Journal of Experimental Biology and Agricultural Sciences, October - 2023; Volume – 11(5) page 809 – 814

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Identification and antibiotic susceptibility profiles of anaerobic bacteria isolated from patients with acne vulgaris

Salma Walid[1], Geetha Subramaniam[1*], Lalita Ambigai Sivasamugham[1], Wong Ling Shing[1], Preamala Gunabalasingam[2], Nurfara Ain Binti Ramli[2], Nithiya Visayaragawan[2], Gan Li Lian[3], Anshoo Agarwal[4]

[1]INTI International University, Persiaran Perdana BBN, Putra Nilai, 71800 Nilai, Negeri Sembilan, Malaysia
[2]Department of Dermatology, Hospital Tuanku Jaafar Seremban, Ministry of Health Malaysia
[3]Clinical Research Center, Hospital Tuanku Jaafar Seremban, Ministry of Health Malaysia
[4]Department of Pathology, Northern Border University, 9280 Arar, Saudi Arabia

Received – June 05, 2023; Revision – August 06, 2023; Accepted – November 03, 2023
Available Online – November 30, 2023

DOI: http://dx.doi.org/10.18006/2023.11(5).809.814

KEYWORDS

Acne vulgaris

Antibiotic susceptibility profiles

Anaerobic bacterial isolates

Human Health

ABSTRACT

Commensal bacteria like the Staphylococcal species are part of the skin microbiota, which helps maintain healthy skin. However, certain factors can lead to these commensals becoming opportunistic pathogens capable of causing diseases like acne vulgaris. Topical and systemic antibiotics have been the main treatment for acne. However, long-term antibiotic usage could result in the emergence of resistant bacterial strains and treatment failure. This study evaluated the antibiotic susceptibility patterns of anaerobic bacteria isolated from clinical acne samples. Skin swabs were collected from 50 acne patients and cultured under anaerobic conditions. The resulting bacterial isolates were identified using biochemical tests and 16S rRNA gene sequencing. The antibiotic susceptibility patterns of the confirmed isolates were determined using the disc diffusion assay for eight commonly prescribed antibiotics for acne treatment. Sequencing results revealed that *S. epidermidis* was the most isolated bacterial species (68%, n=34), followed by *S. aureus* (8%, n=4). However, a significant proportion of bacterial isolates were susceptible to all eight tested antibiotics, which is unusual. On the other hand, 26% (n=13) of the tested bacterial species isolates were found to be resistant to clindamycin, while 36% (18) were resistant to erythromycin and 20% (n=10) were to tetracycline. Since there has been limited research regarding the antibiotic resistance patterns of anaerobic acne-associated bacteria in Malaysia, this study can help shed some light on suitable local prescription practices and raise awareness about the cautious use of antibiotics in treating acne vulgaris.

* Corresponding author
E-mail: geetha.subramaniam@newinti.edu.my (Geetha Subramaniam)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

1 Introduction

Many of the bacteria found on the skin are not only ubiquitous but are also harmless. These bacteria help to create an environment that suppresses the growth of pathogenic bacteria. The involvement of the microbiome in numerous skin disorders, such as acne vulgaris, has become the focus of research in recent decades, particularly when it comes to anaerobic and facultative anaerobic bacteria since anaerobic bacteria are a common cause of infections, some of which can be serious and life-threatening. This is compounded by the fact that these bacteria are frequently underestimated due to inefficiencies in collecting and transit protocols and a lack of isolation and susceptibility testing in many clinical microbiology laboratories (Pauzenberger et al. 2019). The microbiome offers genetic variation, influences disease symptoms and manifestations, and impacts dynamic metabolic processes and immunomodulatory competence (Mawardi et al. 2021).

However, factors such as genetics, as well as alterations in the chemical and physical environment of the skin, including changes in pH, hormonal imbalance, or abrasion, may result in a disproportionate growth of commensal bacteria such as *Cutibacterium acnes* and *Staphylococcus* spp., which causes an imbalance in the normal skin microflora and can therefore lead to a localized and/or systemic infection and disease.

