy and combining antibiotics with non-antibiotic agents such as benzoyl peroxide or topical retinoids to reduce resistance development. Recent studies have also explored the use of probiotics alongside systemic antibiotics to help preserve microbial diversity and reduce disturbances to the skin and gut microbiomes.

3.6.2 Retinoids and Sebum Regulation

Retinoids remain one of the most effective therapeutic options for acne because they target several key mechanisms involved in disease development. Among them, isotretinoin (13-cis retinoic acid) is considered the gold standard treatment for severe nodular and treatment-resistant acne. Its primary action is to dramatically reduce the size and activity of sebaceous glands, leading to a marked decrease in sebum production. Since C. acnes relies on sebum as its primary nutrient source, reducing sebum creates an environment that is less favourable for bacterial growth and biofilm formation. Beyond lowering the overall bacterial burden, isotretinoin also appears to influence the composition of the skin microbiome. Microbiome studies have demonstrated that treatment is associated with restoration of a healthier microbial balance, including a reduction in the dominance of pro-inflammatory C. acnes phylotype IA1 and an increase in microbial diversity. These findings suggest that isotretinoin promotes skin health not only through its effects on sebaceous gland function but also by indirectly re-establishing microbial homeostasis. Topical retinoids, including adapalene, tretinoin, and tazarotene, provide similar benefits through different mechanisms. They normalize keratinocyte differentiation and reduce follicular hyperkeratinization, preventing the formation of microcomedones that serve as reservoirs for bacterial colonization. By improving follicular turnover and reducing obstruction, topical retinoids create a less favorable environment for persistent biofilm formation and chronic inflammation.

3.6.3 Probiotic and Postbiotic Formulations

Increasing evidence supports the concept of the gut–skin axis, highlighting the close relationship between intestinal microbial health, systemic immunity, and inflammatory skin diseases such as acne. This understanding has stimulated considerable interest in probiotics and postbiotics as microbiome-based therapeutic strategies. Probiotics are live beneficial microorganisms that help restore microbial balance and regulate immune responses. Clinical trials have demonstrated that oral probiotic strains such as Lactobacillus paracasei MSMC 39-1 and Lacticaseibacillus rhamnosus can reduce acne severity, decrease inflammatory lesion counts, and improve cytokine profiles in patients with acne vulgaris. These beneficial effects are thought to result from modulation of both gut microbial communities and systemic inflammatory pathways. Topical probiotic formulations have also shown encouraging results. Preparations containing Lactobacillus-derived extracts or live bacterial cultures have been reported to reduce both inflammatory and non-inflammatory acne lesions by strengthening the skin barrier, suppressing pathogenic bacteria, and regulating local immune responses. These products may help restore a balanced skin microbiome without causing the widespread microbial disruption associated with antibiotics. In addition to probiotics, researchers are investigating postbiotics, which are bioactive compounds produced during probiotic metabolism rather than the living organisms themselves. These include bacteriocins, short-chain fatty acids (SCFAs), antimicrobial peptides, enzymes, and other microbial metabolites that possess anti-inflammatory and antimicrobial properties. Because postbiotics are non-living, they generally exhibit greater stability, longer shelf life, and fewer safety concerns than live probiotic preparations. Although early clinical studies are promising, differences in probiotic strains, formulations, dosage, and treatment duration currently limit direct comparisons between studies, emphasizing the need for larger, standardized clinical trials.

3.6.4 Bacteriophage Therapy Targeting Cutibacterium acnes

Bacteriophage therapy represents one of the most innovative microbiome-targeted approaches currently under investigation for acne management. Bacteriophages, commonly known as phages, are viruses that specifically infect bacterial cells. Unlike broad-spectrum antibiotics, phages selectively eliminate their target bacteria while preserving the surrounding beneficial

microbial community, making them attractive candidates for precision antimicrobial therapy. Naturally occurring C. acnes-specific phages are more frequently detected on healthy skin than on acne-affected skin, suggesting that these viruses may naturally regulate pathogenic bacterial populations and contribute to microbial homeostasis. Experimental studies have shown that topical application of phage cocktails targeting C. acnes significantly reduces bacterial colonization, neutrophil infiltration, inflammatory cytokine production, and lesion severity in animal models of acne. Clinical translation has also begun. Early-phase clinical trials evaluating topical phage formulations, including the BX001 phage gel, have demonstrated favorable safety profiles and significant reductions in C. acnes abundance without disrupting the broader skin microbiome. Despite these encouraging findings, several challenges remain before phage therapy becomes widely available. These include the narrow host specificity of phages, the possibility of bacterial resistance to phages, manufacturing complexities, and the need for standardized regulatory pathways. Nevertheless, bacteriophage therapy represents a promising alternative to conventional antibiotics in the era of increasing antimicrobial resistance.

