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# Determination of antibiotic susceptibility and Minimum Inhibitory Concentration (MIC) of the *Propionibacterium acnes* to the prevalent antibiotics in the treatment of Acne vulgaris

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ABSTRACT

*Propionibacterium acnes* is propounded as one of the significant agent in the occurrence of acne. Due to development of the antibiotic resistance, most of the acne vulgaris treatments are faced with failure. In order to determine the antibiotic resistance of the *P. acnes* strains isolated from the patients with acne vulgaris, this research has been carried out. 70 samples collected using microbial culture technique were studied to identify *P. acnes*. Antibiotic susceptibility of the strains isolated by antibiogram method and serial dilution to suitable antibiotics including Erythromycin, Clindamycin and Tetracycline was shown to be the preferred antibiotics in the acne treatment. Of total 70 studied samples, 14 positive samples (20%) were identified for the presence of *P. acnes*. The frequency of antibiotic susceptibility to Erythromycin, Clindamycin and Tetracycline was determined to be 50%, 64.28% and 85.71%, respectively. Range of MIC for Erythromycin, Clindamycin and Tetracycline were 0.125-64 µg/ml, 0.25-16 µg/ml and 0.063-16 µg/ml, respectively. The frequency of antibiotic resistance of the *P. acnes* to Erythromycin and Clindamycin was high. Since *P. acnes* is significantly sensitive to Tetracycline, it is recommended that this antibiotic to be used for the treatment of acne.

## 1. Introduction

Acne vulgaris is a multifactor disorder of Sebaceous follicle observed in 85% of individuals in ages ranged from 18 to 24 years old (Voils et al., 2005). Acne lesions are divided into two non-inflammatory (open and closed comedones) and inflammatory (Papule, pustule and nodule) forms (Ray et al., 2013). Blockage of sebaceous pores, increase of sebum production, inflammation and *P. acnes* are factors supposed to play a role in the formation of acne (Feldman et al., 2004; Riddle et al., 2007). Intensity of the acne correlates with rate of the Sebum production (Jappe, 2003). For this reason, accumulation of acne is higher in parts of the body where sebaceous glands are plentiful, including neck, thorax, arms and back (Feldman et al., 2004; Malhotra et al., 2012).

*P. acnes* is a gram positive, polymorphic, non-motile, without spore, facultative anaerobic and slow growth (Eugenia Portillo et al., 2013; Perry and Lambert, 2006). Stimulation of immune system and rate of acuteness of the acne correlates with the production of extra cellular enzymes and other biological products produced

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by the bacterium (Jappe, 2003). *P. acnes* plays a role in the appearance of inflammation through production of lipase enzyme and digestion of triglyceride produced in the sebaceous glands and change it into free fatty acids (Voils et al., 2005).

More than five decades antibiotic has been used for the treatment of acne (Riddle et al., 2007). By anti-bacterial and anti-inflammatory activity, antibiotics are effective on the improvement of the acne lesions (Feldman et al., 2004; Malhotra et al., 2012). Macrolide antibiotics and Tetracycline are counted as the first-line treatment for acne (Feldman et al., 2004; Riddle et al., 2007). These antibiotics, due to solubility in the fat, are absorbed by the skin and penetrate into micro comedones and influence on the acne treatment (Jappe, 2003; Riddle et al., 2007). Furthermore, Erythromycin and Tetracycline are effective on the appearance of inflammatory response via decreasing of the production of bacterial lipase enzyme (Voils et al., 2005).

Through irrevocable connection to 50S subunit of the bacterium's ribosome, Erythromycin and Clindamycin stop the protein synthesis. Tetracycline attach to 30S subunit of the bacterium's ribosome and inhibiting the protein synthesis (Webster and Graber, 2008; Malhotra et al., 2012). Establishment of antibiotic resistance to Clindamycin and Erythromycin occurs due to appearance of mutation in *23S rRNA* gene, and establishment of antibiotic resistance to Tetracycline happens due to appearance mutation in *16S rRNA* (Jappe, 2003; Nakase et al., 2014). Due to immethodical usage of antimicrobial drugs without physician's prescription and, also, lack of performance of antibiogram test before beginning of treatment to select the most appropriate antibiotic, the antibiotic resistance of the bacterium species is increasing. With the aim of selection of the most appropriate antibiotic for the treatment of acne, this research has been carried out in order to confront with antibiotic resistance.

## 2. Materials and Methods

### *2.1. Sample collection*

The current research was carried out cross sectional analysis. In this study, 70 patients with acne vulgaris referred to the skin clinic in Tonekabon, Mazandaran, Iran, between January 2014 and February 2015 were studied.

The samples were collected from the face (forehead, cheek and chin) of the studied individuals. Sampling site was disinfected by 70% ethanol. In order to sampling from each individual, a sterile cotton swab placed in the test pipe containing 2 ml of PBS was used (Srikanth et al., 2015). For the purpose of discharging the closed comedones and papules by lancet, a scratch was established on the surface of lesion, and the interior contents were discharged by little pressure of hand.

