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Effect of the Skin Microbiota on Acne

Introduction

Acne is the most common skin disease among adolescents and young adults. It is a chronic inflammatory disease of pilosebaceous units that affects the skin, most commonly affecting the face. Acne develops when these specialized follicles undergo pathologic alteration which results in the formation of both inflammatory and non-inflammatory lesions on the skin, skin reddening and pustule formation (Julianti et al., 2017). When acne is chronic, it develops into acne vulgaris, a serious skin disorder involving the hair follicles and sebaceous glands (Xu and Li, 2019). Acne is especially prevalent during adolescence. During adolescence, 85%-90% of people experience acne. This period coincides with the onset and transition through puberty. The physical and biochemical properties of the skin are influenced by puberty that leads to acne formation (Schneider et al., 2022). Although acne is considered a disease of teenagers, research has uncovered that its prevalence in adults is increasing (Yildirim et al., 2022). One study showed that the mean age of acne patients increased from 20.5 years in 1984 to 26.5 years in 1994 (Gouden et al., 1997).

Acne has been known to have a drastic impact on social life and quality of life of humans. Acne can cause stigmatization, a discrediting mark setting a person off from others. This can disrupt and prevent normal interpersonal relationships, resulting in social discrimination and alienation. This is due to acne lesions, although not life-threatening, these lesions are very troublesome due to their visibility and mainly being on the face. The skin lesions can cause various reactions in people including fear, disgust, and aversion. In many patients, acne is linked to severe psychiatric comorbidities and can even lead to suicidal thoughts and attempts

(Szepietowska et al., 2022). The Dermatology Life Quality Index (DLQI) has found that acne patients have a higher score than psoriasis patients, meaning quality of life is increasingly impaired.

The skin is colonized by a diverse community of microorganisms that are shaped by many factors. Chemical and physical parameters, skin topography, and microbe-microbe interactions all influence the type of microbes present on the skin. Acne usually starts when hormonal changes cause the enlargement and obstruction of sebaceous glands in the skin. This obstruction causes the accumulation of sebum, which is followed by abnormal proliferation of bacterial populations (Elman et al., 2003). *Staphylococcus* and *Cutibacterium* are the most abundant and ubiquitous genera that colonize the human skin. These microbes are found in almost all parts of the skin ecosystem but have preferential niches. For example, some species of *Staphylococci* are located in areas of high humidity, while *Cutibacterium acnes* is found in more sebaceous areas (Ahle et al., 2022). Specifically, *Staphylococcus epidermis* and *Propionibacterium acnes* play major roles in the formation of acne (Julianti et al., 2017). Yeasts also inhabit the skin and can cause acne development along with these bacteria. One of these acne-inducing yeasts is *Malassezia furfur* which can influence sebum production (Xu and Li, 2019).

Staphylococci are typically Gram-positive, facultative anaerobic round shaped bacteria that form bunches. *Staphylococcus epidermidis* is a coagulase-negative, staphylococci that colonizes the skin (potentially opportunistic pathogen). *S. epidermidis* is divided into three main clusters (A, B, C) and is assigned to different sequence types. *S. epidermidis* sequence types have been previously linked to nosocomial infections, suggesting it could have pathogenic potential. Some staphylococcal strains including *S. epidermidis* can produce short chain fatty acids, and

some strains also produce bacteriocins. These prevent the colonization and spread of *C. acnes* and other disease-associated bacteria (Ahle et al., 2022).

Studies have shown that *S. epidermidis* can convert glycerol to four short-chain fatty acids: acetate, butyrate, lactate, and succinate. Butyrate and succinate have exhibited anti-inflammatory effects by directly killing *C. acnes* or lowering the secretion of proinflammatory cytokines. *S. epidermidis* produces succinic acid via fermentation, harbors the early secreted antigen 6kDa (ESAT-6) secretion system with antibacterial activity, or secretes staphylococcal lipoteichoic acid to lower the inflammation associated with *C. acnes* (Huang et al., 2022). Therefore, the presence of *S. epidermidis* is a natural skin defense against acne. Using probiotics to increase *S. epidermidis* on the skin can yield better outcomes for acne patients (Goodarzi et al., 2020). On the other hand, *S. epidermidis* is involved in acne formation because acne vulgaris is characterized by increases in *S. epidermidis* abundance, based on the various types and strains that could be present (O'Neill and Gallo, 2018).

Staphylococcus aureus also causes folliculitis, resembling acne (Durdu and Ilkit, 2013). Also, *S. aureus* is found at higher rates in patients with acne compared to patients with healthy skin, but the exact mechanism of acne formation is not explicit (Kumar et al., 2016).

Propionibacterium acnes is a Gram-positive, facultative, anaerobic rod that is another major colonizer of human skin (Achermann et al., 2014). This bacterium is abnormally proliferated when there is an accumulation of sebum. *P. acnes* attracts inflammatory cells, causing the formation of red, painful pustules of acne. These lesions are able to heal but can cause permanent scarring. *P. acnes* produce porphyrins, mainly coprophyrins. Visible light in the blue range induces a photo-destructive effect on *P. acnes* that can decrease acne severity. This is

due to blue-violet light triggering excitation of coproporphyrins that produce peroxide, able to kill the bacteria (Elman et al., 2003).