3.6.5 Skin Microbiome Transplantation (Skin FMT)

Skin microbiome transplantation, sometimes referred to as skin microbiota transplantation (SMT) or skin FMT, is an emerging therapeutic strategy inspired by the success of fecal microbiota transplantation in treating gastrointestinal disorders. The approach involves transferring beneficial microbial communities from healthy donor skin to individuals with microbial dysbiosis in an effort to restore microbial balance and improve skin health. Experimental studies have demonstrated that donor-derived microbial preparations can successfully colonize recipient skin, modify microbial composition, and influence local immune responses. In acne vulgaris, this strategy aims to re-establish a healthy microbial ecosystem by increasing the abundance of beneficial C. acnes phylotype II (particularly RT6), restoring protective populations of Staphylococcus epidermidis, and enhancing overall microbial diversity. Rebuilding this balanced microbial community may reduce chronic inflammation, improve barrier function, and decrease the recurrence of acne lesions. Although skin microbiome transplantation remains in the early stages of research and has not yet entered routine clinical practice, it represents one of the most exciting developments in microbiome-based dermatology. Ongoing research is focused on identifying suitable donor microbiomes, optimizing microbial delivery systems, ensuring long-term microbial stability, and establishing safety and regulatory standards. As these challenges are addressed, skin microbiome transplantation may become an important component of personalized acne management in the future.

3.7 FUTURE DIRECTIONS AND RESEARCH GAPS

Although significant advances have been made in the characterisation of the acne skin microbiome, there are still a number of gaps that are critical. To determine the temporal causality of dysbiosis and acne onset, first, longitudinal microbiome analyses of patients during pre-acne adolescence, active disease, and remission are required. Cross-sectional data cannot answer the question of whether the loss of phylotypic diversity occurs before or after the development of visible lesions. Second, the microbiome-cosmetic interface is a poorly researched yet commercially and clinically important field. There is a growing trend of cosmetic formulations using prebiotics, postbiotics, or microbiome-modulating active ingredients, although hard evidence of their effectiveness and mechanisms of action is missing. Third, individualised microbiome-based acnes therapy, in which the personal 16S rRNA or metagenomic profiles are used to choose specific interventions, is a conceptually strong yet operationally underdeveloped framework. Microbiome profiling needs to be standardised in terms of sampling techniques, analytical pipelines and clinical interpretation thresholds before it can be scaled to clinical workup. Fourth, the role of the skin virome, especially C. acnes bacteriophages, in follicular microbial ecology should be systematically studied; the natural phage community can be an unexploited source of microbiome-regulating activity. Lastly, the gut-skin axis, with the effects of dietary glycaemic load, dairy consumption, and gut microbiome composition on skin microbiome dynamics in acne, needs to be clarified by integrated multi-

omics cohort studies.

3.8 CONCLUSION

The skin microbiome has become a core, not peripheral, player in the pathophysiology of acne vulgaris. The current paradigm has changed to a model of C. acnes overgrowth to intra-species phylotypic imbalance and general microbial dysbiosis. The loss of C. acnes phylotypic diversity, pro-inflammatory phylotype IA1 ribotypes dominance, and S. epidermidis-mediated homeostasis disruption, and biofilm formation all contribute to the establishment of prolonged follicular inflammation by TLR-mediated and adaptive immune responses. Complex molecular techniques such as complete-length 16S rRNA sequencing, shotgun metagenomics, and single-cell technologies are increasingly revealing the microbial composition of acness at a new level of detail. The paradigm shift of microbiome preservation is therapeutically manifested in the new armamentarium of probiotics, postbiotics, bacteriophage therapy, and skin FMT, which are all indicators of a shift towards not broad-spectrum microbiome disruption. Such strategies have specific potential in an era of increasing antibiotic resistance. Future studies incorporating longitudinal microbiome monitoring, individualised profiling, and multi-omics studies will be necessary to translate the science of microbiomes into effective, personalised acne treatment. The skin microbiome is no longer a passive participant in the pathogenesis of acne- it is a controllable disease driver and an entry point to the next generation of dermatological therapeutic interventions.

