*Available online at http://www.journalcra.com*

*International Journal of Current Research*
*Vol. 7, Issue, 12, pp.24779-24784, December, 2015*

**INTERNATIONAL JOURNAL OF CURRENT RESEARCH**

**ISSN: 0975-833X**

## RESEARCHARTICLE

# DETERMINATION OF MICROBIAL AGENTS OF ACNE VULGARIS AND ANTIBIOTIC RESISTANCE OF *PROPIONIBACTERIUMACNES* STRAINS ISOLATED FROM ACNE PATIENTS AND HEALTHY CONTROLS

**[1]Engy Mohamed, [2,3,*]SamaaTaha, [4]Mona Atwa, [5]Gehan El-Hadidy and [6]Hesham Nada**

[1,4,6]Department of Dermatology, Faculty of Medicine, Suez- Canal University, Ismailia 41522, Egypt
[2,5]Department of Microbiology and Medical Immunology, Faculty of Medicine, Suez- Canal University, Ismailia 41522, Egypt
[3]Department of Microbiology, College of Medicine, Taif University, Al-Taif, Saudi Arabia

**ARTICLE INFO**

***Article History:***
Received 28th September, 2015
Received in revised form
10th October, 2015
Accepted 05th November, 2015
Published online 30th December, 2015

***Key words:***
Acne vulgaris,
*Propionibacterium Acne*,
Antibiotic Resistance.

**ABSTRACT**

Our case-control study was conducted at dermatology clinics and microbiology laboratory at Suez-Canal University Hospitals, Ismailia, Egypt to determine the microbial agents involved in the acne vulgaris, the prevalenceof *P. acnes* strains isolated from acne patients and healthy control groups and their antibiotic susceptibility patterns. Thirty eight acne patients (study group) were assessed according to the Acne Global Severity Scale (AGSS).The control group included 20 healthy subjects with matched age, gender and swab location of the study group. The samples were obtained by a sterile swab and then inserted into a transport medium. In the microbiology lab; microbial agents and *P. acnes* were isolated, identified and antibiotic susceptibility of each isolate was determined by disk diffusion method. According to AGSS,34.2% of the patients had mild acne, 42.1% had moderate acne, 15.8% had severe acne and 7.9% of them had very severe acne. The most frequent detected microorganisms in patients and controls were *S. epidermidis* (71.1% versus 35%, respectively), followed by *P. acnes* (18.4% versus 10%, respectively) and then *S. aureus* (10.5% versus 5%, respectively).There was significantly higher incidence of *P. acnes* in moderate (57.1%) and severe (28.6%) cases of acne in comparison to mild cases (14.3%) (p=0.0005). The most sensitive antibiotic was doxycycline (76.3%), followed by azithromycin (65.8%), tetracycline (60.5%), erythromycin (52.6%) and lastly clindamycin (50%). Selection of appropriate antibiotics is highly important in order to decrease antibiotic resistance rates and treatment failure.

***Copyright © 2015 Engy Mohamed et al.*** *This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.*

**Citation**: **Engy Mohamed, SamaaTaha, Mona Atwa,Gehan El-Hadidy, Hesham Nada 2015**. "Determination of microbial agents of acne vulgaris and antibiotic resistance of*propionibacteriumacnes* strains isolated from acne patients and healthy controls", *International Journal of Current Research,* 7, (12), 24779-24784.

## INTRODUCTION

Acne vulgaris is a chronic inflammatory disorder of pilosebaceous units that affects more than 85% of adolescents and young adults, but can also persist into older adult (Hanna *et al*. 2003; Ghodsi*et al*. 2009).Several 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 (P.) acnes*, and the production of inflammation (Zouboulis 2009).The mechanisms by which inflammatory lesions arise are poorly understood. Bacterial involvement has been implicated.