Recently, *P. acnes* has been renamed *Cutibacterium acnes* to account for genomic adaptive changes, although the name *P. acnes* is still taxonomically valid. Specific lipase genes were identified that encodes for triacylglycerol lipase and lyso-phospholipase that are able to degrade sebum lipids (Platsidaki and Dessinioti 2018). Adult acne patients harbor an 'acne microbiome' dominated by specific strains of *Cutibacterium acnes*. Their acne severity is also associated with the loss of *C. acnes* diversity. Acne-associated strains of *C. acnes* contain extra virulence related genes, produce more porphyrins, trigger a proinflammatory response, and contain more antibiotic resistance elements. One virulence gene in acne-associated strains is *ytp*A that contributes to host tissue degradation and inflammation by encoding for a lysophospholipase. Also, *acs*A and *rcs*B are genes in acne associated strains that are involved in biofilm formation (Cavallo et al., 2022). Porphyrins are pro-inflammatory metabolites that are crucial in vitamin B12 synthesis. Vitamin B12 supplementation in humans suppresses B12 synthesis in *C. acnes* and increases host inflammatory response and bacterial porphyrin biosynthesis. *C. acnes* strains that are associated with acne respond to B12 supplementation by increasing porphyrin production, unlike healthy skin-associated *C. acnes* strains. The time-frame of when the development of the 'acne microbiome' occurs and when the shift in *C. acnes* strain composition occurs during puberty is unclear (Schneider et al., 2022).

C. acnes has a highly inflammatory effect that initiates the release of lymphocytes, neutrophils, and macrophages. These cause follicular damage, and the leakage of fatty acids, lipids, and bacteria into the dermis. This causes inflammatory lesions such as pustules, cysts, papules, and nodules to form onto the skin. The neutrophils that are released further contribute to

the acne inflammation by damaging the follicular epithelium (Fox et al., 2016). *C. acnes* produces the short-chain fatty acids, propionate and valerate through glycerol fermentation. These short-chain fatty acids have a proinflammatory effect on epidermal keratinocytes by inhibiting histone deacetylase activity, promoting cytokine expression in response to Toll-like receptor (TLR) ligands for TLR2 or TLR3. Propionate and valerate drive the inflammatory response by enhancing the release of IL-6 and IL-8 from sebocytes after activation of TLR-2 by macrophage-activating lipopeptide-2 (Huang et al., 2022).

C. acnes has been identified on the skin and in sebaceous glands in multi–species biofilm communities. A biofilm is a cluster of microbes that contains three essential components: the microbial cells, a surface to which the cells adhere, and a self-produced extracellular polymeric matrix in which the cells are embedded and they form larger communities. Bacteria within biofilms may exist as persister cells in a sessile state. These bacteria can communicate through "quorum sensing" and are metabolically protected and inert. This biofilm protects the bacteria within it from antibiotic therapy by limiting the penetration of effective antimicrobial concentrations (Dessinioti and Katsambas, 2022).

Streptococcus agalactiae is a Gram- positive bacterium found on human skin that is predicted to be involved in the pathogenesis and progression of *C. acnes*. This is because *S. agalactiae* CAMP factor (that enhances the beta-hemolysis of *S. aureus)* acts as a pore-forming toxin. *S. agalactiae* has pore-forming toxins such as beta-hemolysin/cytolysin and CAMP factor that mediate their entry into host epithelial and endothelial cells by forming pores in the host cell. The beta-hemolysin/cytolysin is encoded by the *cylE* gene and allows it to form an inflammatory response in the host cell. The CAMP factor is encoded by the *cfb* gene and leads to cytolysis (Kumar et al., 2016).

There are other bacterial groups that differ between acne skin and healthy skin. A study by Shi et al. (2021) cultured the skin of acne patients and also people with healthy skin. They found that compared to healthy skin, the skin of people with acne had increased *Firmicutes* and reduced *Proteobacteria* and *Acinetobacter*. This same study also found 5 operational taxonomic units (OTUs) exclusively on the skin of participants with acne. OTU535601 (*Lachnospiraceae*), OTU4460604 (*Clostridiales)*, OTU3217705 (*Moraxellaceae*). OTU1066814 (*Prevotella*), and OTU455671 (*Lactococcus*) were the top 5 most abundant species found on the acne participant's skin that were not found on healthy skin. These OTUs have been studied before showing that *Moraxellaceae* contribute to mucosal inflammation. In a previous study, participants with atopic dermatitis had significant enrichment of *Prevotella* (Shi et al., 2021). With this information in mind, these top 5 most abundant species found on the skin of participants with acne may be key bacterial factors for the development of acne vulgaris (Shi et al., 2021).

Pseudomonas aeruginosa and *Escherichia coli* are Gram-negative bacteria that can cause acne-like lesions called Gram-negative folliculitis. Gram-negative folliculitis most commonly occurs in patients who have had inflammatory acne for long periods of time and have been treated with long term-antibiotics. Acne patients with Gram-negative folliculitis typically have severe seborrhea with oily skin with perioral and perinasal papules and pustules. Gram-negative folliculitis is the replacement of the Gram-positive resident flora on the facial skin by Gram-negative rods. These Gram-negative rods colonize in the sebaceous follicles, causing inflammation. This leads to papules and pustules that very closely resemble acne vulgaris (Böni and Negrhoff, 2003).

