The susceptibility of pathogens associated with acne vulgaris to antibiotics

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ABSTRACT

BACKGROUND Acne vulgaris is a pilosebaceous disorder. Bacterial activity and inflammation both influence an acne formation. Antibiotics suppress the bacterial activities and elicit anti-inflammatory effects. The overuse of antibiotics may lead to a resistance in bacteria. This study was aimed to provide an overview of bacteria that may cause acne and determine their susceptibility to antibiotics.

METHODS This was a cross-sectional study sampling from 93 patients with acne in Ciptomangunkusumo Hospital. Comedones were extracted and cultured on Brucella blood agar, under aerobic and anaerobic conditions at 35°C for 24–48 hours. Bacterial identification was performed using Vitek®, and susceptibility test using E-test. Data interpretation was based on the Clinical and Laboratory Standards Institute 2015.

RESULTS *Staphylococcus epidermidis* (50.5%), *Propionibacterium acnes* (11.0%), and *Staphylococcus aureus* (7.7%) were identified. Bacteria were not found in 69.2% and 1.1% of samples in anaerobic and aerobic cultures, respectively. *P. acnes* was susceptible to doxycycline (100%) and minocycline (100%), while 10% was resistant to erythromycin, clindamycin, and tetracycline. *S. epidermidis* was susceptible to minocycline (100%); but resistant to erythromycin (65.2%), clindamycin (52.2%) and tetracycline (32.6%). The susceptibility of *S. epidermidis* to doxycycline was 89.1%, which was lower than that of *P. acnes* (100%). *S. aureus* was found to be sensitive to minocycline (100%), doxycycline (71.4%), clindamycin (71.4%), and tetracycline (71.4%); but it was resistant to erythromycin (42.9%).

CONCLUSIONS Doxycycline and minocycline showed 100% effectiveness for *P. acnes*. The isolated bacteria were more susceptible to doxycycline compared to tetracycline. The use of clindamycin and erythromycin needs to be limited as most *S. epidermidis* isolates were resistant to both.

KEYWORDS acne vulgaris, antibiotics, *Propionibacterium acnes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*

Acne vulgaris is a skin disease associated with the inflammation of the pilosebaceous unit that is especially commonly found in teenagers.[1] Clinical features of acne include comedones, papules, pustules, and nodules. Even though acne tends to be self-limiting, this skin abnormality can cause potential atrophic and hypertrophic scar formation that may decrease the patient's quality of life and cause psychological disorder.[1,2] Data from hospital outpatient visits from the cosmetic dermatology division of Cipto Mangunkusumo Hospital showed that in 2014 there were 1,525 new acne cases. It was the second most common disease from the dermatovenereology outpatient department. The pathogenesis of acne formation is a combined multifactorial process stemming from the hyperproliferation of follicle, sebum hypersecretion, bacterial colonization, and inflammation. *Propionibacterium acnes* was thought to

be the most important causative agent of acne lesions but is now regarded as one of several interconnected factors in the pathophysiological phenomenon. The role of *P. acnes* in comedogenesis has not yet been entirely understood. It is assumed that *P. acnes* may play a role in comedogenesis by secreting lipase that hydrolyzes the triglycerides of the sebum into free fatty acids and glycerol products, which are likely to be comedogenic.[3]

Genetic, racial, hormonal, and environmental factors such as climate, temperature, and humidity; cosmetics, the consumption of food and drugs; as well as stress can also affect pathogenesis.[1] The main species of microbes that are involved in acne pathogenesis are *P. acnes* (anaerobic bacteria), *Staphylococcus* sp. (aerobic bacteria), and *Pityrosporum* sp.[4]