REFERENCES

1. Tan, J. K., & Bhate, K. (2015). A global perspective on the epidemiology of acne. British Journal of Dermatology, 172(Suppl 1), 3–12.
2. Bhate, K., & Williams, H. C. (2013). Epidemiology of acne vulgaris. British Journal of Dermatology, 168(3), 474–485.
3. Grice, E. A., & Segre, J. A. (2011). The skin microbiome. Nature Reviews Microbiology, 9(4),
4. Byrd, A. L., Belkaid, Y., & Segre, J. A. (2018). The human skin microbiome. Nature Reviews Microbiology, 16(3), 143–155. https://doi.org/10.1038/nrmicro.2017.157
5. O'Neill, A. M., & Gallo, R. L. (2018). Host-microbiome interactions and recent progress into understanding the biology of acne vulgaris. Microbiome, 6(1), 177. https://doi.org/10.1186/s40168-018-0558-5
6. Dreno, B., Dekio, I., Baldwin, H., Demessant, A. L., Dagnelie, M. A., Khammari, A., & Corvec, S. (2024). Acne microbiome: From phyla to phylotypes. Journal of the European Academy of Dermatology and Venereology, 38(4), 657–664. https://doi.org/10.1111/jdv.19540
7. Niedźwiedzka, A., Micallef, M. P., Biazzo, M., & Podrini, C. (2024). The role of the skin microbiome in acne: Challenges and future therapeutic opportunities. International Journal of Molecular Sciences, 25(21), 11422. https://doi.org/10.3390/ijms252111422
8. Dreno, B., Dagnelie, M. A., Khammari, A., & Corvec, S. (2020). The skin microbiome: A new actor in inflammatory acne. American Journal of Clinical Dermatology, 21(Suppl 1), 18–24. https://doi.org/10.1007/s40257-020-00531-1
9. Barnini, S., Conte, M., Campa, M., Favali, C., Tempesta, S., Florio, W., & Lupetti, A. (2022). Skin dysbiosis and Cutibacterium acnes biofilm in inflammatory acne lesions of adolescents. Scientific Reports, 12(1), 21045.
10. Walsh, T. R., Efthimiou, J., & Dreno, B. (2016). Systematic review of antibiotic resistance in acne: An increasing topical and oral threat. Lancet Infectious Diseases, 16(3), e23–e33. https://doi.org/10.1016/S1473-3099(15)00527-7
11. Yu, T., Xu, X., Liu, Y., et al. (2024). Multi-omics signatures reveal genomic and functional heterogeneity of Cutibacterium acnes in normal and diseased skin. Cell Host & Microbe, 32(7), 1129–1146.e8.
12. Eguren, C., Navarro-Blasco, A., Corral-Forteza, M., Reolid-Pérez, A., Setó-Torrent, N., García-Navarro, A., & Navarro-López, V. (2024). A randomised clinical trial to evaluate the efficacy of an oral probiotic in acne vulgaris. Acta Dermato-Venereologica, 104, 33206.
13. Sathikulpakdee, S., Kanokrungsee, S., Vitheejongjaroen, P., Kamanamool, N., Udompataikul, M., & Taweechotipatr, M. (2022). Efficacy of probiotic-derived lotion from Lactobacillus paracasei MSMC 39-1 in mild to moderate acne vulgaris: Randomised controlled trial. Journal of Cosmetic Dermatology, 21(10), 5092–5097. https://doi.org/10.1111/jocd.14971
14. Yerushalmy-Feler, A., Ron, R., London-Ron, N., Harari, M., Oved, K., & Yankilevich, P. (2023). Topical phage therapy in a mouse model of Cutibacterium acnes-induced acne-like lesions. Nature Communications, 14(1), 1005. https://doi.org/10.1038/s41467-023-36694-8
15. Golembo, M., Puttagunta, S., Rappo, U., Weinstock, E., Engelstein, R., Gahali-Sass, I., & Zak, N. B. (2022). Development of a topical bacteriophage gel targeting Cutibacterium acnes

for acne-prone skin and results of a phase 1 cosmetic randomised clinical trial. JEADV Clinical Practice, 2(2), e93. https://doi.org/10.1002/ski2.93