***Corresponding author: Samaa Taha,***
*Department of Microbiology and Medical Immunology, Faculty of Medicine, Suez- Canal University, Ismailia 41522, Egypt.*

Several major organisms were isolated from the surface of the skin and the pilosebaceous duct of patients with acne including *P. acnes* and *Staphylococci (S.)* species (James 2005).The production of pro-inflammatory mediators like interleukin (IL)-1 and tumor necrosis factor (TNF)-α, as well as many lipases may play a role in the pathogenesis of the disease. *P. acnes* are a Gram-positive pleomorphic rod and are traditionally regarded as part of the normal human skin microbiota. *P. acnes* strains are categorized as phylotypes IA, IB, II and III according to sequence comparison of their *tly* and *rec A*genes (Bruggemann 2005; Alexeyev and Jahns 2012). Although it's role is intensely debated, there is increasing evidence that *P. acnes* is a powerful inducer of inflammation and that it plays a crucial role in the pathogenesis of acne in genetically disposed individuals (Dessinioti and Katsambas 2010; Szabo and Kemeny 2011; Fitz-Gibbon *et al*. 2013). On

the other hand, the correlation between *P. acnes* levels and the magnitude of inflammation has not been demonstrated (Farrar and Ingham 2004).Evidence for *P. acnes'* involvement in acne has mainly been derived from culture evaluations utilizing various sampling techniques. *P. acnes* populations had different pathogenic potential, e.g. epidermal and follicular (Bek-Thomsen *et al*., 2008).Antibiotic resistance has a major influence on the choice of therapeutic approach to acne. In patients harboring resistant strains, clinical outcomes are poor (Moon *et al*., 2012). The occurrence of some instances of poor responses to antibiotics suggests that a proportion of Egyptian acne patients harbor resistant strains. Therefore, we aimed to determine the microbial agents involved in acne vulgaris and antibiotic susceptibility patterns of *P. acnes* strains isolated from acne patients and healthy controls. Our study aimed to determine the microbial agents involved in acne vulgaris and antibiotic susceptibility patterns of *p.acnes* strains isolated from acne patients and healthy controls.

## MATERIALS AND METHODS

This case-control study was carried out on 38 acne patients referred to the dermatology clinics of Suez Canal University Hospitals, Ismailia, Egypt from February to June 2014. The acne patients were of both genders and any age group. They were examined visually for the presence and severity of acne based on the Acne Global Severity Scale (AGSS).The patients didn't receive any topical treatment for 2 weeks, or systemic treatment for 1 month prior to skin swab. The control group included 20 healthy control subjects with matched age, gender and swab location of the study group. The control subjects had no concurrent acne. The study was approved by the ethics committee at Suez Canal University. Written informed consent was obtained from all participants or their guardian before enrolled in this study. Microbiological samples were obtained from each patient with inflammatory acne lesions such as papules and pustules. After cleansing the skin on face and trunk with 70% ethanol, the material and pus were extracted from skin lesions, taken by sterile swab sticks, inserted into transport anaerobic thioglycolate medium (Merck, Darmstadt, Germany) and delivered to the microbiology laboratory within 1 hour. In the laboratory, each specimen was inoculated onto two plates of blood agar (Conda, Madrid, Spain), one was incubated in aerobic condition at 37oC for 24 hours and the other incubated an aerobically for a week.

Identification of growing bacteria was performed by standard procedures including the macroscopic appearance of the colonies, Gram staining and biochemical tests such as catalase and coagulase activity. Isolates of Propionibacteriawere preliminary identified by colony morphology, Gram's stain, catalase, and indole tests. Then, suspected colonies of Propionibacteriawere subjected to testing by API 20 A kit (Biomerieux, France) for further confirmation and species identification (Ross *et al*. 2001).Antimicrobial susceptibility testing was determined by culturing in Muller- Hinton media (Conda, Spania) with disk diffusion method and according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (Performance standards for antimicrobial disc susceptibility test 2003). *P. acnes* ATCC6919 was used as a quality control strain for antimicrobial susceptibility testing (Ross *et al*. 2001); the following antibiotic discs were stamped with a disc dispenser or manually placed; Azithromycin (AZM) 15µg, Clindamycin (DA) 2µg, Doxycycline (DO) 30µg, Erythromicine (E) 15µg and Tetracycline (TE) 30µg. All discs were purchased from Oxoid (Nepean, Ontario).Data were managed using Statistical Package of Social Sciences (SPSS) version 20 (IBM SPSS Ver. 20.0). P value of less than or equal (0.05) was considered statistically significant (at 95% level of confidence). Comparisons between patients and control group were made using the t-test or Mann-Whitney U test for continuous endpoints and the Chi-Square or Fisher Exact probability tests for categorical endpoints.

## RESULTS

### Demographic characteristics of the studied populations

Table (1) shows the demographic characteristics of the studied patients and control populations. The mean age of the studied groups were matched without any statistically significant differences (p>0.05). In patients and controls groups, the frequencies of females (55.3% and 60%, respectively) were higher than males (44.7% and 40%, respectively), but there was no statistically significant difference between them (Table 1 and Figure 1).