Infections caused by such opportunistic pathogens have been treated with antibiotics for decades, which has led to the development of antibiotic resistance to routinely used antibiotics, such as erythromycin as well as clindamycin, have been documented in many countries (Walsh et al. 2016; Alkhawaja et al. 2020). Furthermore, via horizontal gene transfer, antibiotic resistance may transfer to other commensal bacteria that colonize the skin, such as S. aureus. As a result, indiscriminate antibiotic prescription could lead not only to the emergence of antibiotic resistance in *C. acnes* but also to the transmission of resistance to other species of bacteria (Alkhawaja et al. 2020).

Despite the prevalence of acne vulgaris among the Malaysian population, little work has been done to evaluate antibiotic resistance patterns of skin bacteria, including *Staphylococcus* spp. isolates. While the association between *C. acnes* and acne has been extensively researched, only a few studies have analyzed the overall bacterial composition of acne patients' skin and their susceptibility to antibiotics commonly used to treat acne conditions. Therefore, this study aimed to determine the antibiotic susceptibility patterns of anaerobic bacteria isolated from clinical acne samples from patients treated at the Dermatology Department of Hospital Tuanku Ja'afar Seremban, Malaysia. This information would be valuable in identifying the current trend of antibiotic resistance among bacterial isolates from acne vulgaris patients and could aid in more definitive and effective treatment of acne vulgaris in Malaysian hospitals.

2 Materials and Methods

2.1 Ethical clearance

Before the commencement of the study, the Research & Ethics Committee of INTI International University (INTI/UEC/2018/001) as well as the Medical Research and Ethics Committee (MREC) (21-1891-61558[2]) under the National Medical Research Register (NMRR-21-1891-61558), granted the ethics approval. Patients who participated in this study were asked to complete an informed consent form using the Patient Information Sheet (PIS) authorized by the MREC before collecting the acne samples.

2.2 Clinical sample collection

Trained dermatologists collected acne samples at the Dermatology Clinic of Hospital Tuanku Jaafar Seremban (HTJS). Patients who were taking antibiotics, undergoing other types of medical treatment, pregnant mothers or below the age of 18 years were excluded from this study. The acne samples were collected from the nodules, papules, and pustules on the patients' jaw, cheeks, and forehead. The external skin area of the acne lesions was swabbed with an alcohol pad before the collection using the Amies transport media with charcoal (Microscience). The acne swabs were delivered to INTI International University's Molecular Biology Laboratory 1 within 24 to 48 hours of collection for processing.

2.3 Isolation and growth of bacterial isolates

The acne swabs were cultured on nutrient agar and incubated under anoxic conditions at 37°C. Colonies with different morphology were sub-cultured to obtain pure cultures for further analysis. Gram staining, catalase test, oxidase test, and subculturing on MSA were performed for preliminary identification of the individual cultures (Shoaib et al. 2020).

2.4 16S rRNA analysis of bacterial isolates

DNA of each bacterial isolate from pure, overnight cultures was extracted in a cell lysis buffer (100mM Tris-HCl, pH8.0, 10mM EDTA, pH8.0, 0.5% Tween-20, 1% Triton-X) using the boiling method (Dimitrakopoulou et al. 2020). The 16S rRNA gene was amplified using universal primers pA (5′-AGA GTT TGA TCC TGG CTC AG-3′) and pH (5′-ACG GGC GGT GTG TAC AAG-3′) with Promega Go-Taq Green Master Mix. PCR amplification was performed in a Biorad thermocycler with the following thermocycling profile: an initial denaturation step at 95^0C for 3 mins, followed by 30 cycles consisting of 95^0C for 30s, 55^0C for

1 min and 72^{0}C for 1 min, as well as a final extension step at 72^{0}C for 10 mins (Alnabati et al. 2021). Following the PCR amplification of the 16S rRNA gene, the PCR products with a size of 1530 bp were partially sequenced at Apical Scientific Sdn. Bhd. using the universal primers pA and pH. BioEdit version 7.2 was used to evaluate the acquired sequencing results, and the obtained sequences were then matched with those in DNA databases using NCBI Blast queries (Souwmiya et al. 2015).