16. Grice, E. A., Kong, H. H., Conlan, S., Deming, C. B., Davis, J., Young, A. C., & Segre, J. A. (2009). Topographical and temporal diversity of the human skin microbiome. Science, 324(5931), 1190–1192. https://doi.org/10.1126/science.1171700
17. Dagnelie, M. A., Corvec, S., Saint-Jean, M., Nguyen, J. M., Khammari, A., & Dreno, B. (2019). Cutibacterium acnes phylotypes diversity loss: A trigger for skin inflammatory process. Journal of the European Academy of Dermatology and Venereology, 33(12), 2340–2348. https://doi.org/10.1111/jdv.15729
18. Dreno, B., Pecastaings, S., Corvec, S., Veraldi, S., Khammari, A., & Roques, C. (2018). Cutibacterium acnes (Propionibacterium acnes) and acne vulgaris: A brief look at the latest updates. Journal of the European Academy of Dermatology and Venereology, 32(Suppl 2), 5–14. https://doi.org/10.1111/jdv.15043
19. Sánchez-Pellicer, P., Navarro-Moratalla, L., Núñez-Delegido, E., Ruzafa-Costas, B., Agüera-Santos, J., & Navarro-López, V. (2022). Acne, microbiome, and probiotics: The gut–skin axis. Microorganisms, 10(7), 1303. https://doi.org/10.3390/microorganisms10071303
20. Castillo, D. E., Nanda, S., & Keri, J. E. (2019). Propionibacterium (Cutibacterium) acnes bacteriophage therapy in acne: Current evidence and future perspectives. Dermatology and Therapy, 9(1), 19–31. https://doi.org/10.1007/s13555-018-0275-9
21. Fitz-Gibbon, S., Tomida, S., Chiu, B. H., Nguyen, L., Du, C., Liu, M., & Kim, J. (2013). Propionibacterium acnes strain populations in the human skin microbiome are associated with acne. Journal of Investigative Dermatology, 133(9), 2152–2160. https://doi.org/10.1038/jid.2013.21
22. Dagnelie, M. A., Montassier, E., Khammari, A., Mounier, C., Corvec, S., & Dreno, B. (2019). Inflammatory skin is associated with changes in the skin microbiome composition on the back of severe acne patients. Experimental Dermatology, 28(8), 961–967. https://doi.org/10.1111/exd.13988
23. Dreno, B., Araviiskaia, E., Berardesca, E., Gontijo, G., Sanchez Viera, M., Xiang, L. F., & Bieber, T. (2016). Microbiome in healthy skin: update for dermatologists. Journal of the European Academy of Dermatology and Venereology, 30(12), 2038–2047. https://doi.org/10.1111/jdv.13965
24. Coenye, T., Spittaels, K. J., & Achermann, Y. (2022). The role of biofilm formation in the pathogenesis and antimicrobial susceptibility of Cutibacterium acnes. Biofilm, 4, 100063. https://doi.org/10.1016/j.bioflm.2021.100063
25. Moradi Tuchayi, S., Makrantonaki, E., Ganceviciene, R., Dessinioti, C., Feldman, S. R., & Zouboulis, C. C. (2015). Acne vulgaris. Nature Reviews Disease Primers, 1, 15029. https://doi.org/10.1038/nrdp.2015.29
26. Zaenglein, A. L., Pathy, A. L., Schlosser, B. J., et al. (2016). Guidelines of care for the management of acne vulgaris. Journal of the American Academy of Dermatology, 74(5), 945–973.e33. https://doi.org/10.1016/j.jaad.2015.12.037
27. Dessinioti, C., & Katsambas, A. (2022). Antibiotics and antimicrobial resistance in acne: Epidemiological trends and clinical practice considerations. Yale Journal of Biology and Medicine, 95(4), 429–443.
28. Reynolds, R. V., Yeung, H., Cheng, C. E., Cook-Bolden, F., Desai, S. R., Druby, K. M., & Tan, J. K. (2024). Guidelines of care for the management of acne vulgaris. Journal of the American Academy of Dermatology, 90(5), 1006.e1–1006.e30. https://doi.org/10.1016/j.jaad.2023.12.017
29. Claesen, J., Spagnolo, J. B., Ramos, S. F., Kurita, K. L., Byrd, A. L., Aksenov, A. A., & Fischbach, M. A. (2020). A Cutibacterium acnes antibiotic modulates human skin microbiota composition in hair follicles. Science Translational Medicine, 12(570), eaay5445. https://doi.org/10.1126/scitranslmed.aay5445
30. Platsidaki, E., & Dessinioti, C. (2018). Recent advances in understanding Propionibacterium acnes (Cutibacterium acnes) in acne. F1000Research, 7, 1953. https://doi.org/10.12688/f1000research.15659.1