Comparasion of Microbiome Composition in Acne Vulgaris Using Metagenomic Shotgun and 16s Rrna

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Abstract

Acne vulgaris is a common skin disease in adolescents and young adults. One factor that plays a role in the development of acne vulgaris is changes in the composition of the microbiome in the skin. The microbiome is the community of microorganisms that live on the surface of the skin and plays an important role in maintaining the ecological balance of the skin. Research on microbiome composition in acne vulgaris has been conducted using various analytical methods, including shotgun metagenomics and 16S rRNA. The aim of this study was to compare the microbiome composition in acne vulgaris using shotgun metagenomics and 16S rRNA. This research method is a literature review while data collection techniques are carried out by library studies obtained from 3 databases Pubmed, Google Scholar and Science Direct. The collected data were then analyzed using qualitative analysis. The results showed that some of the most common bacteria in acne vulgaris, such as Propionibacterium acnes, Staphylococcus epidermidis, and Staphylococcus aureus. In recent years, microbiota screening has been developed using NGS techniques using metagenomic whole genome shotgun and 16S rRNA DNA sequencing analysis. NGS techniques have been able to determine the microbiota of facial skin, and differentiate the bacterial abundance of acne-prone and healthy skin.

Keywords: 16s rRNA, Acnes Vulgaris, Microbiome, NGS

INTRODUCTION

The sebaceous glands in the hair follicles are affected by the chronic inflammatory skin disease known as acne vulgaris (Zhao & Mu, 2020). Acne vulgaris generally develops in areas of the skin rich in lipid secretions and clinically manifests as acne vulgaris, pustules, nodules, and papules are various additional types of skin lesions. Epidemiological studies in different countries of both races show that adolescents have the highest incidence of acne vulgaris, there is little difference in the prevalence of atopic dermatitis (Sacotte & Silverberg, 2018). The majority of mild acne cases in men and women vulgaris, and acne vulgaris. face, chest and back. The causes of acne vulgaris are most often an increase in sebum secretion, hormonal imbalances, and bacterial infections. The cause of bacterial infection for acne vulgaris has been researched for the microbiome and its interaction with innate immunity (O'Neill & Gallo, 2018). The microbiome on the cause of acne vulgaris refers to microorganisms such as bacteria, fungi, and viruses. In previous studies, several bacteria have been found on the skin of acne vulgaris, such as Cutibacterium acnes (previously called *Propionibacterium acnes*), Staphylococcus *epidermidis, and Staphylococcus aureus* (Iskandar, 2013).

With the passage of time, the development of examination technology for microbiota has developed rapidly (Parris & Stewart, 2019). In the last 2 years, microbiota examination has been developed using *Next Generation Sequencing* (NGS) with whole genome shotgun metagenomics and 16S rRNA. The use of NGS has been able to determine the microbiota on the skin of the face, and distinguish the abundance of bacteria from acne vulgaris and healthy skin.

METHODS

This literature review analyzed relevant articles and focused on clinical learning methods that affect critical thinking skills and confidence of nursing students. The articles used in this literature review were articles obtained using 3 databases Pubmed, Google Scholar and Science Direct by entering the keywords "Acnes Vulgaris", "NGS", "Microbiome" and "16s rRNA". Articles obtained with the keywords "Acnes Vulgaris" were 44,300, "NGS" was 1,1800,000, "Microbiome" was 1,360,000 and "16s rRNA" was 878,000. Articles were then filtered based on inclusion and exclusion criteria, the inclusion criteria in this study were articles published in the last 11 years and in Indonesian or English. While the exclusion criteria in this study are articles published more than 11 years and articles in languages other than Indonesian and English. After collecting articles and applying the inclusion criteria, researchers obtained 115 articles. Then the researcher read and sorted articles that were relevant to the research, which obtained 52 articles. The data that has been collected is then coded with the help of the Mendeley application program, and analyzed qualitatively.

RESULTS

About 16 percent of the weight of the human body is made up of the skin, one of the body's largest and most vital organs. The skin serves as a conduit between the body and its surroundings. It performs several crucial physiological processes, including defense against external physical, chemical, and biological assaults., but also prevents excessive water loss from the body and its role in thermoregulation (Adetuy & Oloke, 2022). It is influenced by factors such as genetics, hormones, sebum production, hydration levels, and commensal microbiota within the body (Prescott et al., 2017). The epidermis, dermis, and subcutaneous layers are the three primary parts of the skin tissue. Each layer has certain characteristics and functions.

