

# Bacterial profile and antibiotic resistance from comedone of acne vulgaris patients in West Java, Indonesia



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**Abstract**— Bacterial activity is one of the factors involved in the pathogenesis of acne vulgaris (AV). Previous studies reported that other bacteria found in addition to *P. acnes* in AV lesions, may also play a role in acne pathogenesis. Furthermore, an increase in antibiotic resistance towards these bacteria become problems. The aim of this study was to identify bacteria and determine antibiotic resistance from comedones of AV patients in Dr. Hasan Sadikin General Hospital West Java, Indonesia. A descriptive cross-sectional study was undertaken from January to February 2019. A total of 30 samples were collected from closed comedone, then cultured under aerobic and anaerobic conditions. Species identification was done by Vitek<sup>®</sup> 2 compact. The isolates were tested for resistance to nine antibiotics by disk diffusion methods. The result of this study consisted of 53.6% *P. acnes*, 17.9% *Staphylococcus epidermidis* (*S. epidermidis*), 10.7% *Staphylococcus hominis ssp. hominis* (*S. hominis ssp. hominis*), 7.1% *Staphylococcus capitis* (*S. capitis*), 1.8% for each of *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Staphylococcus warneri*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae ssp. pneumoniae*, and *Enterobacter aerogenes*. The two most common bacteria in one comedone were *P. acnes* concurrently with *S. epidermidis*. The highest antibiotic resistance of all bacteria was against clindamycin (62.5%), azithromycin (60.7%), erythromycin (57.1%), cotrimoxazole (46.4%), tetracycline (28.6%), levofloxacin (25%), doxycycline (23.2%), minocycline (16.1%), and cefadroxil (14.3%), respectively. In conclusion, the three most common bacterias from comedone AV are *P. acnes*, *S. epidermidis*, and *S. hominis ssp. hominis*. Clindamycin is the most resistant antibiotic and cefadroxil is the most sensitive antibiotic.

**Keywords**— acne vulgaris, antibiotic resistance, bacteria, comedone

## 1. Introduction

Acne vulgaris (AV) is a chronic inflammatory disorder of pilosebaceous follicles that affects primarily in adolescents. It is characterized by a pleomorphic eruption of non-inflammatory lesions (closed and open comedones) and inflammatory lesions (papules, pustules, or nodules).[1,2] The pathogenesis of AV is multifactorial, consists of hyperproliferation of follicular infundibulum, excess sebum production, inflammation, and bacterial activity (*P. acnes*).[1] Previous studies reported that other bacteria found in addition to *P. acnes* in comedone, may play a role in the pathogenesis of AV.[2] These organisms include *S. epidermidis*, *Staphylococcus aureus* (*S. aureus*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Streptococcus sp.*, *Enterobacter sp.*, and *Escherichia coli* (*E. coli*). However, the role of these microorganisms in the pathogenesis AV is still controversial.[3,4]

Acne vulgaris is not infectious disease, but bacteria play a role in its pathogenesis, so that antibiotics have been widely used for AV therapy. The widespread and long-term used of antibiotics in treatment of AV lead to antibiotic resistance. The incidence of *P. acnes* resistance increased from 20% in 1978 to 62% in 1996. Antibiotic resistance could affect the therapeutic response and treatment failure.[5] This study was undertaken to identify bacteria both aerobically and anaerobically and also to determine antibiotic resistance from comedones of AV patients in Dr. Hasan Sadikin General Hospital West Java, Indonesia.

## 2. Material and Methods

This study was a descriptive cross-sectional study. The subjects consisted of 30 AV patients. Patients who had comedones on the face were included in this study after signing informed consent. The exclusion criterias were included pregnancy, patients who were treated with hormonal therapy in the last three months, systemic antibiotic in the last two weeks and topical antibiotic in the last one week. The study was approved by The Health Research Ethics Committee, Hasan Sadikin Hospital/Faculty of Medicine, Universitas Padjadjaran, West Java, Indonesia with ethical clearance.