Malassezia is the most abundant fungal organism on human skin. The genus *Malassezia* belongs to the phylum *Basidiomycota*. This is the most prevalent fungal genus of healthy skin,

but *Malassezia* does demonstrate potential to be pathogenic. *Malassezia* species lack fatty acid synthase genes, except *M. pachydermatis*, making these species dependent on exogenous lipids. This explains why this fungal genus is distributed on seborrheic skin areas (Saunte et al., 2020). This fungal genus co-exists with *C. acnes* and induces acne. Studies have shown that the administration of antifungal drugs significantly reduced acne lesions, leading to the suggestion that *Malassezia* was the potential cause of refractory acne. Other studies have shown that *Malassezia restricta* and *Malassezia globose* can be isolated from the skin of young acne patients. *Malassezia* can hydrolyze triglycerides in sebum which produces free fatty acids, possibly affecting the abnormal keratinization of hair follicular ducts. This also causes chemotaxis of polymorphonuclear neutrophils and promotes secretion of pro-inflammatory cytokines (Xu and Li 2019).

Methods to detect these acne causing bacteria and yeasts:

Typically, bacteria and yeasts are cultured and isolated using appropriate bacterial growth media. First, sampling has to be conducted using swabbing of skin and/or acne themselves. Swabs are used and diluents include phosphate buffered saline (PBS, pH 7.2) to elute and surface spread plate the bacteria on culture media including Tryptic Soy Agar and yeast on culture media including Dixon's and Leeming-Notman and Yeast Potato Dextrose Agar (Saunte et al., 2020). Potato dextrose agar is also an option to culture the yeast, however, it is less used because it is difficult to cultivate some of the new *Malassezia* species on this agar (Kaneko et al., 2005). The components of Dixon's and Leeming-Notman that favor growth of this yeast include low pH, and Tween 40 or Tween 60 (Böhmová at al., 2018).

Further biochemical testing can be done that include PCR, RNA, and DNA sequencing to detect the specific species of bacteria or yeast.

Shi et al. (2021) cultured the bacteria from participant's skin to detect acne causing bacteria. They had participants avoid cleaning the skin, makeup, deodorants, and ointments 24 hours before sampling. They used sterile cotton swabs and scrubbed the sampling site 20 times (10 times in one direction and 10 times perpendicular to the first direction). The swabs were placed in PBS solution and stored at -20°C. From this, DNA was extracted followed by PCR amplification of the 16S rRNA and sequencing. The sequencing results were processed by QIIME 1.9.1. USEARCH was used to obtain operational taxonomic units with 97% similarity. The sequencing revealed that *Firmicutes* were increased in participants with acne and *Proteobacteria* was reduced as reported earlier (Shi et al., 2021).

Using swabs to collect and identify skin microbes along with next-generation sequencing alone does not cover the microbes that live deep in pilosebaceous units. Therefore, Huang et al. (2022) conducted three punch biopsies of acne lesions. These biopsies were homogenized and centrifuged. The supernatants were spread on tryptic soy agar (TSA) plates for growth of bacterial colonies. The colonies were isolated and cultured in tryptic soy broth media and then bacterial DNA was extracted. Bacterial identification was conducted by 16S rRNA sequencing. The bacteria identified included *S. epidermidis, C. acnes, and Acinetobacter junii* (Huang et al., 2022).

Yeast identification methods:

Malassezia can be detected by swabbing the skin site, then spreading on Dixon's or Leeming-Notman agar and incubating at 32-35°C under anaerobic conditions. Then observation for colonies that are cream to yellowish and smooth or slightly wrinkled will determine the presence of this genus. Furthermore, the colonies are globose, oblong-ellipsoidal to cylindrical yeast cells ("*Malassezia*"). Then PCR techniques with primers 26S-F and 26S-R to amplify the

rRNA 26S regions, Matrix Assisted Laser Desorption/Ionization – Time Of Flight (MALDI-TOF), and or Raman spectroscopy can be used for fast and accurate fungal identification (Ilahi et al., 2017; Saunte et al., 2020). To prepare the sample for MALDI-TOF, the sample is mixed with a matrix compound and dried onto a metal sample plate. Then it is placed in a high vacuum source chamber in the mass spectrometer ("Protein Chemistry Lab"). In order to determine the presence of Malassezia using MALDI-TOF, the samples can be compared to the Bruker database dedicated to fungi (4111 spectra) (Diongue et al., 2018). To prepare the sample for Raman spectrometry, the sample can be filtered, centrifuged, or immunocapture (Jahn et al., 2019).