2.5 Antibiotic susceptibility of bacterial isolates

Antibiotic susceptibility of the bacterial isolates was tested using 8 different antibiotic discs including cefoxitin (30µg), clindamycin (2µg), doxycycline (30µg), erythromycin (15µg), levofloxacin (5µg), minocycline (30µg), tetracycline (30µg), as well as trimethoprim-sulfamethoxazole (12.5/23.75 µg). A single colony of the pure bacterial isolates was inoculated into 3 mL of nutrient broth (OXOID) and incubated under anaerobic conditions for 16-18 hours at 37˚C. Approximately 100 µL of the culture was dispensed into 3 mL of sterile water, vortexed, and compared to a 0.5 McFarland standard. Fifty microliters of the culture with confirmed turbidity were lawned on Mueller-Hinton agar using a sterile wooden applicator and dried for about one minute. The antibiotic discs were placed on the surface of the agar, while a sterile empty disc immersed in sterile water that acts as the negative control was placed on the agar using sterile forceps and gently pressed . This procedure was performed in triplicates in a laminar airflow cabinet . The agar plates with the antibiotic discs were then incubated for 24 hours at 37℃ under anaerobic conditions, and the antimicrobial activity of each disk was determined by measuring the diameter of the zone of inhibition. The results were interpreted as resistant, intermediate, or sensitive according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI 2021).

2.6 Statistical analysis

The diameter of the zone of inhibition was analyzed for significant differences using the one-way ANOVA to compare the mean differences between groups influenced by one independent variable and to understand if there was any effect on the dependent variable or any significant difference from each other. Significance was accepted at $P \leq 0.05$.

3 Results

3.1 Anaerobic bacteria isolated from acne samples

Out of 50 sequenced isolates, 68% (n=34) were identified as *Staphylococcus epidermidis*, followed by *S. aureus* 8% (n=4) and *S. marcescens* 6% (n=3), with each isolate matching with sequences that had Query Cover of 100 percent and E- value of 0.0. The lower the E-value, the more significant the match. Furthermore, a 100% query cover indicates the target sequence in the database spans the whole query sequence (Figure 1).

3.2 Antimicrobial susceptibility of bacterial isolates

A significant proportion of bacterial isolates were susceptible to all eight tested antibiotics. However, 26% (n=13) of the tested bacterial species isolates were resistant to clindamycin, while resistance to erythromycin was detected in 36% (n=18) of bacterial isolates. Resistance to cefoxitin was detected in 6% (n=3) of isolates, with 2% (n=1) exhibiting intermediate susceptibility. Only 2% (n=1) of bacterial isolates were found to be resistant to sulfamethoxazole-trimethoprim as well as doxycycline, while resistance to levofloxacin was detected in 2% (n=1) of isolates. Out of the 50 isolates screened, 20% (n=10) of isolates exhibited resistance to tetracycline, and 4% (n=2) of bacterial isolates exhibited intermediate susceptibility to tetracycline. The susceptibility rates of the tested isolates are depicted in Figure 2.

