Antibiotic Susceptibility Patterns of Propionibacterium Acnes Isolated from Acne Vulgaris in Assiut University Hospitals, Egypt

Ihsan Abdel Sabour Hassan, Mona Amin Hassan, Mona Sallam Embarek*, Dalia A Attallah, Mohamed Ahmed El Mokhtar, Ghada Mohammed Alaa Eldin

1Department of Microbiology and Immunology, Faculty of Medicine, Assiut University, Egypt.
2Department of Microbiology and Immunology, Faculty of Medicine, Assiut University, Egypt.
3Department of Microbiology and Immunology, Faculty of Medicine, Assiut University, Egypt.
4Department of Dermatology, Venereology and Andrology, Faculty of Medicine, Assiut University, Egypt.
5Department of Microbiology and Immunology, Faculty of Medicine, Assiut University, Egypt.
6Department Women Health Hospital, Assiut University Hospitals, Egypt.

ABSTRACT

Background: Antibiotics are frequently used to treat acne patients either as bactericidal or anti-inflammatory agents. However, with the increased use of antibiotics, resistant strains of Propionibacterium acnes began to emerge and have been associated with a poor treatment outcome. Objectives: Detection of staphylococcal and Propionibacterium acnes strains in cases of acne vulgaris in Assiut university hospitals, Egypt and antibiotic susceptibility patterns of Propionibacterium acnes isolates. Methodology: Microbiological samples were obtained from one hundred patients with inflammatory acne lesions. Samples were cultured on blood agar and mannitol salt agar media under aerobic conditions at 37°C for isolation of staphylococcal strains, and on blood agar under anaerobic conditions at 37°C for 3 to 7 days for isolation of Propionibacterium acnes. Bacteria were identified by colonial morphology, standard biochemical tests, and API 20A test for identification of Propionibacterium acnes isolates. Antibiotic sensitivity testing of Propionibacterium acnes strains was done against clindamycin, erythromycin, doxycycline, trimethoprim/sulfamethaxazole, tetracycline and levofloxacin. Results: Staphylococcal strains were detected in 55% of acne cases, while Propionibacterium acnes were detected in 35% of cases. Most Propionibacterium acnes isolates were sensitive to levofloxacin (80%), followed by doxycycline (51.4%), tetracycline, trimethoprim/sulfamethaxazole (20.0% for each) while showed highest resistance rates to clindamycin (85.7%) and erythromycin (82.9%). Conclusion: Levofloxacin was the most effective antibiotic for Propionibacterium acnes followed by doxycycline, while Erythromycin and clindamycin were the least effective antibiotics for Propionibacterium acnes.

INTRODUCTION

Acne vulgaris is a chronic inflammatory multifactorial, pleomorphic skin disease of the pilosebaceous follicles (PSFs) that affects more than 85% of adolescents and young adults¹, and is characterized by a variety of non-inflamed (open and closed comedones) and inflamed (macules, papules, pustules, and nodules) lesions ².

*Corresponding author:
Mona Sallam Embarek Mohamed
Email: monasallam2000@yahoo.com
Department of Microbiology and Immunology, Faculty of Medicine, Assiut University, 71516 Assiut, Egypt.
Tel.: +20882413500/2411899 Fax: +20882333327

Four major factors are involved in the pathogenesis of acne vulgaris including increased sebum production, hypercornification of the pilosebaceous duct, an abnormality of the microbial flora (especially colonization of the duct with Propionibacterium acnes), and the production of inflammation ³. Although acne is not infectious, three major organisms have been isolated from the pilosebaceous ducts of acne patients including Staphylococcus epidermidis, Malassezia furfur and Propionibacterium acnes (P. acnes) ⁴. P. acnes is a non-spore-forming, gram-positive, anaerobic, pleomorphic rod whose end products of fermentation include propionic acid ⁵. P. acnes is considered an opportunistic pathogen, causing a range of infections as well as being associated with a number of inflammatory conditions. It
is primarily recognized for its role in acne vulgaris where it is thought to contribute to the inflammatory phase of the condition. Over several decades, topical and systemic antibiotics have been the mainstay of treatment of mild to moderate acne vulgaris by inhibiting the growth of P. acnes and their production of pro-inflammatory mediators. The antibiotics also work in immunomodulation and other anti-inflammatory activities. P. acnes is known to be sensitive to a wide range of antibiotic classes including macrolides, clindamycin, tetracycline, quinolones, penicillins and cephalosporins, but resistant to aminoglycosides, mupirocin and metronidazole. Resistance of P. acnes to antibiotics was first reported in the United States in 1979.