The epidermis is the outermost layer of the skin, which is the layer that is directly opposite the external environment of the skin (Gilaberte & Juarranz, 2016). The epidermal layer includes 5 (Figure 1), namely layers (Ogonowska & Nakonieczna, 2021):

Basal layer / stratum germinativum

The basal layer (basal layer) separates the deepest layer from the dermis, and hemidesmosomes connect it to the basal membrane. In this layer, the cells are rectangular in shape parallel to the columnar mitotic stem cells that continuously produce keratinocytes. This layer also contains melanocytes (Putri, 2018).

a. Stratum spinosum

It contains irregular polyhedron cells with cytoplasmic processes and is sometimes referred to as the "spine". Dendritic cells can be found on this layer.

b. Stratum granulosum

It has 35 layers of cells, containing keratinized cells in the shape of diamonds grains and flat grains. Keratohyalin the keratinous precursors seen in granules eventually aggregate, cross-bind, and form bundles. Glycolipids, which are released onto the surface of cells and act as glue to hold cells together, are present in flat granules.

a. Stratum lucidum

A thin clear layer made of eleidin, a byproduct of keratohyalin transformation, is present on the thicker skin found on the palms and soles of the feet, which has 23 layers of cells.

b. Stratum corneum

Having a 2030 cell layer, is the upper layer composed of keratin-dead keratin keratinocytes and stratum corneum, known as nucleated squamous epithelial cells. This is the layer with the greatest change in thickness, especially for the corneum. Inside this layer, the dead keratinocytes secrete defensin. Defensin is part of the skin's first line of defense.

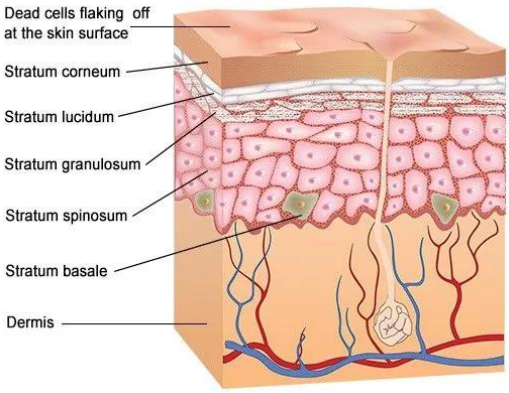

Figure 1. The epidermal layer consists of 5 layers, each of which has a role for the body's first defense system

Sebum is a substance resulting from holocrine secretion obtained from sebocytes undergoing a maturation process followed by specific cell death (Clayton et al., 2020). Sebum consists of neutral nonpolar cell and lipid remains, predominantly comprised of fatty acids and triglycerides (57.5% of total lipids), *esters of wax (26%)*, cholesterol (12%) and squalene (4.5%). [4] The sebaceous glands (KS) account for most of the skin's surface lipids through its main product, sebum, which helps maintain moisture and prevent skin drying. A uni or multilobular form of sebaceous glands that are typically connected to hair follicles that combined with the hair follicles make up a structure known as a pilosebaseic unit.

The *natural moisturizing factor* (NMF) is produced by flat granules, consisting of amino acids and their metabolites, which are byproducts formed from the breakdown of filagrin. NMF was found inside SC cells and provided humectant (water-binding) qualities at SC (Figure 2). NMF consists of highly soluble chemicals in water (Gunnarsson et al., 2021): Consequently, it may soak up a lot of water, even when the humidity level is low. This allows SC to maintain high moisture content even in dry environments. NMF also provides an aqueous environment that is important for enzymes that require such conditions to function.

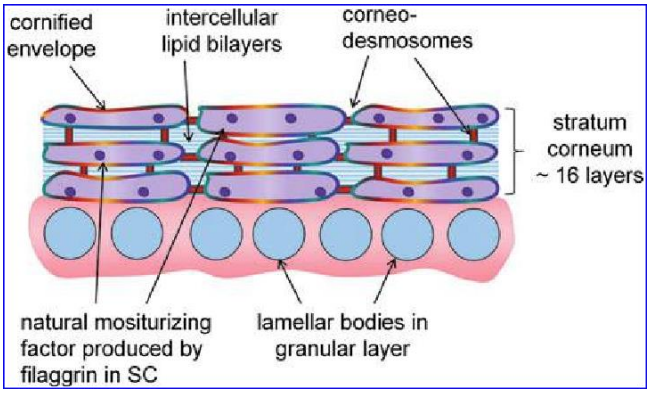

Figure 2. NMF in the stratum corneum maintains a high water content for the enzyme to function