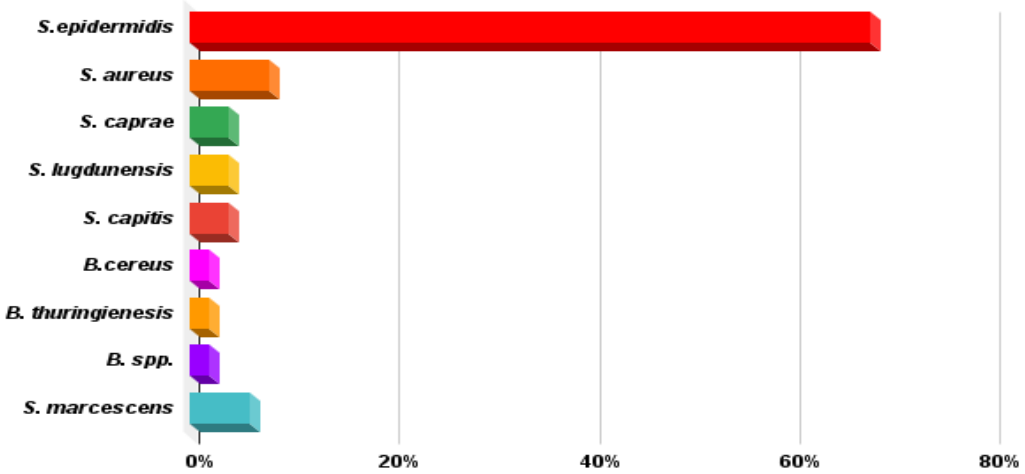

Figure 1 The proportion of anaerobic bacterial species isolated from acne samples based on the sequencing data

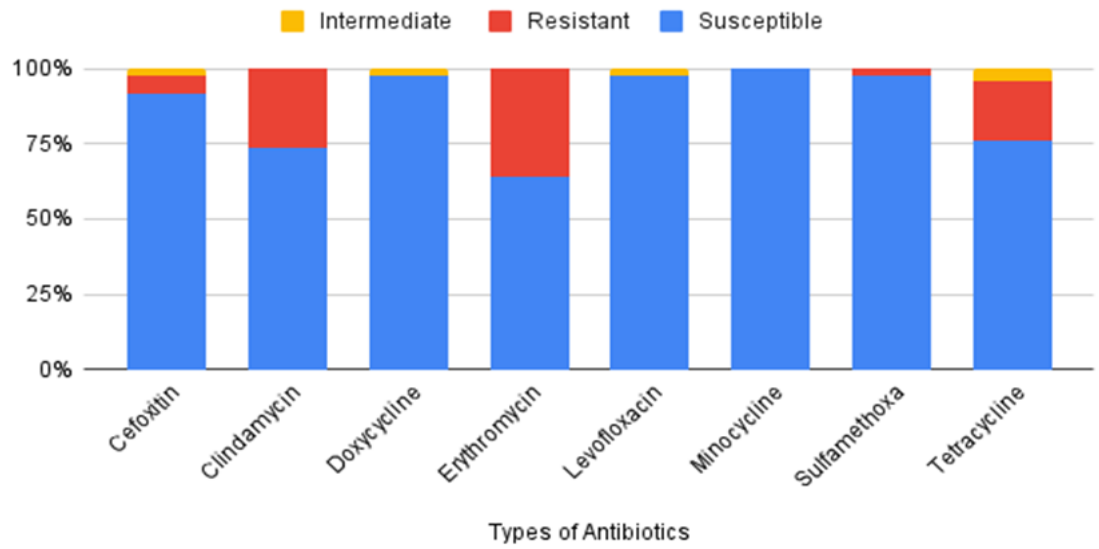

Figure 2 Antibiotic susceptibility rates of bacterial isolates against eight types of antibiotics

4 Discussion

The 16S rRNA gene sequencing showed that the anaerobic cultures of the acne samples were comprised predominantly of Staphylococcus epidermidis, followed by *S. aureus* and *Serratia marcescens*. This result is consistent with the findings reported by Alkhawaja et al. (2020) and Sitohang et al. (2019). Conversely, a study by Legiawati et al. (2023) reported that *C. acnes* was the most isolated pathogen from acne vulgaris, followed by *S. epidermidis*. However, a comparison cannot be made since *C. acnes* was not reported in this study. Due to the high isolation rate and growing understanding of the human skin microbiome, questions concerning the role of skin commensals, including *S. epidermidis*, in the physiopathology of acne have arisen. *S. epidermidis* has been observed to increase inflammatory acne. Furthermore, an imbalance of skin commensals in favour of *S. epidermidis* may aggravate sebaceous unit inflammation, although the specific pathway that triggers this inflammation has not yet been elucidated (Tabri 2018). Conversely, several studies have reported an antagonistic role played by *S. epidermidis* against *C. acnes*, in which *S. epidermidis* limits the *in vivo* proliferation of *C. acnes* by producing succinic acid, a fatty acid fermentation product that possesses antimicrobial activity (Claudel et al. 2019). The relative proportion of *S. aureus* isolated in this study was significantly lower than that of *S. epidermidis*, which is concurrent with the abovementioned studies. This is because there is no reported causal link between *S. aureus* and the development of acne vulgaris, but rather an indirect relationship whereby *S. aureus* can cause secondary infections of acne lesions in some patients (Totte et al. 2016).