**Table 1. Demographic characteristics of the studied patients and control populations**

| Variables | Patients (n=38) | Control (n=20) | p-value |
|---|---|---|---|
| Age (years) | | | |
| Mean ±SD | 21.0±6.5 | 22.1±4.9 | 0.51 |
| Range | 13-36 | 14-34 | |
| Gender | | | |
| Male | 17 (44.7%) | 8 (40.0%) | 0.73 |
| Female | 21 (55.3%) | 12 (60.0%) | |

Insignificant p-value >0.05.

**Table 2. Disease characteristics of the studied patients**

| Variables | Patients n=38) |
|---|---|
| Disease duration (years) | |
| Mean ±SD | 6.03±3.4 |
| Range | 1-12 |
| Acne Global Severity Scale (AGSS) | |
| Mild | 13 (34.2%) |
| Moderate | 16 (42.1%) |
| Severe | 6 (15.8%) |
| Very severe | 3 (7.9%) |
| Antibiotic treatment | |
| No previous antibiotic treatment | 16 (42.1%) |
| Systemic doxycycline | 5 (13.2%) |
| Systemic erythromycin | 3 (7.9%) |
| Topical erythromycin | 5 (13.2%) |
| Topical clindamycin | 4 (10.5%) |
| Combined treatment | 5 (13.2%) |

### Disease characteristics of the studied patients

Table (2) and Figures (2 and 3) show the disease characteristics of the studied patients. The mean disease duration of the studied patients was 6.03±3.4 years with a range of 1-12 years.

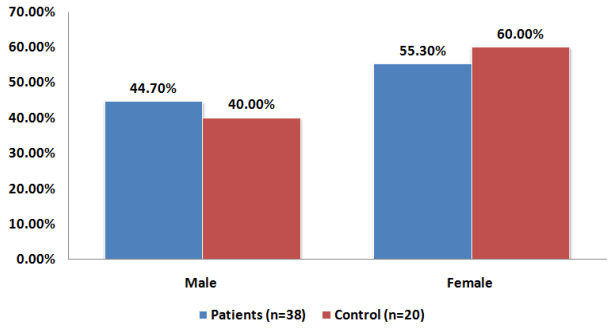


**Figure 1. Gender distribution in the studied patients and control populations**

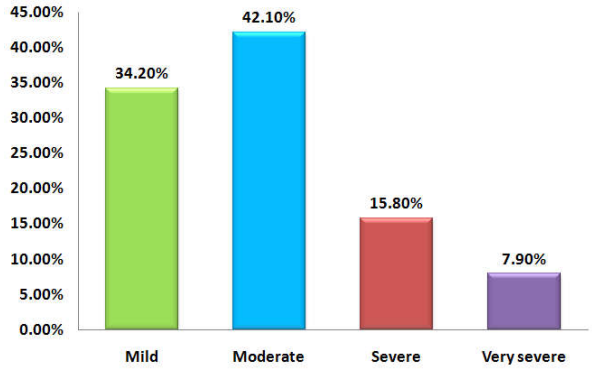


**Figure 2. Distribution of the studied patients according to Acne Global Severity Scale**

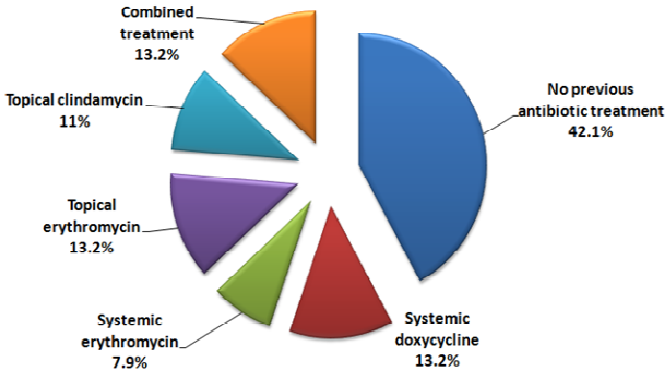


**Figure 3. Distribution of the studied patients according to previous antibiotic treatment**

According to AGSS,34.2% of the patients had mild acne, 42.1% had moderate acne, 15.8% had severe acne and 7.9% of them had very severe acne (Table 2). Regarding antibiotic treatment, 42.1% of the patients didn't receive any previous antibiotic treatment, 13.2% administered systemic doxycycline, 7.9% administered systemic erythromycin, 13.2% applied topical erythromycin, 10.5% applied topical clindamycin and 13.2% of them had combined treatment.