Acne Vulgaris

Acne vulgaris is a chronic inflammatory skin disease of the sebaceous glands of the hair follicles (Oh, 2021). Acne vulgaris generally develops in areas of the skin rich in lipid secretion and clinically manifests as papules, pustules, nodules, and various other forms of skin lesions (Leung & Hon, 2021). Epidemiological studies in different countries and races show that adolescents have the highest incidence of acne vulgaris, there is a slight difference in the incidence of acne vulgaris between men and women, most cases of mild acne vulgaris, and acne vulgaris face, chest and back.

According to American studies, men and women with an age range of 20–29 years experienced an incidence of acne vulgaris of 42.5 percent and 50.9 percent, respectively. According to a survey of 693 Korean children aged 7 to 12 years, the average incidence of acne vulgaris was 36.2% overall, 23.8% in younger kids (ages 7-9), and 47.4% in older kids (10 to 12 years). In the Nigerian student study, 68.8% of students had acne vulgaris and 50.9% of the population was female. Indonesian Cosmetic Dermatology study group (Perdoski) stated that acne vulgaris ranks 3rd in the 3rd most diseases out of the number of visitors to the Department of Skin and Venereal Health Sciences in Hospitals and Skin Clinics (Deliana et al., 2019). The Department of Skin and Venereal Health Sciences FKUI / RSUPN dr. Cipto Mangunkusumo - Jakarta in Indonesia stated that the incidence of acne vulgaris is 95-100% of men and 83-85% of women between the ages of 16 and 17, while in adulthood, women account for 12 percent and males for 3 percent of those who suffer from acne vulgaris (Qonnayda & Sutini, 2022).

Some of the causes of acne vulgaris or acne vulgaris that have been known are (Mahto, 2017):

a. Sebum

Acne vulgaris is thought to occur most frequently as a result of changes in sebum. Acne vulgaris can develop as a result of increased sebum output, and mounting evidence indicates that alterations to the sebum component are directly associated to the development of acne vulgaris (Lynn & Dellavalle, 2016).

b. Hormone levels

Acne vulgaris sufferers have higher levels of testosterone and *5α-Dihydrotestosterone* (DHT) than healthy-skinned individuals, indicating a link between androgens and the occurrence of acne vulgaris. No clear distinction in sebum levels was found between patients Non-obese and hairless women with acne vulgaris and healthy skin (Hashim & Al-Salihi, 2015). However, total testosterone, free testosterone, and progesterone levels are higher in people with acne vulgaris. Significantly greater amounts of free testosterone and sex hormone-binding globulins are found in patients with severe acne vulgaris.

c. Bacterial infections

Propionibacterium acnes is a skin colonization bacterium that is thought to be closely related to the development of acne vulgaris because it can increase sebum secretion and inflammation (McLaughlin et al., 2019). It is a *lipophilic Bacillus* bacterium that was originally isolated from the skin of acne vulgaris patients. *P. acnes* improves T cell division, stimulates the release of pro-inflammatory T cell factors, and stimulates *Toll-like receptor* (TLRs)-2 macrophages to increase.

d. Inflammatory Response

In the mechanism of development of acne vulgaris, the immune response is associated with high levels of expression of IL1a, TLR, and IL2 receptors in the sebaceous gland unit of the hair follicles (Zouboulis et al., 2020). Curative prurigo scarring lesions show high levels of *T cell skin-homing*, macrophage infiltration, high human leukocyte antigen expression and capillary proliferation during regression, and delayed hypersensitivity reactions involved in prurigo's inflammatory mechanisms.

e. Other factors.

Diet, lifestyle, and other factors are also linked to the emergence of acne vulgaris (Heng et al., 2022). Recently, scientists have examined the relationship between acne vulgaris and blood lipid levels. In comparison to healthy persons, acne vulgaris patients who were not obese and who had no hair had significantly higher levels of both total cholesterol (TC) and low-density lipoprotein cholesterol (LDLC) (AppA1) are. Cholesterol levels of highdensity Acne sufferers had considerably lower levels of apolipoprotein (AppA-1) and lipoprotein cholesterol (HDL-C) (Li et al., 2017). Similar results were that levels of TC, LDLC and lipoprotein (LP) were significantly higher in male and female patients with severe acne vulgaris than in unaffected individuals. This correlation provides a new way of thinking to learn more about the etiology of acne vulgaris and its treatment mechanisms.