In our study, 6% of swab samples were cultivated with a Gram-negative bacteria (*S. marcescens*), which is not a typical skin commensal. Long-term antibiotic treatment alters the typical follicular microbial community, making the presence of Gram-negative bacteria more prevalent (Sun and Chang 2017). Gram-negative folliculitis should be suspected in acne vulgaris patients who have been on long-term antibiotics and have primarily inflammatory lesions, such as pustules, nodules, or papules (Tan et al. 2017).

The disk diffusion assay carried out revealed that all 8 tested antibiotics were effective against a significant majority of the tested bacterial strains, suggesting that the rate of resistance in this particular study was much lower than that of similar studies (Ruchiatan et al. 2023; Gamil et al. 2023). The greatest proportion of isolates exhibiting resistance were mainly against 2 antibiotics, i.e. clindamycin and erythromycin. Both the topical antibiotics clindamycin and erythromycin are the most prescribed antibiotics for *Acne vulgaris*. As such, resistance to these antibiotics is frequently observed in patients undergoing a long-term (approximately 6 to 8 weeks) of monotherapy using one of these antibiotics (Alkhawaja et al. 2020).

Alkhawaja et al. (2020) documented the incidence of cross-resistance to both clindamycin and erythromycin in both *S. epidermidis* and *S. aureus*. The presence of the erm(X) resistance gene, which confers resistance to the macrolide, Lincosamide, and Streptogramin B (MLS) group of antibiotics, is suspected to be the cause of cross-resistance between erythromycin and clindamycin. This explains why antibiotic resistance patterns in isolates from the same patient are similar, implying gene transfer between species.

Gram-positive bacteria develop resistance to macrolides through ribosomal RNA methylation, enzymatic inactivation of the antibiotic, and active reflux. Erythromycin ribosome methylases (*Erm*) are

enzymes that methylate rRNA and are encoded by the *erm* gene. In resistant bacteria, the *erm* proteins dimethylate the A2058 residue of 23S rRNA, located within the ribosome's V-domain. This prevents the antibiotic from binding, resulting in the macrolide losing its effectiveness and causing the bacteria to become resistant to the macrolide antibiotic (Munita and Arias 2016). In terms of enzymatic inactivation of macrolides, esterases and phosphotransferases are synthesized to inhibit antibiotics by interacting with them, preventing antibiotics from attaching to the bacteria's binding sites. Active efflux confers resistance to macrolides in bacteria by actively pumping out the antibiotics, reducing the antimicrobial's overall efficiency (Munita and Arias 2016).

In the patients of this current study, the resistance to the tetracyclines was lower than resistance to erythromycin and clindamycin. Further analysis revealed that tetracycline had the highest resistance among the three antibiotics in the tetracycline group, with a single isolate showing intermediate susceptibility to doxycycline and no resistance to minocycline. This lack of resistance to minocycline and reduced resistance rate to the tetracycline group compared to erythromycin and clindamycin appears to corroborate findings by Sutcliffe et al. (2020).

Many patients participating in this study have received treatment with benzoyl peroxide (BPO). Benzoyl peroxide, a commonly utilized acne treatment, is a potent non-antibiotic antibacterial agent with keratolytic, anti-inflammatory, and wound-healing qualities (Yang et al. 2020). A cross-sectional study by Sardana et al. (2016) comparing different routes of acne treatment, such as antibiotics and benzoyl peroxide, reported that the treatment group with benzoyl peroxide had the least number of resistant strains. Therefore, it could be postulated that benzoyl peroxide treatment resulted in a significant proportion of the tested isolates being susceptible to the antibiotics used in this study.