Antibiotics have important roles in the treatment of acne because it can suppress bacterial activities and provide an anti-inflammatory effect.[1,2] Antibiotics can be used in topical and systemic preparations. Oral antibiotics are only used on moderate-severe acne and are administered for 6–8 weeks with a maximum of 12–18 weeks, and combined with topical antibiotics on mild-severe acne.[2] The clinical practice guidelines of Cipto Mangunkusumo Hospital in 2012 recommended the use of either 50–100 mg doxycycline twice a day, 50–100 mg minocycline twice per day, or 150–300 mg clindamycin 2–3 times per day. These may be the first line of oral antibiotics to treat moderate and severe acne. Cotrimoxazole, erythromycin, and trimethoprim are administered as alternative oral antibiotics. The Dermatology Association of Indonesia recommended using either 500 mg tetracycline twice per day, 50–100 mg doxycycline twice per day, 50–100 mg minocycline twice per day, or 150–300 mg clindamycin 2–3 times per day as the drugs of choice in acne treatment. The acne guidelines of Cipto Mangunkusumo Hospital and the Dermatology Association of Indonesia are based on the updates from the global alliance to improve the outcomes.[2] Erythromycin and clindamycin are considered safe for use in pregnant woman.[5]

Recently, the use of antibiotics in acne therapy has encountered problems due to the increasing prevalence of antibiotic-resistant strains of bacteria. The escalation of antibiotics resistance in *P. acnes* has had a global impact.[1] Eady et al[6] revealed 73 cases of resistance in *Propionibacterium* sp. to erythromycin (47.94%) and tetracycline (20.55%) in France, Germany, Japan, Australia, United States of America, and England. The first study to provide data on bacterial resistance in acne was conducted at Cipto Mangunkusumo Hospital in 2006 by Barira et al.[7] The antibiotics susceptibility test in their study was done using the disc diffusion method, which is not recommended by the Clinical and Laboratory Standards Institute (CLSI) for testing anaerobic bacteria.[8,9]

Staphylococcus species are also involved in acne pathogenesis. There have been no studies on the susceptibility of *Staphylococcus epidermidis* and *Staphylococcus aureus*[4] to antibiotics in Cipto Mangunkusumo Hospital. Therefore, this study was aimed to provide a general overview of *P. acnes, S. epidermidis, and S. aureus* as well as their susceptibility to antibiotics. The results of this study may be useful in decisions on choosing the appropriate drug for the treatment of acne.

METHODS

Subjects

Skin samples were taken from a total of 93 consecutive acne patients, aged between 15 to 39 years old with moderate to severe acne at the Department of Dermatovenereology, Cipto Mangunkusumo Hospital between November 2015 and January 2016. The most common grade of acne was moderate acne vulgaris (92.3%). The minimum sample size according to the formula for descriptive study sample size was 91 subjects. It is the largest number calculated from each bacteria positivity from an earlier study by Barira et al.[7] The ethical approval was granted under the number 011/UN2.F1/ETIK/2015 from the Faculty of Medicine, Universitas Indonesia.

Study design

This was a cross-sectional study using a consecutive sampling method from patients with moderate-severe acne at Cipto Mangunkusumo Hospital. Specimens were taken from comedones on the face using a sterilized comedone extractor. Then, using a sterilized cotton bud, the specimens were split into aerobic and anaerobic conditional treatments in jars containing thioglycolate medium. An AnaeroGen® Compact sachet was placed into each anaerobic jar for the isolation of anaerobic bacteria. No sachets were used for the isolation of aerobic bacteria.

Table 1. A summary of the demographic data, clinical characteristics, and antibiotic history of interventions (n=91)