Prevention methods:

One of the most crucial steps for preventing acne is cleansing the skin every morning and night. Cleansing involves the elimination of dirt, dead cells, accumulated skin products, and sweat from the skin. It is performed by cleansers such as soaps, cleansing bars, foaming cleansers, scrubs, gels, and particulates. Cleansers contain surfactants, agents composed of molecules with hydrophilic heads and lipophilic tails that emulsify and remove dirt, microorganisms, and oil. However, surfactants can have problems such as insolubility in hard water, increase in skin pH with use, and a drying and irritating effect on the skin. Changes in skin pH can lead to problems because the acid mantle of the skin is a bacteriostatic medium and inhibits bacterial growth. Cleansers can also bind to the epidermis and disrupt the barrier, causing it to lose its ability to hold water. Cleansing too harshly can lead to disruption of the skin barrier, increased transepidermal water loss, irritated skin, increased bacterial colonization, and increased comedonal formation. Because of this, a good cleanser removes oil and debris, but does not remove constitutive lipids such as ceramides and cerebrosides that prevent transepidermal water loss (Goodman 2009).

Cleansers used for acne prevention should be ‘soap-free’, ‘acidic’ or ‘pH-balanced’, and free of abrasives or alcohols. People with oily skin but otherwise robust skin should use a highly rinseable cleanser without a residual moisturizer. This includes sodium lauryl sulfate sodium-based cleansers that foam and lather as well as synthetic cleansers that are mild and have low irritancy potential. For people with other skin types such as dry/combination, older, sensitive, or sun-damaged skin, a non-comedogenic moisturizing liquid cleanser is recommended. This cleanser will work without overdrying the skin. For dry irritated skin, light lotion and wash-off cleansers are best because they contain emollients that leave a surface film. Robust and oily skin should use medicated and exfoliating cleansers that contain antibacterial agents and benzoyl peroxide (Goodman 2009).

Moisturizing is an important step because it prevents and alleviates skin irritation by soothing the skin and slowing the evaporation of water. Moisturizers are emollients that contain primarily occlusives, humectants, and lubricants. Humectants work by promoting the retention of moisture. Occlusives are filmogenic compounds such as natural polyesters, fatty alcohols, hydrocarbons, waxes, and silicones. Moisturizers should be lightweight and include UV protection during the day and moisturizers at night can be heavier (Goodman 2009).

In order to prevent the formation of acne, facial and hair products that contain oils should be avoided. These products lead to the pores being clogged and acne forming. Instead, oil-free or noncomedogenic products should be used. Also, repeatedly scrubbing the face with soaps, detergents, and other agents can cause trauma to the underlying comedones of the skin, increasing inflammation. If a patient is hoping to prevent acne, they should refrain from aggressively rubbing the face. Other factors that contribute to pore occlusion include tight

clothing and headgear. Therefore, these articles should be avoided to prevent acne (Knutsen-Larson et al., 2012).

One option for the prevention of acne is regulating diet. There is abundant evidence to support the consumption of omega-3 fatty acids reducing acne. This is due to omega-3 fatty acids decreasing, the insulin-like growth factor-1, IGF-1, which is implicated in sebum production and follicular occlusion. Insulin-like growth factor-1 decreases nuclear levels of the metabolic forkhead box class O transcription factor, leading to activation of the mammalian target of rapamycin complex 1. Mammalian target of rapamycin complex 1 is connected to cell proliferation and metabolism. In acne, mammalian target of rapamycin complex 1 mediates lipid synthesis, hyperplasia of keratinocytes, and sebaceous gland hyperproliferation. Omega-3 fatty acids also reduce inflammatory acne lesions by inhibiting the synthesis of proinflammatory leukotriene B (Baldwin and Tan 2021).

Low glycemic index and low glycemic load diets are effective at preventing acne. Glycemic index of a food is the rise in blood glucose level, relative to pure glucose, 2 hours after the consumption of that food. Glycemic load measures the food's ability to cause blood glucose levels to rise, which accounts for the carbohydrate in the food. A low Glycemic load diet is able to reduce the free androgen index and increase insulin-growth factor binding protein-3, which directly impacts the keratinocyte hyperplasia and apoptosis. Both a low glycemic index diet and glycemic load diet decrease IGF-1 levels, decreasing overall sebum production (Baldwin and Tan 2021).

Abstaining from milk can be an effective way to prevent acne. Acne flares in patients consuming milk could be related to whey proteins and casein via insulinotropic and IGF-1 pathways. Studies have shown that milk consumption increases IGF-1 levels and casein

stimulates IGF-1 to a greater extent. This indicates why other dairy products such as butter and cheese have not demonstrated acne-causing effects (Baldwin and Tan 2021).

Avoiding cigarette smoking has shown to help prevent the formation of acne. Patients that smoke cigarettes have been shown to have increases in acne development. Studies have found a dose-dependent relationship between daily cigarette use and acne disease severity (Knutsen-Larson et al., 2012).

Treatment Options

There are various available treatments for acne; however, there are many patients that fail to respond adequately or develop problematic side effects. Topical treatments are available, but they can cause irritation to the skin. These tropical treatments include comedolytic agents, antibiotics, and various anti-inflammatory drugs. Oral antibiotics are used to treat acne, but 40% of acne bacteria are insensitive to oral antibiotics. When using oral antibiotics for acne, the regimen should not exceed three months (Platsidaki and Dessinioti, 2018). Antibiotics targeting *P. acnes* have been routinely used in acne treatment over the past four decades. Macrolides including Clindamycin and erythromycin, as well as tetracyclines are most widely prescribed by physicians to treat acne and to inhibit *P. acnes* (Xu and Li, 2019). Monotherapy with topical antibiotics is not recommended for acne due to the development of antibiotic resistance (Zaenglein et al, 2016).