The samples were collected from closed comedone by sterilized comedo extractor after cleaning using antiseptic technique. The samples were inoculated individually on blood agar, Mac-Conkey's agar, and Fluid Thioglycollate Medium, then incubated both aerobically and anaerobically at 37°C. Species identification was done using Vitek<sup>®</sup> 2 compact (Biomerieux, France). All the isolates were tested for antimicrobial resistance to tetracycline, doxycycline, minocycline, clindamycin, erythromycin, azithromycin, levofloxacin, cotrimoxazole, and cefadroxil by disk diffusion methods. The results of resistance were interpreted using Clinical and Laboratory Standard Institute guidelines.

## 3. Outcomes

The characteristic and bacterial profile are shown in Table 1 and Table 2. Most of the study participants were 10 to 25 years old (83.3%), with a predominance of females (86.7%). According to Lehmann criteria, 50% was mild, 46.7% was moderate, and 3.3% was severe. The majority of patients had duration of AV between 1 to 5 years (70%) and 76.7% patients had history of previous acne treatment.

All the samples from 30 study participants were found bacteria with a total of 56 isolates. Four (13.3%) samples showed only an anaerobic growth and 26 (86.7%) samples showed mixed growth. The most common mixture bacteria were *P. acnes* concurrently with *S. epidermidis*. *P. acnes* (53.6%) was the predominant anaerobic bacteria, while *S. epidermidis* (17.9%) was the predominant aerobic bacteria, followed by *S. hominis spp. hominis* (10.7%).

The distribution of antibiotic resistance are described in Table 3. The highest antibiotic resistance of all isolates was clindamycin, followed by azithromycin, erythromycin, cotrimoxazole, tetracycline, levofloxacin, doxycycline, minocycline, and cefadroxil. Among of 30 *P. acnes* strains isolated, the highest resistance was seen with 66.7% for cotrimoxazole, followed by 60% for erythromycin, 56.7% for clindamycin and azithromycin, 23.3% for levofloxacin, 16.7% for tetracycline, and 10% for each of doxycycline, minocycline, and cefadroxil. In addition, clindamycin, azithromycin, and erythromycin were the most resistance antibiotics among of ten *S. epidermidis* strains, while clindamycin and azithromycin were the most resistance antibiotics for six *S. hominis spp. hominis* strains.

## 4. Discussion

The characteristics of this study showed 30 AV patients, mostly 26 females (86.7%) and the highest age group was 10 to 25 years (83.3%). Biswas *et al.* [6] also reported that 10 to 20 years old was the highest age group. Darwish *et al.* [7] also observed in their study that female were more affected by AV than male. This due to hormonal imbalance in female.

This study showed that *S. epidermidis* was the predominant aerobic bacterial isolates and *P. acnes* was the predominant anaerobic bacterial isolates. The bacterial species found in this study were *P. acnes*, *S. epidermidis*, *S. hominis ssp. hominis*, *Staphylococcus capitis* (*S. capitis*), *S. aureus*, *Staphylococcus haemolyticus* (*S. haemolyticus*), *Staphylococcus warneri* (*S. warneri*), *P. aeruginosa*, *K. pneumoniae spp. pneumoniae*, and *Enterobacter aerogenes*. In the study by Hasan. [8] the most frequent bacteria in comedone AV was *P. acnes*, followed by *S. epidermidis*, and *S. aureus*. Another study done by Biswal *et*

*al.* [9] reported that *P. acnes* was the predominant bacteria, followed by *S. aureus* and *S. epidermidis* in the comedone AV. Our study support the previous study, namely in addition to *P. acnes* also found other bacteria in comedone AV. In our study, the two most common bacterias were *P. acnes* concurrently with *S. epidermidis*. In the study of Biswal *et al.* [9] 13.75% samples showed mixture growth (aerobic and anaerobic growth) and were mostly caused by growth of *P. acnes* and *S. epidermidis*.