Characteristics	n (%)
Age (years), median (min–max)	20 (16–39)
Gender	
Male	18 (19.8)
Female	73 (80.2)
Education	
Low education	7 (7.7)
Moderate education	66 (72.5)
High education	18 (19.8)
Job	
Unemployed	4 (4.4)
Student	59 (64.8)
Office worker	23 (25.3)
Worker with physical activities	5 (5.5)
Status	
Single	71 (78)
Married	18 (19.8)
Widow/widower	2 (2.2)
Duration of acne vulgaris (months)	
Median (min–max)	18 (4–36)
History of antibiotics	
No	44 (48.4)
Yes	47 (51.6)
1 antibiotic	32 (35.1)
>1 antibiotics	15 (16.5)
Diagnosis	
Mild–moderate acne vulgaris	84 (92.3)
Severe acne vulgaris	7 (7.7)
Antibiotic compliance	
No	31 (65.9)
Yes	16 (34.0)
Oral antibiotic	
Doxycycline	15 (100)
Topical antibiotic	
Clindamycin	38 (80.8)
Others	9 (19.2)
Antibiotic administration route	
Topical	32 (69.6)
Oral and topical	15 (30.4)
Duration of antibiotics consumption (week), median (min–max)	2 (0–6)
Duration from antibiotic consumption with examination date (week), median (min–max)	3 (0–36)

Isolation, identification, and susceptibility test

Bacterial isolation, identification, and the antibiotic susceptibility test were carried out at the Department of Microbiology, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital. The Gram stain was performed directly on the specimens. After placing the specimens in Brucella blood agar and Brucella-Kanamycin blood agar, a metronidazole disc (50 µg) was placed on each plate in duplicate (in aerobic and anaerobic conditions). Thereafter, the plates were incubated under aerobic and anaerobic conditions at 35°C for 24–48 hours. Identification of aerobic and anaerobic bacteria was performed using the Vitek® system. The susceptibility test was conducted using E-test (MIC Test Strip, Liofilchem Diagnostic) which consisted of the tetracycline group (tetracycline, doxycycline, minocycline) and macrolides group (erythromycin, clindamycin). Each antibiotic has a minimum inhibitory concentration (MIC) range from 0.016 to 256 µg/ml. The susceptibility of the microorganisms was interpreted according to the standard breakpoints determined by the CLSI 2015,[8] and according to the method used in an earlier study by Nakase et al[9] for other bacteria species.

Data analysis

The data was processed and arranged into distribution tables and cross tables using statistical package for the social sciences (SPSS) version 21.0.

RESULTS

Analysis of bacteria in aerobic and anaerobic culturing conditions

There were a total of 93 subjects; however, two subjects were excluded from the study because there was no bacterial growth occurred in neither aerobic nor anaerobic culturing conditions. Details on the characteristics of the subjects are presented in Table 1.

Microorganisms isolated from acne patients grew well under both aerobic and anaerobic conditions. In aerobic conditions, there was a significant growth of *S. epidermidis* (50.5% of all cultures under aerobic conditions). *S. aureus* were found in 7.7% of aerobic cultures. Other species were also found in 40.7% of samples, which consisted of *Staphylococcus hominis* (13.2%), *Klebsiella pneumoniae* (3.3%), and *Staphylococcus haemolyticus* (2.2%). *P. acnes* were

successfully cultured in 11% of anaerobic samples. Anaerobic bacteria other than *P. acnes* were also found in this study (19.8%), which consisted of *Atopobium vaginae* (5.5%), *Clostridium bifermentans* (3.3%), *Clostridium difficile* (2.2%), *Clostridium* group (2.2%), and unidentified bacteria (2.2%). Other bacteria that were isolated from each culture are *Clostridium perfringens, Clostridium subterminale, Clostridium sporogenes*, and *Clostridium innocuum*.

Antibiotics resistance and susceptibility of *P. acnes, S. aureus,* and *S. epidermidis* to antibiotics

Bacterial susceptibility testing was done using E-Test Strip (Liofilchem®) under both aerobic and anaerobic conditions. From the total of 91 specimens, there were 10 specimens with *P. acnes* growth, 46 specimens with *S. epidermidis* growth, and seven specimens with *S. aureus* growth. Table 2 shows the resistance patterns of the bacteria against several antibiotics.

In this study, some bacteria were found to be susceptible to doxycycline or minocycline. About 10% of *P. acnes* cultures were resistant to erythromycin, clindamycin, and tetracycline. All *P. acnes* isolates were susceptible to doxycycline and minocycline.