### *2.2. Culture*

Collected samples were transferred to the test pipe containing the Brain Heart Infusion Broth culture medium (Merck- Germany) (Shannon et al., 2006), and following the addition of sterile paraffin (Merck- Germany) in order to establish anaerobic conditions (Srikanth et al., 2015), they were incubated in 37˚C for 24 to 48 hours (Shannon et al., 2006). Then, a loop of the bacterial suspension, on the sterile conditions, was taken from the liquid culture medium and cultured on the plate containing Brain Heart Infusion Agar (Merck- Germany). The cultured plates were placed into the anaerobic jar and incubated under 37˚C for 3 to 4 days (Moshir et al., 2002).

### *2.3. Phenotypic identification*

For the purpose of initial identification of the studied bacterium, gram staining was prepared from the questionable colonies. After observation of the bacterium morphology and preparation of the pure culture, variety of diagnostic tests, including catalase, oxidase, indole, motility, $SH_2$ and fermentation of glucose and maltose were used for the purpose of final identification of the considered bacterium (Zandi et al., 2011). Considering the results achieved from the conducted diagnostic tests, the samples showed attribute of the desired bacterium were studied in terms of the antibiotic sensitivity (Cauich et al., 2001).

### *2.4. Antibiogram*

0.5 McFarland Standard solution of *P. acnes* was used. 200 µl of the provided bacterial suspension were transferred to the Mueller

Hinton Agar culture medium (Merck- Germany) and cultured by the sterile L-shaped rod. In the sterile conditions, disk of Erythromycin (15μg), Clindamycin (2μg) and Tetracycline (30μg), (Padtan Teb- Iran) was placed on the culture medium. The cultured plates were placed into the anaerobic jar and incubated in 37˚C for 48 hours. After observation of the inhibition zone, diameter of the zone was measured. By comparison of the zones' diameter with Clinical and Laboratory Standards Institute (CLSI), results were determined in sensitive and resistant forms (Cockerill et al., 2012).

*2.5. MIC determination*

Serial dilution technique was used to determine the Minimum Inhibitory Concentrations (MIC) of antibiotics in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. Mueller Hinton Broth culture medium (Merck-Germany) and antibiotics (Sigma Aldrich-Germany) was used for determining MIC. (Ghane et al., 2011). The MIC was determined after incubation under anaerobic conditions in 37˚C for 24 to 48 hours. The MIC results were interpreted according to CLSI guidelines (Cockerill et al., 2012).

*2.6. Statistical analysis*

The results were analyzed on the basis of demographic information, including gender, age, time disease, family and treatment background. In order to interpret the results, SPSS statistical software (SPSS version 18) and chi-square independence test were used.

## 3. Results

Of total 70 collected samples, 49 cases were female and 21 cases were male with the age range of 14 to 32 years old. Of 70 studied samples, 14 positive samples (20%) of the *P. acnes* were identified. As observed in table.1, rate of calculated P-value related to the studied age groups is equal to 0.013, therefore it can be expressed that there is a significant relationship between rate of frequency of *P. acnes* and age of patients. Significant relationship was not observed between the frequency of *P. acnes* and other studied factors.

In order to determine of antibiotic resistance, Kirby Bauer test was carried out on the 14 positive samples of the *P. acnes*. MIC values are shown in table 2. Susceptibility to Erythromycin was shown by 50% of the isolates. MICs ranged between 0.125- 64 (μg/ml). Of the clinical isolates, 64.28% were susceptible to Clindamycin. Clindamycin MICs for all isolates were in the range of 0.25- 16 (μg/ml). Most *P. acnes* isolates were susceptible to Tetracycline, rate of susceptibility to Tetracycline was determined to be 85.71% and MICs ranged between 0.063-16 μg/ml.

## 4. Discussion

Frequency of resistant to antibiotics for *P. acnes* is increasing with high speed. Immethodical consumption of the antibiotic, usage of single-drug treatments, usage of antibiotic with a limited influence spectrum (Patel et al., 2010; Dreno et al., 2004), establishment of mutation under pressure of selective antibiotic and transfer of the resistance gene between microorganism are the factors which play role in development of the antibiotic resistance (Humphrey, 2012). Also, being exposed an antibiotic leads to the appearance of the crossing resistance to other antibiotics (Collignon, 2002).

In recent years, macrolides, especially Erythromycin were regarded as the first-line treatment for acne (Gannon et al., 2011). By increase antibiotic resistance to Erythromycin, Tetracycline was replaced. Usage of Tetracycline in the pregnant women, children who are under 13 years old and summer season, due to establishment of sensitivity to light, accompanied by limitation (Feldman et al., 2004; Gannon et al., 2011). One of complications of consumption of Clindamycin antibiotic is occurrence of the digestive problems and establishment of the pseudomembranous colitis in patients (Riddle et al., 2007).