*Propionibacterium acnes* is a bacterium that plays a role in the pathogenesis of AV. It produces lipase, chemotactic factors, metalloprotease, proinflammatory cytokines, and biofilm formation.[2] *P. acnes* produces free fatty acid from triglyceride that induce comedogenesis.[1] It also produces biofilm that lead to *P. acnes* adhere to follicular wall and bind to corneocyte resulting in microcomedone. This condition shows that *P. acnes* plays a role from the formation of microcomedone, not only in the inflammation of AV.[2]

The role of *S. epidermidis* in the pathogenesis of AV remains controversial.[4] According to Pathak *et al.* [10] *P. acnes* and *S. epidermidis* were the most found bacteria around 70 to 82% in AV patients compared to healthy populations. *S. epidermidis* is thought to inhibit the progression of AV, because it produces antimicrobial peptides such as phenol-soluble modulins, epidermin, and epilancin. Biofilm that produced by *S. epidermidis* provides the anaerobic conditions to grow *P. acnes*. Lipase and delta haemolysin are virulence factors produced by *S. epidermidis* in the development of AV.[4]

In this study, isolates of *P. acnes* significantly were resistant to cotrimoxazole, erythromycin, clindamycin, and azithromycin than to the other antibiotics tested. Moon *et al.* [11] in Korea showed similar result that *P. acnes* were resistant significantly to erythromycin and clindamycin. In the study by Zandi *et al.* [12] in Iran reported that all *P. acnes* strains were susceptible to doxycycline. Contrast to our study, the prevalence of *P. acnes*-resistant was low to doxycycline (10%). In our study, *S. epidermidis* strains were resistant significantly to erythromycin, azithromycin, and clindamycin. A similar result also found in the study by Moon *et al.* [11] that showed the highest antibiotic resistance toward *S. epidermidis* was erythromycin and clindamycin.

## 5. Conclusion

There are ten species of bacteria from comedone AV with the three most common bacterias are *P. acnes*, *S. epidermidis*, and *S. hominis spp. hominis*. Clindamycin is the most resistant antibiotic, meanwhile cefadroxil is the most sensitive antibiotic.

## 6. References

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**Table 1. Characteristic of Study Participants**

| Category                                   | Study Participant (n=30) |      |
|--|--------------------------|------|
|  | n                        | %    |
| <b>Gender</b>                              |                          |      |
| Male                                       | 4                        | 13.3 |
| Female                                     | 26                       | 86.7 |
| <b>Age</b>                                 |                          |      |
| 10-25 years old                            | 25                       | 83.3 |
| >25 years old                              | 5                        | 16.7 |
| <b>AV duration</b>                         |                          |      |
| <1 year                                    | 2                        | 6.7  |
| 1-5 years                                  | 21                       | 70   |
| >5 years                                   | 7                        | 23.3 |
| <b>Severity grading (Lehmann criteria)</b> |                          |      |
| Mild                                       | 15                       | 50   |
| Moderate                                   | 14                       | 46.7 |
| Severe                                     | 1                        | 3.3  |
| <b>History of treatment</b>                |                          |      |
| Yes  | 23                       | 76.7 |
| No   | 7                        | 23.3 |