S. epidermidis was more resistant to erythromycin, but more sensitive to minocycline. The resistance patterns of *P. acnes* and *S. epidermidis* were similar, with both being resistant to erythromycin. No bacteria were found to be resistant to minocycline. The susceptibility of *S. epidermidis* against doxycycline (89.1%) was slightly lower than that of *P. acnes* (100%). However, erythromycin resistance was higher in *S. epidermidis* (65.2%) than in *P. acnes* (10%). We also found that *S. epidermidis* was resistant to clindamycin (52.2%) and tetracycline (32.6%).

There were seven specimens with *S. aureus* growth. *S. aureus* was found to be more resistant to erythromycin but more susceptible to minocycline. There were no bacteria found that has resistance to both minocycline and tetracycline.

DISCUSSION

Acne is a chronic skin disease that not only occurs in teenagers but also in adults.[2] The pathogenesis of acne is affected by the balance of hormones, which can be less balanced during adolescence. Clinical features of acne may include comedones, papules, pustules, and nodules. Even though acne may be self-limiting, it can potentially cause atrophic and hypertrophic scarring that may decrease patient's quality of life.[1,2] It is important for dermatologists to treat acne effectively because it can manifest into acute outbreaks or in a slow onset, prolonged-relapse. For acne treatment, there is increasing evidence that treating acne with the combination of topical and oral antimicrobial agents is effective as a first-line therapy for acne that

Table 2. The resistance patterns of *P. acnes*, S. *aureus,* and S. *epidermidis* against five antibiotics

Microorganisms	Antibiotics	Susceptible (%)	Intermediate (%)	Resistance (%)
P. acnes (n=10)	Doxycycline	100	0	0
	Tetracycline	90	0	10
	Minocycline	100	0	0
	Clindamycin	90	0	10
	Erythromycin	90	0	10
S. aureus (n=10)	Doxycycline	71.4	14.3	14.3
	Tetracycline	71.4	28.6	0
	Minocycline	100	0	0
	Clindamycin	71.4	14.3	14.3
	Erythromycin	42.9	28.6	28.6
S. epidermidis (n=46)	Doxycycline	89.1	6.5	4.3
	Tetracycline	63.0	4.3	32.6
	Minocycline	100	0	0
	Clindamycin	32.6	15.2	52.2
	Erythromycin	34.8	0	65.2

P. *acnes=Propionibacterium acnes*; S. *aureus=Staphylococcus aureus;* S. *epidermidis=Staphylococcus epidermidis*

is inflammation and non-inflammation related.[1,2,10,11] The use of oral antibiotics will lead to the emergence of antibiotic resistance in the commensal floras across all sites in the body. Thus it can only be recommended in moderate to severe acne.[2,4]

Inflammation is one of the mechanisms and factors influencing the formation of acne. It also occurs through a delayed-type hypersensitivity response with the production of protease, hyaluronidase, and chemotactic factors. Symptoms may worsen due to the formation of reactive oxygen species and lysosomal enzymes. *P. acnes* can bind to the toll-like receptor-2 (TLR-2) on monocytes around sebaceous follicles. Then, monocytes release cytokines, commonly interleukin-1α (IL-1α), interleukin-8 (IL-8), interleukin-12 (IL-12), and tumor necrosis factor-α (TNF-α), which can trigger inflammation. As an immune response, there is a release of antimicrobial peptides (histone H4 and cathelicidin). Cathelicidin interacts with β-defensin and psoriasin. In response to *P. acnes*, monocytes differentiate into macrophages ($CD209^+$) and dendritic cells ($CD1b^+$).[1,2]