Since then, antibiotic-resistant P. acnes has been reported in other parts of the world with different rates. This study aimed to isolate and determine the antibiotic susceptibility patterns of P. acnes and staphylococci from cases of acne vulgaris in Assiut university hospitals.

**METHODOLOGY**

**Study design**

This is a prospective observational study involving one hundred patients who visited the Dermatology Clinic at the Department of Dermatology, Venerology and Andrology, Assiut University Hospital, Egypt and clinically diagnosed as facial inflammatory acne. Patients with acne were classified into mild, moderate and severe according to Lehmann et al. Sample collection and bacterial identification:

Inflammatory acne lesions were punctured with a hypodermic needle and the contents were collected with a comedone extractor under complete aseptic condition. The contents were taken by sterile disposable cotton swabs. Samples were then inserted into cooked meat broth (Hi media) as transport media and sent to the Department of Microbiology and Immunology, Faculty of medicine, Assiut university where bacteriological analysis were conducted. Samples were cultured on blood agar and mannitol salt agar (Biolab, Hungary) at 37°C under aerobic conditions for isolation of staphylococci and on blood agar at 37°C under anaerobic conditions in anaerobic jars for 3 to 7 days for isolation of P. acnes. Staphylococci were identified according to colonial morphology, Gram staining, and standard biochemical tests. Colonies of P.acnes were morphologically identified according to Engelkirk and Engelkirk; 1 to 2 mm in diameter, circular, convex, glistening, and opaque. Some strains produced a narrow zone of hemolysis. Further identification of P.acnes was done by Gram staining, indole test, catalase test, and API20A system (Biomerieux ® Sa) according to manufacturer’s instructions.

**Antibiotic susceptibility patterns of P. acnes isolates**

Antibiotic sensitivity tests were performed against clindamycin, erythromycin, doxycycline, trimethoprim-sulfamethaxazole, tetracycline, and levofloxacin by Disc Diffusion method. Interpretation of the results was done according to CLSI as susceptible, intermediate or resistant.

**Statistical analysis**

Statistical analyses were performed with the SPSS 20.0 version software. Variables were described by number and percentage (N, %), or mean and standard deviation (Mean ± SD) as appropriate. Chi2 test was used to compare between categorical variables. A P value < 0.05 was considered statistically significant.

**RESULTS**

During the study period; from December, 2013 until February, 2015, skin swabs were taken from inflammatory papular and pustular lesions of one hundred patients (27 males and 73 females with mean age 20.4±4.1 years) with facial acne vulgaris. Determination of acne severity revealed that 22% of the patients have mild acne, 65% have moderate acne and 13% have severe acne. Thirty nine patients were under treatment with either topical or systemic antibiotics or both and 61 patients did not have previous antibiotic treatment or stopped treatment for at least one month. Exacerbating factors were found in 91 patients including; menstruation (78%), stress (56%), sun exposure (44%), excessive salt (29%), chocolate (16%), and milk (6%) consumption. Staphylococci spp. were the most common isolates identified in 55% of cases (44% Staph. epidermidis, 7% Staph. aureus, and both in 4%). P. acnes were detected in 35% of cases (figure 1). No growth was observed in 10 cases.

**Antibiotic susceptibilities of P. acnes isolates by Disc diffusion method**

Most P.acnes isolates were sensitive to levofloxacin (80%), followed by doxycycline (51.4%), tetracycline, trimethoprim/sulfamethaxazole (20.0% for each), (p<0.001). On the other hand, P.acnes showed high resistance rates to clindamycin (85.7%), followed by erythromycin (82.9%), trimethoprim /sulfamethaxazole (80.0%), tetracycline (62.9%), (p<0.001) (Table 1).