In this study the rate of antibiotic resistance to Erythromycin, Clindamycin and Tetracycline was determined to be 50%, 35.71% and 14.28%, respectively. Rate of resistance to antibiotics of Erythromycin, Clindamycin and Tetracycline were 28.6%, 57.1% and 57.1%, respectively (Moshir et al., 2002). In 2005, a study was carried out by Oprica and Nord in Europe in

order to determine antibiotic sensitivity of the *P.acnes*. The obtained results showed that 17.1% of strains of the *P. acnes* were resistant to Erythromycin, 15.1% of those were resistant to Clindamycin and 2.6% of those were resistance to Tetracycline (Oprica and Nord, 2004). In 2011, another study was conducted by Zandi et al, in Kerman aiming at survey of the antibiotic resistance of the *P. acnes* isolated from the patients with acne. Antibiotic resistance of the isolated strains to Erythromycin was determined to be 12.1% and for Clindamycin was 10.3% and for Tetracycline was 5.2% (Zandi et al., 2011). In 2013, a study was carried out by Mendoza et al, in Columbia for the purpose of determination of the antibiotic sensitivity of the *P. acnes* isolated from the patients with acne. Rate of antibiotic resistance to each one of antibiotics of Erythromycin, Clindamycin and Tetracycline was determined to be 35%, 15% and 8%, respectively (Mendoza et al., 2013).

Available similarities and differences among the results achieved from the current studies represent existence of various antibiotic resistance patterns. Reason for these discrepancies may be due to the difference in the antibiotic pattern, dose and time span of the antibiotic's consumption. A survey of results achieved from the available studies suggests high antibiotic resistance to Erythromycin and Clindamycin. Instead, *P. acnes* in that manner, is of a good sensitivity to Tetracycline antibiotic.

In order to decrease antibiotic resistance level and the correct treatment of acne, antibiogram test is suggested for the patients with long-term treatment history who don't respond to treatment appropriately. A companying of edible antibiotic with topical consumption of Benzoyl peroxide increases therapeutic effects of the antibiotics. Also, usage of non-antibiotic treatments such as Azelaic acid and Topical retinoids is suggested.

## Conclusion

The frequency of antibiotic resistance of the *P. acnes* to antibiotics of Erythromycin and Clindamycin is in a high level. In return, *P. acnes* is sensitive to Tetracycline noticeable quantity, it is recommended that this antibiotic is to be used for the treatment of acne.

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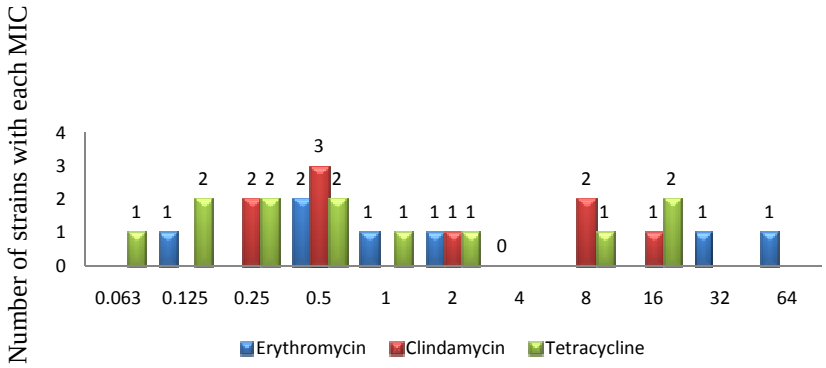
**Table.1** Demographic information of the studied patients and relative and absolute frequency of the *P. acnes* in terms of each one of the mentioned factors

| Index | Status | Frequency | Percentage frequency | Positive culture | Percentage positive culture | P-value |
|---|---|---|---|---|---|---|
| **Gender** | Female | 49 | 70% | 10 | 20.4% | 0.587 |
| | Male | 21 | 30% | 4 | 19% | |
| **Age (years)** | 10- 15 | 6 | 8.57% | 0 | 0% | 0.013 |
| | 15- 20 | 32 | 45.71% | 9 | 28.1% | |
| | 20- 25 | 18 | 25.71% | 3 | 16.7% | |
| | 25- 30 | 12 | 17.14% | 0 | 0% | |
| | 30- 35 | 2 | 2.86% | 2 | 100% | |
| **Time Disease (years)** | Random | 37 | 52.86% | 7 | 18.9% | 0.844 |
| | 1- 5 | 20 | 28.57% | 5 | 20.8% | |
| | 5- 10 | 11 | 15.71% | 2 | 28.6% | |
| | 10- 15 | 2 | 2.86% | 0 | 0% | |
| **Family Background** | Yes | 37 | 52.86% | 5 | 35.7% | 0.151 |
| | No | 33 | 47.14% | 9 | 57.1% | |
| **Treatment Background** | Yes | 29 | 41.43% | 6 | 42.9% | 0.904 |
| | No | 41 | 58.57% | 8 | 41.1% | |

**Table.2** Susceptibility of *P. acnes* to antibiotics

| Antibiotics | Sensitive % (N) | Range (µg/ml) | MIC50 (µg/ml) | MIC90 (µg/ml) |
|---|---|---|---|---|
| **Erythromycin** | 50 (7) | 0.125- 64 | 1 | 32 |
| **Clindamycin** | 64.28 (9) | 0.25- 16 | 0.5 | 8 |
| **Tetracycline** | 85.71 (12) | 0.063- 16 | 0.5 | 16 |




**MIC (µg/ml)**

**Figure.1** Diagram of MIC values

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