**Table 2. Bacterial Profile of Study Participants**

| Growth of Bacteria  | Study Participant (n=30) |      |
|---|--------------------------|------|
|   | n                        | %    |
| <b>Anaerobic bacteria</b>   |                          |      |
| <i>Propionibacterium acnes</i>  | 30                       | 100  |
| <b>Aerobic bacteria</b>   |                          |      |
| <b>Gram positive</b>  |                          |      |
| <i>Staphylococcus epidermidis</i>   | 10                       | 33.3 |
| <i>Staphylococcus hominis ssp hominis</i>                                 | 6                        | 20   |
| <i>Staphylococcus capitis</i>   | 4                        | 13.3 |
| <i>Staphylococcus aureus</i>  | 1                        | 3.3  |
| <i>Staphylococcus haemolyticus</i>  | 1                        | 3.3  |
| <i>Staphylococcus warneri</i>   | 1                        | 3.3  |
| <b>Gram negative</b>  |                          |      |
| <i>Pseudomonas aeruginosa</i>   | 1                        | 3.3  |
| <i>Klebsiella pneumoniae spp pneumoniae</i>                               | 1                        | 3.3  |
| <i>Enterobacter aerogenes</i>   | 1                        | 3.3  |
| <b>Mixed growth</b>   |                          |      |
| <i>Propionibacterium acnes &amp; Staphylococcus epidermidis</i>           | 10                       | 33.3 |
| <i>Propionibacterium acnes &amp; Staphylococcus hominis ssp hominis</i>   | 6                        | 20   |
| <i>Propionibacterium acnes &amp; Staphylococcus capitis</i>               | 4                        | 13.3 |
| <i>Propionibacterium acnes &amp; Staphylococcus aureus</i>                | 1                        | 3.3  |
| <i>Propionibacterium acnes &amp; Staphylococcus haemolyticus</i>          | 1                        | 3.3  |
| <i>Propionibacterium acnes &amp; Staphylococcus warneri</i>               | 1                        | 3.3  |
| <i>Propionibacterium acnes &amp; Pseudomonas aeruginosa</i>               | 1                        | 3.3  |
| <i>Propionibacterium acnes &amp; Klebsiella pneumoniae spp pneumoniae</i> | 1                        | 3.3  |
| <i>Propionibacterium acnes &amp; Enterobacter aerogenes</i>               | 1                        | 3.3  |

**Table 3. Antibiotic Resistance Profile in Comedone Acne Vulgaris**

| No | Bacteria                             | Σ Colony | Antibiotic resistance (n) |       |       |       |       |     |       |       |       |
|----|--------------------------------------|----------|---------------------------|-------|-------|-------|-------|-----|-------|-------|-------|
|    |                                      |          | CD                        | AZ    | ER    | CT    | CS    | LV  | DK    | MN    | CF    |
| 1  | <i>P. acnes</i>                      | 30       | 17                        | 17    | 18    | 20    | 5     | 7   | 3     | 3     | 2     |
| 2  | <i>S. epidermidis</i>                | 10       | 7                         | 7     | 7     | 4     | 5     | 5   | 3     | 3     | 3     |
| 3  | <i>S. hominis spp. hominis</i>       | 6        | 5                         | 5     | 3     | 1     | 4     | 0   | 3     | 1     | 1     |
| 4  | <i>S. capitis</i>                    | 4        | 2                         | 2     | 1     | 0     | 0     | 1   | 1     | 1     | 1     |
| 5  | <i>S. aureus</i>                     | 1        | 1                         | 1     | 1     | 0     | 0     | 0   | 0     | 0     | 0     |
| 6  | <i>S. haemolyticus</i>               | 1        | 1                         | 0     | 0     | 0     | 1     | 0   | 1     | 1     | 0     |
| 7  | <i>S. warneri</i>                    | 1        | 0                         | 0     | 0     | 0     | 0     | 0   | 0     | 0     | 0     |
| 8  | <i>P. aeruginosa</i>                 | 1        | 0                         | 0     | 0     | 0     | 0     | 1   | 0     | 0     | 0     |
| 9  | <i>K. pneumoniae spp. pneumoniae</i> | 1        | 1                         | 1     | 1     | 0     | 0     | 0   | 1     | 0     | 0     |
| 10 | <i>Enterobacter aerogenes</i>        | 1        | 1                         | 1     | 1     | 1     | 1     | 0   | 1     | 0     | 1     |
|    | Total                                | 56       | 35                        | 34    | 32    | 26    | 16    | 14  | 13    | 9     | 8     |
|    |                                      |          | 62.5%                     | 60.7% | 57.1% | 46.4% | 28.6% | 25% | 23.2% | 16.1% | 14.3% |



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