**Antibiotic susceptibility patterns of P. acnes isolates in relation to severity of acne, age of the patients, disease duration, and previous antibiotic therapy**

Antibiotic susceptibility patterns of P.acnes isolates revealed insignificant differences between sensitivities to antibiotics in all grades of acne (table 2). As regards age of the patients; no significant difference was detected when antibiotic susceptibility patterns of P. acnes isolated from patients with age less than 20 years (n=14) compared with those from patients with age 20 years or more (n=21) (figure 2). Moreover, statistically
insignificant differences were detected when antibiotic susceptibility patterns of *P. acnes* isolated from patients with disease duration less than 2 years (*n=11*) with those from patients with disease duration equal to or greater than 2 years (*n=24*) (figure 3). Comparison of the antibiotic susceptibility patterns of *P. acnes* isolated from patients under antibiotic therapy (*n=11*) with those from untreated patients (*n=24*) revealed statistically insignificant differences as regards any of the studied antibiotics (figure 4).

### Table 1: Antibiotic susceptibility patterns of *P. acnes* isolates (*n=35*)

<table>
<thead>
<tr>
<th>Susceptibility patterns</th>
<th>Sensitive</th>
<th>Intermediate</th>
<th>Resistant</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>28</td>
<td>80.0</td>
<td>2</td>
<td>5.7</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>5</td>
<td>14.3</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>3</td>
<td>8.6</td>
<td>2</td>
<td>5.7</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>7</td>
<td>20.0</td>
<td>6</td>
<td>17.1</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethaxazole</td>
<td>7</td>
<td>20.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>18</td>
<td>51.4</td>
<td>9</td>
<td>25.7</td>
</tr>
<tr>
<td><strong>P. value</strong></td>
<td></td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

### Table 2: Antibiotics susceptibility patterns of *P. acnes* isolates according to the severity of acne.

<table>
<thead>
<tr>
<th>Susceptibility pattern</th>
<th>Mild (<em>n=7</em>)</th>
<th>Moderate (<em>n=24</em>)</th>
<th>Severe (<em>n=4</em>)</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S (%)</td>
<td>I (%)</td>
<td>R (%)</td>
<td>S (%)</td>
<td>I (%)</td>
<td>R (%)</td>
</tr>
<tr>
<td>LVX</td>
<td>6(85.7)</td>
<td>0(0)</td>
<td>1(14.3)</td>
<td>19(79.2)</td>
<td>2(8.3)</td>
<td>3(12.5)</td>
</tr>
<tr>
<td>ERY</td>
<td>2(28.6)</td>
<td>0(0)</td>
<td>5(71.4)</td>
<td>3(12.5)</td>
<td>0(0)</td>
<td>21(87.5)</td>
</tr>
<tr>
<td>CLI</td>
<td>0(0)</td>
<td>2(28.6)</td>
<td>5(71.4)</td>
<td>2(8.3)</td>
<td>0(0)</td>
<td>22(91.7)</td>
</tr>
<tr>
<td>TET</td>
<td>0(0)</td>
<td>1(14.3)</td>
<td>6(85.7)</td>
<td>6(25)</td>
<td>5(20.8)</td>
<td>13(54.2)</td>
</tr>
<tr>
<td>SXT</td>
<td>0(0)</td>
<td>0(0)</td>
<td>7(100)</td>
<td>6(25)</td>
<td>0(0)</td>
<td>18(75)</td>
</tr>
<tr>
<td>DOX</td>
<td>3(42.9)</td>
<td>3(42.9)</td>
<td>1(14.3)</td>
<td>12(50)</td>
<td>5(20.8)</td>
<td>7(29.2)</td>
</tr>
</tbody>
</table>

**Abbreviations:** LVX: Levofloxacin, ERY: Erythromycin, CLI: Clindamycin, TET: Tetracycline, SXT: Trimethoprim/sulfamethaxazole, DOX: Doxycycline

**P1:** Comparison between sensitivities of *P. acnes* to antibiotics

**P2:** Comparison between intermediate sensitivities of *P. acnes* to antibiotics

**P3:** Comparison between resistances of *P. acnes* to antibiotics

NA: Not applicable comparison. * Significant difference at *P*<0.05

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**Fig. 1:** API 20A for identification of *P. acnes* isolates
**DISCUSSION**

In the present study one hundred patients with acne vulgaris were studied. We found that menstruation in female (78%) and stress in both sex (56%) were the most frequent exacerbating factors. This is in agreement with Lucky et al. who found about 70% of women complain of a flare 2-7 days premenstrually and he believed that related to a premenstrual changes in the hydration of pilosebaceous glands. In addition, Zhang et al. reported that there is connection between acne and stress. He explained this observation as corticotrophin...
releasing hormone level changes in response to stress and it has a role in regulating sebaceous gland function. Sun and diet especially salt, chocolate and milk also were found to exacerbate acne in the present study but to a lesser extent which is agreement with previous reports 16,17. 