The most frequent bacteria isolated from comedone lesions are *S. epidermidis, S. aureus,* and *P. acnes*. Resistance genes in *S. epidermidis* are more easily obtained and transmitted. These genes have an important role as reservoirs of antibiotic resistance.[4,12] The other bacteria, for instance, *S. aureus,* is usually a commensal bacterium of human skin. However, it can also act a pathogen and cause infection. *S. aureus* can adapt easily to its environment.[7] In this study, *S. epidermidis* was the most commonly isolated bacteria species (50.5%). Barira et al[7] and Moon et al[13] found smaller proportions of *S. epidermidis* (30%) and (28%), respectively. Sylvia et al[14] found the bacteria in larger proportions (63.6%). Differences in *S. epidermidis* proportions may differ due to differences in methods of specimen collection and the fact that it is a commensal bacterium in comedone.[12] In 2012, Moon et al[13] found that *S. epidermidis* was the most common bacteria found in Korean acne patients. Though it is known that the bacteria can aggravate inflammation, its pathogenesis is still unclear.[13] In this study, *S. epidermidis* was most susceptible to minocycline and was resistant to erythromycine. It is believed that *S. epidermidis* acts as a reservoir of resistance-related genes for antibiotics.[15] This was proven by our study that showed resistance to erythromycin (65.2%), clindamycin (52.2%), and tetracycline (32.6%) are found in higher proportions in *S. epidermidis*.

S. aureus was found in 7.7% of subjects. This result was similar to that of Sylvia et al,[14] (i.e., 9.1%) with a slightly different proportion. A recent study showed that *S. aureus* is involved in acne pathogenesis.[4] *S. epidermidis* is capable of transferring resistance genes via plasmids to *S. aureus*.[4] However, in this study *S. aureus* did not seem to be a potential acne promoter. It was found to be the most susceptible bacteria to minocycline but the most resistant to erythromycin. *S. aureus* showed higher susceptibility to minocycline (100%) than tetracycline (71.4%). This result was similar to the results from Khorvash et al,[16] which showed that the susceptibility of *S. aureus* to tetracycline was 69.4%.

P. acnes can increase inflammation associated with acne. It was predicted to be the most prevalent species. However, it was present in only 11% of subjects. This result stands in stark contrast to some earlier reports. Barira et al[7] found that *P. acnes* was present in 38% of samples, while Sylvia et al[14] found that 78.8% of samples were positive for *P. acnes*. The low prevalence of *P. acnes* in this study was likely related to a larger proportion of *Staphylococcus* sp. This microbe has the ability to adapt to environmental conditions easily and inhibit other bacteria.[4,12] From the 10 specimens that were able to grow, seven new *P. acnes* specimens were obtained after inoculating into thioglycolate twice. However, González et al[17] isolated *P. acnes* from all specimens. In their study, the specimens were taken from both inflammatory lesions (papules and pustules) and non-inflammatory lesions (open and closed comedone). These were then directly embedded into the appropriate medium without using thioglycolate broth. This method increases the isolate rate of *P. acnes*.[2,3] The number of samples with no growth in anaerobic cultures was higher than that of, aerobic cultures due to difficulties in growing anaerobic bacteria, even where the procedure for samples collection was carried out properly.

Each microbe has different resistance characteristic and susceptibility to antimicrobials. Antibiotics have a direct anti-inflammatory effect through inhibiting enzyme activity, chemotaxis, and complement path activation.[4,17] Several factors that can affect the characteristics of resistance to antibiotics; these include the type of antibiotic, the relationship between the bacteria and antibiotics, host characteristics, environment, and the antibiotic usage regime. [4,9,12] MIC is the lowest antibiotic concentration

that is needed to kill resistant bacteria, antibiotic levels higher than MIC should kill those bacteria.[4,12]