The macrolide class of antibiotics work by binding to the 50S subunit of the bacterial ribosome. This halts protein synthesis by the bacteria and causes death (Vázques-Laslop and Makin 2018). Clindamycin 1% solution or gel is the preferred topical antibiotic to combat acne. Topical erythromycin in 2% concentration is available but has reduced efficacy in comparison with clindamycin (Zaenglein et al, 2016). However, long-term use of oral macrolides for acne

treatment increases the macrolide-resistant *P. acnes* strains. Several regions of the world have reported increasing levels of *P. acnes* resistance to macrolides and clindamycin. This resistance is due to point mutations G2057A, A2058G, and A2059G in the domain V of 23S rRNA and the presence of the *erm(X)* gene (Xu and Li, 2019). The *erm(X)* gene codes for erythromycin resistant rRNA methyltransferases. The *erm(X)* gene is acquired from a *Corynebacterium* transposon Tn5432 (Dessinioti and Katsambas, 2022). These enzymes give rise to macrolide, lincosamide, and streptogramin B cross resistance (Mitcheltree 2021). In order to combat this antibiotic resistance, it is recommended to use topical antibiotics in combination with benzoyl peroxide or retinoid acne treatment. This reduces the total number of *P. acnes* strains on the skin and lowers the antibiotic resistance of *P. acnes* to erythromycin and clindamycin (Xu and Li, 2019). Tetracyclines are another class of antibiotics that are frequently used to treat acne. Tetracyclines are very active against the majority of *P. acnes* isolates. This class of antibiotics works by binding to the 30S subunit of the bacterial ribosome and inhibiting protein synthesis. Tetracyclines also have anti-inflammatory effects by inhibiting chemotaxis and metallo-proteinase activity (Zaenglein et al, 2016). One problem with using this antibiotic to treat acne is that resistance to this antibiotic is rising. This increased resistance is due to a G1058C mutation in *P. acnes* 16S rRNA gene. Of the tetracycline class, minocycline is the most effective agent for acne treatment due to it having a lower resistance rate (Xu and Li, 2019). The safest dose for minocycline in an extended-release form is 1 mg/kg. Doxycycline is most effective between 1.7 to 2.4 mg/kg dose range (Zaenglein et al, 2016).

As mentioned above the main problem with prescribing antibiotics to treat acne is the rising antimicrobial resistance. This is a growing issue as indicated by the Centers for Disease Control and Prevention's estimate that antimicrobial resistance infections cause one death every

fifteen minutes in the United States (Dessinioti and Katsambas, 2022). Emerging resistant strains are caused by the overuse of topical and systemic antibiotics, the long treatment courses of antibiotics used for acne, and the availability of over-the-counter antibiotics preparations in some countries. Current acne guidelines recommend antibiotics for no longer than three months; however, the reported length of antibiotic treatment is much longer. A study from the US MarketScan Commercial Claims and Encounters database saw that of the 29,908 patients prescribed oral antibiotics, over half were treated for over three months (Dessinioti and Katsambas, 2022).

It has been proposed to formulate antibiotics with sucrose to eliminate the risk of generating *C. acnes*. This is because sucrose is a selective fermentation initiator that can intensify the fermentation of *S. epidermidis*, but not *C. acnes.* By including sucrose as an adjuvant in antibiotics, *S. epidermidis* would be able to increase its growth. This would help eliminate *C. acnes* because the short-chain fatty acids that *S. epidermidis* produce directly kill *C. acnes* (Wang et al., 2016).

Because of the adverse effects of monotherapy with antibiotics, prescribing antibiotics in combination with benzoyl peroxide formulations is recommended. Benzoyl peroxide has anti-propionibacterium effects irrespective of antibiotic susceptibility. It may also enhance the penetration and concentration of topical antibiotics in acne lesions. Benzoyl peroxide has been found to act in synergy with topical antibiotics against some resistant strains and may reverse the selectivity of topical clindamycin resistant *C. acnes* strains (Dessinioti and Katsambas, 2022). Benzoyl peroxide 5% gel treatment has been shown to reduce the surface and follicular *C. acnes* after as little as two days of treatment. It is recommended to use a topical benzoyl peroxide

course for at least five to seven days between antibiotic courses to reduce the emergence of resistant strains. (Dessinioti and Katsambas, 2022).