As regards to the distribution of bacteria in acne patients in the present study we found that Staphylococci species (55%) were the most frequent bacteria in all grades of acne mainly Staph. epidermidis (44%) followed by Staph. aureus (11%). P. acnes were detected to a lesser extent (35%) from acne lesions.

Our finding was in agreement with previous data that stated Staph. epidermidis was the most common bacteria isolates (36%), whereas P. acnes was isolated from 33%, 30%, and 32% of acne patients, respectively 18-20. On the other hand, other studies reported higher percentages of P. acnes in acne patients 9,21. The differences in the geographical regions may affect the bacteria involved in acne vulgaris which could explain this discrepancy in the distribution of bacteria in acne 22. Antibiotics are frequently used to treat acne patients either as bactericidal or anti-inflammatory agents 23. However, with the increased use of antibiotics, resistant strains of P. acnes began to emerge and are increasing worldwide from one region to another 24. It has also been associated with a poor treatment outcome 25.

As regards to the antibiotic susceptibility of P. acnes; our study found that most P. acnes isolates showed high sensitivity rates to levofloxacin (80%) followed by doxycycline (51.4%), while erythromycin (14.3%) and clindamycin (8.6%) were the least sensitive antibiotics. On the other hand, the most resistant antibiotic to P. acnes were clindamycin (85.7%) followed by erythromycin (82.9%) and trimethoprim/ sulphamethaxazole (62.9%). Our result is in agreement with a previous report that found that P. acnes were highly resistant to trimethoprim/ sulphamethaxazole, erythromycin and clindamycin and being very susceptible to levofloxacin and tetracycline 26.

Moreover, Moon et al. 19 found the resistance of P. acnes to clindamycin (30%) and erythromycin (26%) were more frequent than other antibiotics tested. Resistance to tetracycline, doxycycline, trimethoprim/sulphamethaxazole and levofloxacin were 3.3%, 6.7%, 6.7% and 0%, respectively. In addition, a previous report 21 detected high susceptibility of P. acnes to doxycycline (73.5%), while resistance of P. acnes was more frequent to clindamycin (66.3%) followed by erythromycin (49%) which is in agreement with our results. Although the exact cause of P. acnes resistance to antibiotics is not fully understood, the chronicity of the disease, antimicrobial administration route, prolonged use of antibiotics, poor treatment compliance, and easy access to the therapeutic agents without medical supervision are factors that contributed to antibiotic resistance 21. As regards the antibiotic susceptibility patterns of P. acnes in relation to the age of patients and disease duration, no significant differences were detected in our study. This is in agreement with a previous report 21.

Furthermore, Moon et al. 19 stated that, although antimicrobial resistance especially to erythromycin and clindamycin tend to be higher in patients under 25 years and with disease duration more than 2 years, it did not reach statistical significance. On the other hand, another report found higher antibiotic resistance in patients with acne vulgaris with disease duration more than 2 years 9. They explained this observation by the fact that patients with longer duration of acne are more prone to antimicrobial therapy.

Eady et al. 3 believed that the resistant strains appear 12-24 weeks after initiating the antibiotic therapy and may last after the discontinuation of the antimicrobial agents for P. acnes. In the present study, no significant differences were detected as regards antibiotics susceptibility patterns of P. acnes isolates in relation to previous antibiotic therapy for acne. This is in agreement with early data 26,27 that found statistical significant differences on comparing susceptibility patterns of P. acnes isolated from patients with previous antibiotic therapy with those who did not receive previous antibiotics.

On the other hand, another data 21,28 found greater antibiotic resistance in patients who had treatment history. This discrepancy with our results may be attributed to the shorter period of antibiotic therapy in the present study. The isolation of resistant P. acnes strains from patients with no previous antibiotic therapy indicates that factors other than antibiotic treatment may play a role in emergence of resistance 29.

CONCLUSION

From our results, we concluded that levofloxacin was the most effective antibiotic for P. acnes followed by doxycycline, while Erythromycin and clindamycin were the least effective antibiotics for P. acnes. This can change the strategy of treatment of acne.

REFERENCES

17. Ferdowsian HR, and Levin S. "Does diet really affect acne ?". Skin Therapy Lett. 2010; 15 (3); 1-2, 5