The skin microbiota has been researched in recent years. With the beginning of culture-based research, there is a shortage of only a few desirable microbes and quantitatively (Chowdhari et al., 2022). Physiological changes in the microbiota determine the role in the potential causes and therapies of acne vulgaris. State-of-the-art analytical tools are needed in studies to determine whether the microbiota plays a role in causing or aggravating acne conditions. The role of the skin microbiota in the pathogenesis and development of acne has been studied for decades (Fitz-Gibbon et al., 2013), but has not been fully explained. hampered by bias-based ability to Even today, sample selection, uniform dissolution, and analytical methods remain challenging problems for low-bias sequence-based methods.

Skin Microbiota

As the largest organ, the skin has biotic components other than *host* cells, namely commensal microorganisms that are beneficial and serve as protectors to help protect the skin from pathogenic invasion. The skin is also an ecosystem for several types of bacteria, fungi and viruses, with their respective roles in protection against pathogens, immune system processes, and the formation of natural skin products (Barnard & Li, 2017). To control the development and growth of microorganisms on the skin is also helped by the activity of

antimicrobial peptides (AMP). AMP is produced by a variety of cells in the epidermis and dermis, including keratinocytes, sebocytes, and immunocytes such as neutrophils, mast cells, monocytes, T cells, and dermal adipocytes. AMP generally provides semi-specific and very strong defenses against various microbes that disrupt the membrane. The production of AMP and antimicrobial lipids regulates the growth of the microbiota by being actively regulated by the sebaceous glands and ecrin glands. Cathelicidin AMP and the filaggrin protein also regulate the penetration of bacteria deeper into the epidermis.

Some bacteria and the skin's immune system interact mutualistically (Nakatsuji & Gallo, 2021). For instance, S. epidermidis creates a distinctively structured lipoteichoic acid that prevents the introduction of molecular pattern-related damage after tissue damage from activating pro-inflammatory signals excessively. This way, while the commensal is shielded from excessive acute inflammation, the host gains from regulated wound healing. The innate immune system is strengthened by a lipopeptide generated by S. epidermidis that protects the host against pathogenic infections by raising AMP synthesis in human keratinocytes and mast cells.

DISCUSSION

NGS for Metagenomics

Over time, sequencing techniques have become an option for studying the skin microbiota, as it can see to a degree of microbiota abundance that cannot be done by culture and isolation. Examination using *Next Generation Sequencing* (NGS) there are 2 ways, namely metagenomic shotgun and *targeted sequencing*. *Whole Genome Sequencing* (WGS) of metagenomics works by characterizing a microbe by quantifying taxonomic diversity, by determining the *richness* and *abudance* of a microbial community. Taxonomic diversity functions as profiling a microbial community and ensuring the commonality of two or more communities. Taxonomic diversity also provides information on the biological function of such microbes. Quantification of taxonomic diversity by analyzing marker genes, grouping sequences into groups (*binning*), and rearranging sequences into distinct genomes. In *targeted sequencing* only the desired target is preserved, for example by using 16s rRNA, 18s rRNA, and ITS regio. The target will go through PCR amplification and sequencing is carried out. Each region is desirable there is a variable region that allows the identification of different groups of organisms. It has the disadvantage of not being able to identify up to the species level and not being able to know the function directly of this method.

Metagenomic Analysis Pipeline Options

Metagenomic Analysis Pipeline Options are options or choices in implementing the metagenomic analysis process (de Vries et al., 2021). Metagenomics is the study of genetic material derived from various microorganisms present in a particular environment (Rahayu et all, 2020). In metagenomic analysis, DNA or RNA data from environmental samples are sequenced and analyzed to identify and analyze the microorganisms present in those samples (Marzuki, 2018). Metagenomic Analysis Pipeline Options include various software and analysis tools that can be used to analyze metagenomic data (Tamames & Puente-Sánchez, 2019). Some of these options include software for assembly, comparison, classification, and functional analysis of metagenomic data. Each option may have certain advantages and disadvantages, and the selection of the right analysis pipeline will greatly affect the results and interpretation of the metagenomic data (Boulund & Kristiansson, 2018).