P. acnes growth and multiplication are most affected by acne inflammation. Antibiotics were given to reduce the incidence of *P. acnes* on the skin surface and under the follicle, they can also reduce the concentration of free fatty acids.[1,2] Poor outcomes in acne treatment may come from the use of an inappropriate dose of antibiotics.[2] Another trait that benefits antibiotic resistance is its ability to form biofilm, which can change the bacterial phenotype.[4,12,18] From other studies, it is known that *P. acnes* is highly resistant to erythromycin; and most of the strains are cross-resistant to clindamycin as a result of 23S RNA.[4] The resistance trait of *P. acnes* is also based on genetics of the patient and the severity of acne.[1,4] This study showed that *P. acnes* was susceptible to doxycycline and minocycline. This result was similar to the results of Barira et al[7] which showed 94.7% susceptibility to minocycline and doxycycline. We conclude that the proportion of these two antibiotics was not different than those without antibiotic history, thus the susceptibility of *P. acnes* to doxycycline and minocycline very high. *P. acnes* is generally susceptible to tetracycline. Unfortunately, the usage of tetracycline is common as a systemic antibiotic and may lead to resistance. Nevertheless, it is still unclear whether the prior antibiotic treatment history may affect the resistance.[15]

Ten percent of *P. acnes* were resistant to erythromycin, clindamycin, and tetracycline. This result is similar to Barira et al[7] who found that *P. acnes* was most resistant to erythromycin (63.2%), followed by clindamycin (57.9%), and tetracycline (47.4%).[6] Eady et al[6] found 73 resistant strains of *P. acnes* in France, Germany, Japan, Australia, USA, and England (47.94% resistant to erythromycin, 20.55% resistant to tetracycline, and 31.51% resistant to both). However, due to the development of resistance, studies on antibiotic susceptibility should be updated and repeated every 5 to 10 years across countries.[16] Therefore, antibiotics resistance needs to be monitored periodically over time.

The first line oral antibiotics for acne vulgaris were included in this study, which are minocycline and doxycycline. The usage of doxycycline and minocycline is greater than the other antibiotics because these have low molecular weight, are highly soluble, and serve as permeable drugs. The use of minocycline is preferred due to its enhanced tissue penetration, but it should be dose-limited because of its acute vestibular adverse side-effects and its hyperpigmentation effect.[10] Antibiotic resistance encompasses the effects of the use of antibiotics and those prescribed for acne used on other organisms that are more pathogenic. The bacterial flora on the body has a memory and retains resistant variants, even after antibiotic therapy has been discontinued.[2,4] It was found that patients with acne treated with antibiotics had 2.15 times higher risk of developing upper respiratory infection.[2] The use of antibiotics should be limited because it may cause collateral damage. The effectiveness of treatment should target multiple pathogenic factors instead of primarily focused on treating the bacteria.[13] Oral antibiotics can only be used on moderate to severe acne, and should not be used as monotherapy. Usage should be discontinued after 12–18 weeks.[11] The use of topical benzoyl peroxide in combination with oral antibiotics can help eradicate the antibiotic resistance of *P. acnes* and improve the overall efficacy of antibiotic treatment. Oral antibiotics usage should be stopped until control is achieved. If repeated treatment is necessary, it is recommended that the same oral antibiotic be used.[19] However, this needs to be verified in future studies.[6]

A limitation of this study is the relatively small number of samples. Even though, first, we evaluated 93 consecutive acne patients, there was no bacterial growth from 69.2% and 1.1% of samples in the anaerobic group and aerobic group, respectively. Thus only the ones with bacterial cultures could be isolated. Furthermore, there were only 10 specimens with *P. acnes* growth and seven specimens with *S. aureus* growth which did not reach the minimum CLSI criteria (i.e., at least 30), which means the susceptibility pattern could not be analyzed. The susceptibility test was only based on this study and could not reflect the isolate population in general. A bigger sample number will lead to more isolates so that these results are more valid.

P. acnes was susceptible to both doxycycline and minocycline, which showed 100% effectiveness. All isolated bacteria were more susceptible to doxycycline compared to tetracycline. The use of clindamycin and erythromycin needs to be limited since most of the *S. epidermidis* isolates were resistant to both agents. Therefore, periodical resistance monitoring over time is suggested in the future.

Conflicts of Interest

The authors confirm there is no conflict of interest in this study.