Topical retinoids are another form of treatment that can be used for acne. Topical retinoids work by controlling the formation of microcomedones, reducing the formation of existing comedones and lesions, decreasing sebum production, and normalizing desquamation of the epithelium. These topical retinoids are able to target the microcomedones and suppress comedone formation. They also possess anti-inflammatory properties. Some suggest that topical retinoids be the first choice of treatment for most acne types. One side effect of this treatment is acne flare up within the first few weeks of treatment. One commonly used topical retinoid is tretinoin, a form of vitamin A. It is effective because it regularizes desquamation of the epithelium, which prevents obstruction of pilosebaceous units. This treatment also possesses anti-inflammatory properties and has been used as acne treatment for decades. Adapalene is another treatment that is a synthetic retinoid analogue. This treatment is most commonly used as a first line topical retinoid treatment for acne vulgaris. It works by normalizing the cell differentiation of the follicular epithelium and preventing comedone formation. Adapalene also has anti-inflammatory action on the acne lesions. Tazarotene is a synthetic acetylenic pro-drug that is transformed to tazarotenic acid in keratinocytes. It is a newer retinoid for acne treatment that affects keratinocyte differentiation and proliferation in the epithelial tissue. Because this treatment can cause skin irritation, it is regarded as a second line treatment after tretinoin or adapalene (Fox et al., 2016).

Oral isotretinoin is an isomer of retinoic acid that has been used to treat acne. It has been used for over thirty years and has proven to be very effective. It works by decreasing sebum production, acne lesions, and acne scarring. It is mainly used for moderate acne that is treatment

resistant, or acne that produces physical scarring or significant psychosocial distress. Isotretinoin is commonly given at a starting dose of 0.5mg/kg each day for the first month. This dosage increases to 1.0mg/kg/day thereafter as tolerated by the patient. In some severe acne cases, lower starting doses with or without the use of oral steroids may be needed. This medication is highly lipophilic and is best absorbed when it is taken along with food (Zaenglein et al, 2016). Many people also use oral isotretinoin and have a high possibility of severe side effects (Elman et al., 2003). Of these side effects, the most prevalent side effects involve the mucocutaneous, musculoskeletal, and ophthalmic systems, typically mimicking symptoms of hypervitaminosis. Patients taking isotretinoin have reported changes in mood. This includes depression, suicidal ideation, and suicide. Although there is no evidence-based link between the medication and these symptoms, the prescribing physician should monitor for these symptoms. Another concern for patients on isotretinoin is bone demineralization and premature epiphyseal closure that is associated with long-term oral retinoid intake. Patients on this medication have higher rates of colonization with *S. aureus* leading to increased rates of minor skin infections such as folliculitis and furunculosis. One of the most serious side effects of isotretinoin is the risk of retinoic acid embryopathy. This can lead to exposed pregnancies having congenital malformations. This has led to the risk management program, iPLEDGE, being developed. Patients receiving this medication must adhere to iPLEDGE to require patients to abstain from sex or use two contraceptive methods. However, studies have found that one-third of all women of childbearing potential admitted noncompliance with the iPLEDGE requirements (Zaenglein et al, 2016).

When skin lesions resembling acne are caused by the fungus, *Malassezia*, antifungal medication aids in treating acne lesions. Therefore, when patients fail to respond to typical acne medications, in particular those with pruritic, 1 to 2 mm monomorphic pustules and papules, it is

important to consider *Malassezia* as the cause. This occurs most frequently in patients in hot, humid environments or in patients with excessive sweating. In order to treat this fungus, oral ketoconazole can be prescribed as an initial choice. This drug is a good first choice because most *Malassezia* species are sensitive to it. Fluconazole is another antifungal that can be prescribed. Fluconazole is very effective against treating skin lesions due to *M. sympodialis*, *M. slooffiae*, but less active against *M. globose*, and *M. restricta*. Itraconazole is an antifungal that could be used for *M. globose* because it has a high activity against the fungus by altering fungal cell function. It inhibits cytochrome P450-dependent ergosterol synthesis, disturbing membrane function, resulting in growth inhibition and death (Gupita and Foley 2015; Yoshida 1988). If medications show lack of improvement, the medications should be changed because it is likely due to the *Malassezia* resistance to various antifungals (Rubenstein and Malerich 2014).

Many over-the-counter products for acne contain salicylic acid. This is a keratolytic agent whose mechanism of action is to dissolve the intercellular cement that holds the cells of the epithelium together. Salicylic acid is fungistatic and bacteriostatic. Salicylic acid also has a minor anti-inflammatory effect and enhances the penetration of certain substances (Fox et al., 2016). This allows salicylic acid to be an effective over-the counter treatment.

To overcome antibiotic resistance and these side effects, essential oils and medicinal plant extracts present alternative solutions for combating acne. Some research has shown cinnamon to have potential activity against acne. Cinnamon contains cinnamaldehyde, which has anti-inflammatory activity. Cinnamaldehyde works by inhibiting the production of nitric oxide, a compound responsible for inflammatory conditions within the human body. Cinnamon is also able to prevent the production of the pro-inflammatory agent, cyclooxygenase-2. Cyclooxygenase-2 is a key enzyme that interacts in the inflammation process by catalyzing the

rate-limiting steps in the transformation of arachidonic acid to prostaglandins (Cui and Jia 2021). This gives cinnamon antibacterial and anti-inflammatory properties that allow it to attack acne. Honey also has been seen to work as a natural antibiotic and have anti-inflammatory activity. Honey has anti-inflammatory properties that aid it in reducing the redness of acne. Honey also is acidic, preventing the growth of bacteria. Honey releases hydrogen peroxide, an antimicrobial that is able to remove bacteria that leads to acne growth. Honey also is able to scavenge free radicals because it contains natural antioxidants (Julianti et al., 2017).