16S rRNA (amplicon) sequencing analysis

16S rRNA (amplicon) sequencing analysis is a DNA analysis technique used to identify and characterize microorganisms in a sample based on the sequence of their 16S rRNA gene (Tao et al., 2017). The 16S rRNA gene is part of the bacterial and archaeal genome that functions as part of the cellular ribosome (Ferreira-Cerca, 2022). The 16S rRNA sequencing analysis method is one of the most commonly used metagenomic approaches to investigate the structure and diversity of microbiota in various environments, including soil, water, human gastrointestinal tract and others (Jiang et al., 2023). This technique has become an important tool in life science and biomedical studies dealing with microbiota and their role in health, disease and the environment (Avtanski & Reddy, 2023).

The conversion of *raw reads* in the form of fastq file format into feature tables is the first step in amplicon analysis. Raw readings are typically generated from the Illumina platform in *pair-end* 250 bases (PE250) mode. Amplicon *raw pair-end* readings are first sorted by *barcode* order (*demultiplexing*). The pair-end readings are then combined to produce a sequence of amplicons, with the barcode and primary removed (Abate et al., 2023).

To eliminate low-quality amplicon circuits, a quality check is usually required, either by using USEARCH or QIIME. The net amplicon data provided by the sequencing service provider can also be used for further investigation. In amplicon analysis, choosing a representative sequence as a proxy for a species is an important step. Grouping to OTU and denoising to *amplicon sequence variants* (ASVs) are two popular methods for selecting representative sequences. Using the UPARSE algorithm will divide the sequence into OTU based on a 97% similarity. This approach, however, may not be able to detect small variations

between species or strains. DADA2 is a new denoising algorithm, generating ASVs with a more accurate sequence (Liu & Wang, 2023).

By measuring the frequency of feature sequences in each sample, a feature table (OTU/ASV table) can be created. In addition, taxonomy can be assigned to feature sequences, typically at the levels of the kingdom, phylum, class, order, family, genus, and species. This leads to a perspective on the microbiota that is less dimensional. 16S rRNA amplicon sequencing can only be used to determine the general taxonomic arrangement (Odom & Johnson, 2023).

A lot of software, on the other hand, has been created to anticipate useful information. These predictions are based on linking 16S rRNA a series of taxonomic material in the literature that has functional descriptions. The Kyoto Encyclopedia of Gens and Genomes (KEGG) pathway's metagenomic functional composition can be predicted using PICRUSt, which is based on the OTU table of the Greengenes database. Based on the changing OTU/ASV table, the newly created PICRUSt2 software program can directly predict metagenomic functions. Based on the SILVA database, the *Tax4Fun R package* can estimate the functional capacity of the KEGG microbiota (Shao et al., 2023).

The FAPROTAX pipeline provides annotations of functional information based on existing metabolic and ecological activities such as nitrate respiration, iron respiration, plant pathogens, and animal parasites or symbionts, making it valuable for environmental, agricultural, and animal microbiome research. To predict phenotypes such as oxygen tolerance, Gram staining, and pathogen potential using the BugBase database which is an *extended database* from *Green Genes* (Zhang et al., 2023).

Metagenomic analysis of shotguns

Compared to amplicons, Shotgun metagenomes can produce significantly higher resolution taxonomic annotation and directly provide functional gene profiles. *The KneadData* pipeline or the combination of Trimmomatic and *Bowtie2* required quality control and removal of host contamination from *raw-reads* in metagenomic analysis. Trimmomatic is a software tool used for quality control of Illumina sequencing data that can trim sequences, calibrate primers, and low-quality adapters, and readings mapped to the host genome with Bowtie 2 filtered as tainted readings (Choi et al., 2023).

A fundamental stage in shotgun metagenomics is transforming clean data into taxonomic and functional tables using a read-based and/or assembly-based approach. Net reads are aligned with the database and generate feature tables using a read-based approach. MetaPhlAn2 is a widely used taxonomic profile program that performs taxonomic categorization by aligning

metagenome readings to a database of predefined marker genes. Kraken 2 performs accurate k-mer matching to sequences within NCBI's non-redundant database and uses the lowest *common ancestor* (LCA) technique to perform taxonomic categorization (Wright & Langille, 2023).