Conclusion

In this study, *S. epidermidis* was the most commonly isolated bacterium from the acne sample, followed by *S. aureus*. In contrast to previous research, most bacterial isolates were found to be sensitive to all eight antibiotics tested. This is an important result because numerous investigations of a similar nature have found a large proportion of resistant bacteria, which indicates that when physicians cautiously prescribe antibiotics and when patient compliance is satisfactory, antibiotic resistance can be minimized. The use of alternative, non-antibiotic acne treatment, such as benzoyl peroxide, may be another factor in the low resistance rates among isolates found in this study. On the other hand, bacterial resistance was mostly found against clindamycin and erythromycin. It is, therefore, crucial to practice appropriate prescription and use of antibiotics to prevent the emergence of antibiotic and multi-drug-resistant bacteria.

Acknowledgements

We would like to thank the Director General of Health Malaysia for his permission to publish this article. This study was funded by INTI Research Grant 2021: Seed Grant INTI-FHLS-11-02-2021.

Conflict of Interest

None of the authors have a conflict of interest to report. This paper has not been submitted for publication in any other journal.

References

Alkhawaja, E., Hammadi, S., Abdelmalek, M., Mahasneh, N., Alkhawaja, B., & Abdelmalek, S. M. (2020). Antibiotic resistant *Cutibacterium acnes* among acne patients in Jordan: a cross sectional study. *BMC Dermatology*, *20*(1), 17. doi: 10.1186/s12895-020-00108-9.

Alnabati, N. A., Al-Hejin, A. M., Noor, S. O., Ahmed, M. M., Abu-Zeid, M., & Mleeh, N.T. (2021). The antibacterial activity of four Saudi medicinal plants against clinical isolates of *Propionibacterium acnes*. *Biotechnology and Biotechnological Equipment*, *35*(1), 415–424. https://doi.org/10.1080/13102818.2021.1885992

Claudel, J. P., Auffret, N., Leccia, M. T., Poli, F., Corvec, S., & Dréno, B. (2019). *Staphylococcus epidermidis*: A potential new player in the physiopathology of acne? *Dermatology, 235*(4), 287–294. https://doi.org/10.1159/000499858

CLSI (2021). Performance Standards for Antimicrobial Susceptibility Testing. 31st ed. CLSI supplement M100. Wayne PA: Clinical Laboratory Standards Institute; 2021.

Dimitrakopoulou, M. E., Stavrou, V., Kotsalou, C., & Vantarakis, A. (2020). Boiling extraction method VS commercial Kits for bacterial DNA isolation from food samples. *Journal of Food Science and Nutrition Research, 3*, 311-319.

Gamil, H. D., Mustafa, S. A. G., Amer, R. M. M., & Khashaba, S. A. E. (2023). Implications of antimicrobial resistance during acne treatment: review article. *The Egyptian Journal of Hospital Medicine*, *9*, 821-823.

Legiawati, L., Halim, P. A., Fitriani, M., Hikmahrachim, H. G., & Lim, H. W. (2023) Microbiomes in Acne Vulgaris and Their Susceptibility to Antibiotics in Indonesia: A Systematic Review and Meta-Analysis. *Antibiotics*, *12*(1),145. https://doi.org/10.3390/antibiotics12010145

Mawardi, P., Ardiani, I., Primisawitri, P. P., & Nareswari, A. (2021). Dual role of *Cutibacterium acnes* in acne vulgaris

pathophysiology. *Bali Medical Journal*, *10*(2), 486. https://doi.org/10.15562/bmj.v10i2.2358.

Munita, J. M., & Arias, C. A. (2016). Mechanisms of antibiotic resistance. *Microbiology Spectrum*, *4*(2). https://doi.org/10.1128/microbiolspec.vmbf-0016-2015

Pauzenberger, L., Heller, V., Ostermann, R.C., Laky, B., Heuberer, P. R. & Anderl, R (2019). *Cutibacterium acnes* (formerly *Propionibacterium acnes*) contamination of the surgical field during shoulder arthroscopy. *Arthroscopy: The Journal of Arthroscopic & Related Surgery*, *35* (6), 1750–1757. https://doi.org/10.1016/j.arthro.2019.01.024.