Another essential oil that can be used in the treatment of acne is *Origanum vulgare* L. essential oil. It comes from the plant *Origanum vulgare* L., commonly known as oregano. The compounds carvacrol and thymol are isomeric phenolic monoterpenes that are within *Origanum vulgare* L. and are responsible for its main therapeutic properties. Carvacrol and thymol are the two phenols that give oregano essential oils antimicrobial properties. Because carvacrol and thymol are hydrophobic in character, they act by dissolving the hydrophobic section of the bacteria membrane, which increases membrane permeability, and results in a loss of structure in the phospholipid bilayer. This causes functional and structural damage to the cell membrane. Studies have found this essential oil to have a bactericidal effect against *C. acnes.* This essential oil also has anti-inflammatory properties. The carvacrol in the essential oil is able to inhibit NADPH oxidase, lipoxygenase, and reactive oxygen species (Bora et al., 2022). This allows it to reduce the inflammation that acne causes.

Hormonal treatment is also useful during the treatment of acne. Hormone therapy is effective because sebaceous glands are androgen dependent. This treatment is typically given in oral contraceptive pills and is useful for adolescent and adult females. These reduce the sebum production that is initially produced by androgen. These pills increase the synthesis of sex

hormone-binding globulin which in turn decreases the biologically active free testosterone. All contraceptives have the capability to treat hormone related acne, but progestins are preferred because they do not have androgen activity. Because treatment with hormonal anti-androgens takes 3-6 months for results, treatment with this method must last for at least 12 months. An alternative drug is spironolactone, an androgen receptor blocker that is especially effective for use in patients with inflammatory acne (Fox et al., 2016).

Recent developments have been made using high intensity light to reduce acne, that does not have the side effects associated with antibiotics. *P. acnes* is the main bacteria that produces acne as mentioned earlier (see above). *P. acnes* produces porphyrins, mainly coporphyrins. Exposing the porphyrins to special wavelengths in the red or violet-blue light range starts a chemical reaction that produces peroxide. This peroxide is able to kill the *P. acnes*. This reaction is very quick and only takes milliseconds. It also is confined to the bacteria and has no direct effect in the surrounding human tissue. Data shows a greater than 80% response to 420 nm acne phototherapy with a significant reduction of acne lesions after only eight treatments that last fifteen minutes. There also were no adverse effects in any of the patients that underwent this treatment (Elman et al., 2003). This shows that acne therapy by high intensity, narrow band 405-420 nm light is a fast and effective alternative to acne treatment.

5-aminolevulinic acid mediated photodynamic therapy (ALA-PDT) is a treatment able to combat severe acne. This treatment utilizes 5-aminolevulinic acid (ALA) as a photosensitizer, allowing it to be a non-antibiotic therapy for acne vulgaris. This treatment works by direct photodynamic therapy of sebaceous glands, inhibiting sebum production. It also reduces follicular obstruction through an effect on keratinocyte shedding and hyperkeratosis. ALA-PDT is able to conduct photodynamic killing of *C. acnes*, leading to clinical improvements in acne

severity. After treatment with ALA-PDT, there is increased diversity in the follicular microbiota because reduced *Cutibacterium* colonization frees up niche space. Unlike antibiotic therapies, ALA-PDT does not exhibit broad -spectrum antimicrobial activity beyond *C. acnes.* This therapy creates a new balanced, counteracting relationship with increased microbial diversity that may play a role in restoring the intrafollicular microbiome. Multiple treatments may be required due to some *C. acnes* possibly being insensitive to the therapy or declining in function after one treatment. This treatment has no effect on *S. epidermidis*; however, the abundance of *P. fluorescens* increases after treatment. This is beneficial as a study has found that *P. fluorescens* produces a bacteriocin that inhibits the growth of *C. acnes* and *S. epidermidis*. ALA-PDT was found to suppress the function of the skin microbiome, including energy metabolism, DNA replication and sialidase, possibly changing the vitality and virulence of microorganisms. This treatment not only kills *C. acnes,* but also significantly inhibits its function, leading to a reduction of the inflammatory reaction of acne (Tao et al., 2021).

There has been a recent, eco-friendly approach proposed to treat acne. This approach involves the synthesis of TiO2 nanocuboids using the ordered self-assembly of zein biomolecule in shape-controlled hybrid morphologies. This approach uses cylindrical hydrophobic domains of zein (unfolds) and promotes beta sheet formation and simultaneously chemical modification with activated amino acids tryptophan and tyrosine for responsible and effective shape directing agent in nanohybrid synthesis. This method has antimicrobial activity against the acne causing microorganisms *S. aureus, P. aeruginosa, E. coli, and S. agalactiae*. This antimicrobial action is promising for the use in treatment of acne (Badgujar and Kumar, 2021).

Chemical peels are a great complementary treatment for acne rather than a first-line treatment. These work by using facial resurfacing whereby the removal of epidermis stimulates

re-epithelization and rejuvenation of the skin. This method reduces hyperpigmentation and superficial scarring of the skin that is associated with acne (Fox et al., 2016).