The diversity of contributions within and among samples (species contributions to specific functions) can be explored using HUMAnN2, a commonly used functional profile program. MEGAN is a taxonomic and functional analysis program with a cross-platform *graphical user interface* (GUI). In the field of precise research, this customized database can be used for taxonomic and functional annotations, enabling efficient, precise, and fast analysis. Methods based on assembly using programs such as MEGAHIT or metaSPAdes, assemble net readings into contigs. MEGAHIT is a program that quickly assembles large and complicated metagenome data sets with little computer memory, whereas metaSPAdes can create older configurations but require more processing resources. MetaGeneMark or Prokka is then used to identify genes present in the assembled pool. Using methods such as CD-HIT, redundant genes from independently created contig must be eliminated. Finally, alignment-based techniques such as Bowtie2 or alignment approaches such as Salmon can be used to generate gene abundance tables. Metagenomic data sets typically contain millions of genes. These genes should be grouped together into functional annotations such as *KEGG Orthology* (KO), modules, and pathways, which is a kind of dimensionality reduction (Marizzoni et al., 2020).

Metagenomic Application to Acne Vulgaris

Acne is a disease of the sebaceous glands, hair follicles that connect with the oil glands (sebaceous) in the skin (Su & Zhang, 2023). The clinical picture of acne includes seborrhea (excess fat), non-inflammatory lesions (in the form of covered or open blackheads), inflammatory lesions (in the form of papules or pustules), and various scarring. The distribution of acne is mainly in the area with the most sebaceous glands, on the face, neck, upper chest, shoulders, as well as the back. Nodules and cysts form severe nodulocistic acne.

The skin microbiota can also play a role in the cause of acne (O'Neill & Gallo, 2018). According to metagenomic analysis, the amount of Propionibacterium acnes on the skin does not differ much between healthy skin and acne patients, although the population of the strain there are differences between groups. Certain strains of *P. acnes* are associated with acne, while other strains are found to be abundant on healthy skin. Different strains of P. acnes are important at different inflammatory potentials. *P. acnes* type III is the most pro-inflammatory strain and increases the regulation of proteinase-activated receptor 2 (PAR2), TNF, matrix

metalloproteinase 13, and tissue inhibitor of matrix metalloproteinase 2. Certain strains of *P. acnes* can cause infections that aggravate the lesions.

The population structure of P. acnes strains linked to acne and good skin is investigated using a metagenomic method based on the 16s rDNA. Such a strategy has clear advantages over conventional techniques that could unintentionally try to isolate particular strain types. The study's primary goal was to reduce variability caused by variations in skin location by sampling the microbiome of pre-lesion follicles from patients' noses, where lesions are typically absent P. Acne was identified as the dominating species in sebaceous hair follicles, accounting for 87 percent of all clones, after rDNA 16 from sebaceous units from 49 acne sufferers and 52 healthy controls was amplified and cloned Sepidermidis, Propionibacterium granulosum, and Propionibacterium humerusi are among the additional species found. Although there were no statistically significant changes in the relative abundance of P. acnes between acne and control individuals, ribotyping the 16S rDNA sequence revealed distinct strain groups (Table 1).

Table 1. Strains of *P. acnes* are compared healthy skin with acne patients.

Ribotype	Type	$MLST_8$ Clonal Complexes (CC)	N *	Percentage of Clones from Acne	Percentage of Clones from Controls	*p*-Value
RT1	IA_1/IB	CC1; CC3; CC4; CC5	90	48%	52%	0.84
RT2	II	CC6; CC72	48	51%	49%	0.36
RT3	IA_2	CC2	60	40%	60%	0.092
RT4	IA_1	CC3	23	84%	16%	0.049
RT5	IA_1/IC	CC1; CC3; CC107	15	99%	1%	0.0005
RT6	II	CC6	11	1%	99%	0.02
RT7	ND	ND	10	99%	1%	0.12
RT8	IA_1	CC4	5	100%	0%	0.024
RT9	III	CC77	4	99%	1%	0.29
RT10	ND	ND	5	100%	0%	0.024

* N: Number of subjects.

A total of 6 major ribotypes (RT) were mainly associated with acne patients (RT4, RT5, RT7, RT8, RT9, and RT10), and 4 of these ribotypes were statistically significant in acne patients (RT4, RT5, RT8, and RT10). In addition, a subset of type II (RT6) ribotypes showed statistically significant accumulation in healthy skin. A total of 3 ± 2 ribotypes were associated with each individual, and strains isolated from the same individual were more closely related than isolates from other subjects, with clone expansion in the individual's microbiome (Miles-Jay et al., 2023).