Ruchiatan, K., Rizqandaru, T., Satjamanggala, P. R., Tache, N., Cahyadi, A. I., et al. (2023). Characteristics of Biofilm-Forming Ability and Antibiotic Resistance of *Cutibacterium acnes* and *Staphylococcus epidermidis* from *Acne vulgaris* Patients. *Clinical Cosmetic Investigational Dermatology*, *16*, 2457-2465. https://doi.org/10.2147/CCID.S422486

Sardana, K., Gupta, T., Kumar, B., Gautam, H. K., & Garg, V. K. (2016). A cross-sectional pilot study of antibiotic resistance in *Propionibacterium acnes* strains in Indian acne patients using 16S-RNA polymerase chain reaction: A comparison among treatment modalities including antibiotics, benzoyl peroxide, and isotretinoin. *Indian Journal of Dermatology*, *61*(1), 45. https://doi.org/10.4103/0019-5154.174025

Shoaib, M., Muzammil, I., Hammad, M., Bhutta, Z., & Yaseen, I. A. (2020). Mini-Review on commonly used biochemical tests for identification of bacteria. *International Journal of Research Publications*, *54* (1). 10.47119/IJRP100541620201224.

Sitohang, I.B.S., Fathan, H., Effendi, E., & Wahid, M. (2019). The susceptibility of pathogens associated with acne vulgaris to antibiotics. *Medical Journal of Indonesia*, *28*, 21–27. https://doi.org/10.13181/mji.v28i1.2735.

Sowmiya, M., Malathi, J., Swarnali, S., Priya, J. P., Therese, K. L., & Madhavan, H. N. (2015). A study on the characterization of *Propionibacterium acnes* isolated from ocular clinical specimens. *The Indian Journal of Medical Research*, *142* (4), 438. https://doi.org/10.4103/0971-5916.169209

Sun, K.L., & Chang, J. M. (2017). Special types of folliculitis which should be differentiated from acne. *Dermato-Endocrinology*, *9*(1), e1356519. https://doi.org/10.1080/19381980.2017.1356519

Sutcliffe, J., McLaughlin, R., Webster, G., Read, A. F., Drlica, C., Elliott, R., & Stuart, I. (2020). Susceptibility of *Cutibacterium acnes* to topical minocycline foam. *Anaerobe*, *62*, 02169. https://doi.org/10.1016/j.anaerobe.2020.102169.

Tabri, F. (2018). The association between *Staphylococcus epidermidis* and palmitic acid level in patients with acne vulgaris. *Surgical and Cosmetic Dermatology*, *10*(3). https://doi.org/10.5935/scd1984-8773.20191121382

Tan, A.U., Schlosser, B. J., & Paller, A. S. (2017). A review of diagnosis and treatment of acne in adult female patients. *International Journal of Women's Dermatology*, *4*(2), 56-71. doi: 10.1016/j.ijwd.2017.10.006.

Totté, J. E., van der Feltz, W. T., Bode, L. G., van Belkum, A., van Zuuren, E. J., & Pasmans, S. G. (2016). A systematic review and meta-analysis on *Staphylococcus aureu*s carriage in psoriasis, acne and rosacea. *European Journal of Clinical Microbiology & Infectious Diseases*, *35* (7), 1069–1077. https://doi.org/10.1007/s10096-016-2647-3

Walsh, T. R., Efthimiou, J., & Dréno, B. (2016). Systematic review of antibiotic resistance in acne: An increasing topical and oral threat. *The Lancet Infectious Diseases*, *16*(3). https://doi.org/10.1016/s1473-3099(15)00527-7.

Yang, Z., Zhang, Y., Lazic Mosler, E., et al. (2020). Topical benzoyl peroxide for acne. *Cochrane Database of Systematic Reviews*, *2020*(3), CD011154. https://doi.org/10.1002/14651858.cd011154.pub2