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REFERENCES

1. Zaenglein AL, Graber EM, Thiboutot DM. Acne vulgaris and acneform eruptions. In: Goldsmith LA, Katz SI, Gilchrest BA (eds). Dermatology in General Medicine. 8th edition. New York: McGraw Hill. 2012. p. 897–917.
2. Thiboutot D, Gollnick H, Bettoli V, Dréno B, Kang S, Leyden JJ, et al. New insights into the management of acne: an update from the global alliance to improve outcomes in acne group. J Am Acad Dermatol. 2009;60(5 Suppl):S1–50.
3. Webster GF. Overview of the pathogenesis of acne. In : Webster GF, Rawlings AV (eds). Acne and its therapy. New York: Informa;2007:1-7.
4. Dreno B, Martin R, Moyal D, Heniey JB, Khammari A, Seité S. Skin microbiome and acne vulgaris: *Staphylococcus*, a new actor in acne. Exp Dermatol. 2017;26(9):798–803.
5. Pugashetti R, Shinkai K. Treatment of acne vulgaris in pregnant patients. Dermatol Ther. 2013;26(4):302–11.
6. Eady EA, Gloor M, Leyden JJ. *Propionibacterium acnes* resistance: a worldwide problem. Dermatology. 2003;206(1):54–6.
7. Barira S. Proportion of positivity and P. acnes resistance pattern towards oral antibiotics from patients with moderate to severe acne vulgaris [Thesis]. Jakarta: Universitas Indonesia; 2006.
8. CLSI. Performance standards for antimicrobial susceptibility testing; twenty-fifth informational supplement Approved standard M100-S11. M11-A8. Pennsylvania: NCCLS; 2015. p. 102-4.
9. Nakase K, Nakaminami H, Takenaka Y, Hayashi N, Kawashima M, Noguchi N. Relationship between the severity of acne vulgaris and antimicrobial resistance of bacteria isolated from acne lesions in a hospital in Japan. J Med Microbiol. 2014;63(Pt 5):721–8.
10. Leyden JJ, Del Rosso JQ. Oral antibiotic therapy for acne vulgaris. J Clin Aesthet Dermatol. 2011;4(2):40–7.
11. Zaenglein AL, Pathy AL, Schlosser BJ, Alikhan A, Baldwin HE, Berson DS, et al. Guidelines of care for the management of acne vulgaris. J Am Acad Dermatol. 2016;74(5):945–73.
12. Bloemendaal AL, Brouwer EC, Fluit, AC. Methicillin resistance transfer from *Staphylocccus epidermidis* to methicillin-susceptible *Staphylococcus aureus* in a patient during antibiotic therapy. PLoS One. 2010;5(7):e11841.
13. Moon SH, Roh HS, Kim YH, Kim JE, KO JY, Ro YS. Antibiotic resistance of microbial strains isolated from Korean acne patients. J Dermatol. 2012;39(10):833–7.
14. Sylvia L. Association between microorganism from acne lesion [Thesis]. Padang: Universitas Andalas; 2010.
15. Leyden JJ. Antibiotic resistance in the topical treatment of acne vulgaris. Cutis. 2004;73(6 Suppl):6–10.
16. Khorvash F, Abdi F, Kashani HH, Naeini FF, Narimani T. *Staphylococcus aureus* in acne pathogenesis: a case-control Study. N Am J Med Sci. 2012;4(11):573–6.
17. González R, Welsh O, Ocampo J, Hinojosa-Robles RM, Vera-Cabrera L, Delaney ML , et al. In vitro antimicrobial susceptibility of *Propionibacterium acnes* isolated from acne patients in northern Mexico. Int J Dermatol . 2010;49(9):1003–7.
18. Achermann Y, Goldstein EJ, Coenye T, Shirtliff ME. *Propionibacterium acnes*: from commensal to opportunistic biofilm- associated implant pathogen. Clin Microbiol Rev. 2014;27(3):419–40.
19. Del Rosso JQ, Kim G. Optimizing use of oral antibiotic in acne vugaris. Dermatol Clin. 2009;27(1):33–42.