Summary

C. acnes is the primary bacteria that causes acne along with the yeast *Malassezia*. *S agalactiae* may further the progression and prevalence of *C. acnes*. *S. epidermidis* is a Gram-positive bacteria that can inhibit the growth of the acne-causing bacteria, *C. acnes*. Other bacteria can cause folliculitis, which is papules and pustules that closely resemble acne and are common alongside acne. Strict diet, hygiene and therapeutics may help alleviate disease symptoms and control the spread of the "acne microbiome." Understanding the growth characteristics of the acne causing bacteria along with their mode of transmission and persistence will help in the development of appropriate therapeutic agents to control disease incidence of acne development.

Table 1. Main agents associated with acne in humans

Pathogen	Gram-character	Morphology	Treatment	Reference
C. acne	Gram-positive	Rod	Clindamycin (1% lotion applied to area every 12 hours for as long as directed), erythromycin (250 to 500 mg orally twice a day initially, then once a day for maintenance as long as directed), minocycline (1 mg/kg taken orally once daily), benzoyl peroxide 5% gel treatment on skin once daily), ALA-PDT (multiple treatments)	(Mayslich et al., 2021; Zaenglein et al., 2016; Xu and Li, 2019; Dessinioti and Katsambas, 2022; Tao et al., 2021)
S. epidermidis	Gram-positive	Cocci	Oral probiotics that include *S. epidermidis*, Antibiotics that target *C. acnes* with sucrose as adjuvant	(Namvar et al., 2014; Goodarzi et al., 2020; Wang et al., 2016)
Malassezia	Yeast	Club shaped	Ketoconazole (2% cream used daily until conditions have improved), fluconazole (100-200 mg daily orally for 1-4 weeks), itraconazole (100-200 mg daily orally for 1-4 weeks)	(Oakley, 2004; Rubenstein and Malerich, 2014; Gupita and Foley, 2015)

Table 2. Treatment options

Therapeutic	Dose (conc/time)	Mode of action	Target pathogen	Reference
Clindamycin	1% lotion applied to area every 12 hours for as long as directed	Binds to 50S subunit of bacterial ribosome and halts protein synthesis	*C. acnes*	(Vázques-Laslop and Makin, 2018; Xu and Li, 2019; Memon, 2022)
Erythromycin	250 to 500 mg orally twice a day initially, then once a day for maintenance as long as directed	Binds to 50S subunit of bacterial ribosome and halts protein synthesis	*C. acnes*	(Vázques-Laslop and Makin, 2018; Xu and Li, 2019; Erythromycin)
Minocycline	1 mg/kg taken orally once daily	Binds to 50S subunit of bacterial ribosome and inhibits protein synthesis	*C. acnes*	(Zaenglein et al., 2016)
Doxycycline	1.7 to 2.4 mg/kg taken orally once daily	Binds to 50S subunit of bacterial ribosome and inhibits protein synthesis	*C. acnes*	(Zaenglein et al., 2016)
Benzoyl peroxide	5% gel treatment on skin once daily	Releases active free-radical oxygen species, resulting in oxidation of bacterial proteins and death	*C. acnes*	(Dessinioti and Katsambas, 2022; Matin and Goodman, 2022)
Adapalene	.1% lotion applied directly to skin in the evening	Normalizing cell differentiation of follicular epithelium and preventing comedone formation	*C. acnes*	(Fox et al., 2016; Russell, 2000)
Oral Isotretinoin	0.5 mg/kg per day for first month, then 1.0mg/kg per day thereafter	Decreasing sebum production	X	(Zaenglein et al, 2016)

Ketoconazole	2% cream used daily until conditions have improved	Inhibits cytochrome P450 14α-demethylase enzyme, the enzyme responsible for biosynthesis of triglycerides and phospholipids	*Malassezia*	(Gupita and Foley 2015; Suzuki et al., 2016; Sinawe and Cassadesus, 2022).
Fluconazole	100-200 mg orally daily for 1-4 weeks	Increases cellular permeability by inhibiting the synthesis of ergosterol. It does this by interacting with 14-demethylase, a cytochrome P-450 enzyme that is responsible for catalyzing the conversion of lanosterol to ergosterol.	*Malassezia*	(Gupita and Foley 2015; Saunte et al., 2020; Govindarajan et al., 2023)
Itraconazole	100-200 mg orally daily for 1-4 weeks	Inhibits cytochrome P450-dependent ergosterol synthesis, disturbing membrane function, resulting in growth inhibition	*Malassezia*	(Gupita and Foley 2015; Yoshida 1988) (Saunte et al., 2020)
Salicylic acid	Any over-the-counter face wash used twice daily	Dissolve the intercellular cement that holds the cells of the epithelium together	X	(Fox et al., 2016)
Origanum vulgare L. essential oil	0.672 mg/mL applied to skin as needed	Dissolve hydrophobic section of bacterial membrane, resulting in loss of structure of the phospholipid bilayer	*C. acnes*	(Bora et al., 2022)
Oral contraceptive pills	One pill per day for at least 12 months	Reduce sebum production	X	(Fox et al., 2016)
ALA-PDT	multiple treatments	photodynamic killing of *C. acnes*, photodynamic	*C. acnes*	(Tao et al., 2021)

		therapy of sebaceous glands		

Note: X denotes alteration of oil production with no specific target.

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