In the studies Kim (2023) that have been carried out using *ultra-deep* metagenomic shotgun sequencing from samples obtained from 38 acne vulgaris sufferers and 30 healthy people of the same age to find disease- and health-related microbial components in the follicular microbiota. An average of 1.08 gigabase pairs (Gbp) per sample ($6.9x10^7$ bp–$4.8x10^9$ bp) was obtained after removing human DNA sequences and low-quality readings, which were

sufficient to represent microbial diversity from skin samples. Once the readings are mapped and sequencing are cleared into a reference genome set, which includes 1,252 bacteria and 272 fungal genomes from the HMP reference genome database, as well as genomes from *Propionibacterium avidum*, *Propionibacterium granulosum*, *Propionibacterium humerusii*, and *Propionibacterium acnes bacteriophage*. Bacteria are the most common organisms identified in follicles, similar to other areas of the skin, with only a few fungal species reported to have relatively low abundance. *Actinobacteria (95.6%)*, *Firmicutes* (2.3%), *Proteobacteria* (1.2%), *Cyanobacteria* (0.6%), and *Bacteroidetes* (0.6%) were found in the sample (0.2%). The dominance of Actinobacteria follicles is consistent with previous taxonomic studies of sebaceous skin locations. *P. acnes* is the most common and abundant species at the species level. It was identified in all 68 individuals, with a relative abundance of 91.0 percent. *P. acnes* was found in relatively higher abundance in healthy people than in people with acne vulgaris (93.8 percent vs. 88.5 percent) (Dreno et al., 2023).

Although from the analysis of 16s rRNA and metagenomics shotgun it appeared that there was no difference quantitatively *P. acnes* on the skin of patients with acne compared to controls, with gene unit analysis using *operational gene units* (OTU) detected species in control samples and acne patients as additional metagenomic elements, being included in the analysis to distinguish species levels between the 2 groups (Figure 6).

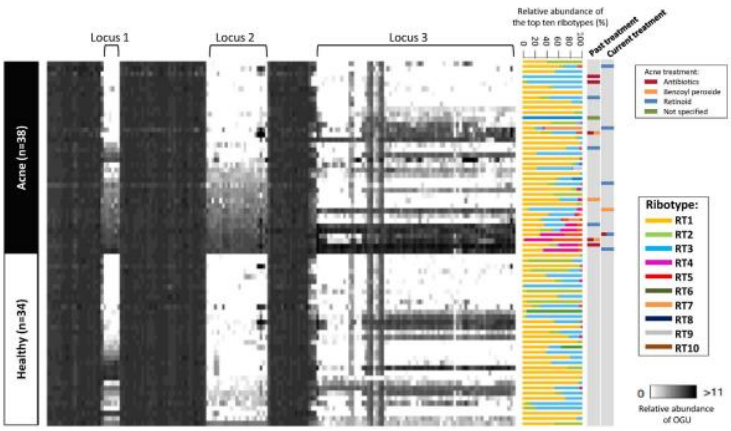

Figure 6. Comparison of the abundance of *P. acnes* OGU

From the analysis, it can be seen that the balance between the ribotype of P.acnes and other species with acnes disease is interrelated. If a balance occurs on the skin between the presence of fewer disease-causing species, then it can have healthy skin. On the contrary, if equilibrium does not occur, and the disease-causing species increase, problems will arise on the skin. In the case of acnes, the occurrence of an overall microbial imbalance. This multi-factor cause of pathogenesis indicates the severity of the course of acne. This could be the development

of more personalized and targeted therapeutic research in acnes treatment and skin health care with a microbiota profile into a probiotic organism for the sake of microbiome balance.

CONCLUSION

In some years it has developed significantly for metagenomic examination of the microbiome, especially for facial skin. Microbiome analysis can be done with NGS techniques using metagenomics *shotguns* that can detect up to the species level and sequencing using 16S rRNA which can determine the abundance of bacteria. For acne vulgaris from microorganisms obtained in the form of fungi, viruses and bacteria. By comparing healthy skin and acne vulgaris, it is found that the most microorganisms are bacteria. The approach using *operational gene unit* (OGU) mapping found that the most bacteria in acne vulgaris is *Propionibacterium acnes*. With age, the microbiome in the skin will be lower compared to healthy skin at productive age. It is hoped that the research that has developed will add to the understanding of microbial mechanisms for the development of the course of acne vulgaris disease and facilitate possible acne vulgaris treatment therapies to balance the skin microbiota and maintain skin health.

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