Table (3) and Figures (4 and 5) show the microbial agents in the samples of the studied patients and control populations.

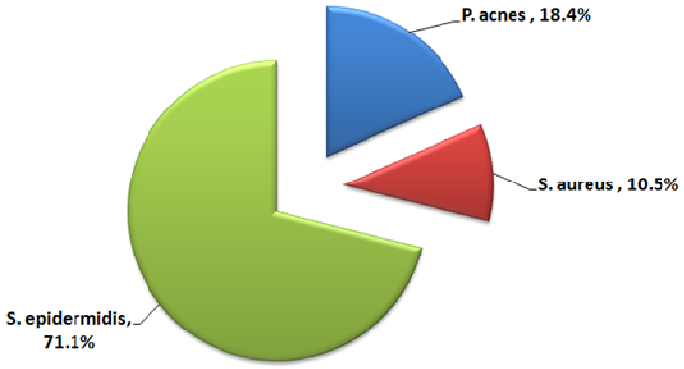


**Figure 4. Distribution of the studied patients according to microbial agents detected in the samples**

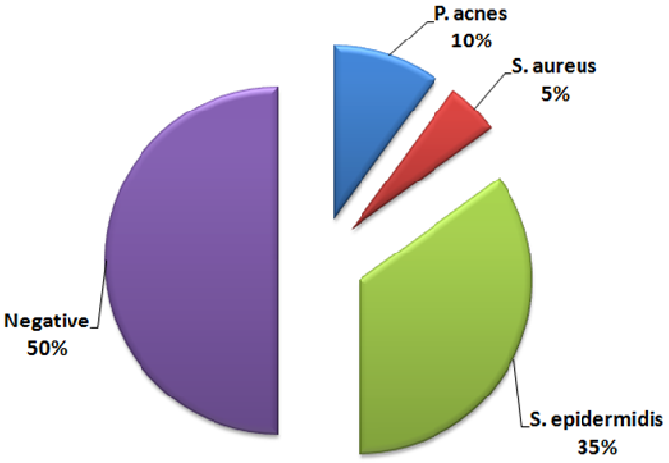


**Figure 5. Distribution of the studied control according to microbial agents detected in the samples**

The most frequent detected microorganism in patients and control was *S. epidermidis* (71.1% versus 35%, respectively), followed by *P. acnes* (18.4% versus 10%, respectively) and then *S. aureus* (10.5% versus 5%, respectively). The frequency of *P. acnes* was significantly higher in patients with acne than in healthy control subjects (OR: 4.8, 95% CI: 0.9–27.2; P = 0.043).

Table (4) shows the relation between isolated *P. acnes* strains in skin swabs and the severity of acne. There was significantly higher incidence of *P. acnes* in moderate (57.1%) and severe (28.6%) cases of acne in comparison to mild cases (14.3%) (p=0.0005). Concerning the antibiotic susceptibility patterns; the most sensitive antibiotic was doxycycline (76.3%), followed by azithromycin (65.8%), tetracycline (60.5%), erythromycin (52.6%) and lastly clindamycin (50%). *S. epidermidis* strains were resistant to doxycycline (29.6%), azithromycin (44.4%), tetracycline (48.1%), erythromycin (63.0%) and clindamycin (66.7%). All *P. acnes* isolates were sensitive to all testedantibiotics. *S. auras* strains were resistant to doxycycline (25.0%), azithromycin (25.0%), tetracycline (50.0%), erythromycin (25.0%) and clindamycin (25.0%) (Table 5).

**Table 4. Relation between isolated *P. acnes* strains in skin swabs and the severity of acne of the studied patients**

| AGSSMicrobial agents | Mild (n=13) | Moderate (n=16) | Severe/very severe (n=9) | p-value |
|---|---|---|---|---|
| *P. acnes* (n=7) | 1 (14.3%) | 4 (57.1%) | 2 (28.6%) | 0.0005** |
| *S. aureus*(n=4) | 1 (25.0%) | 2 (50.0%) | 1 (25.0%) | |
| *S. epidermidis*(n=27) | 11 (40.7%) | 10 (37.1%) | 6 (22.2%) | |

*Significant p-value at ≤0.05, AGSS=Acne Global Severity Scale, P. acnes=propionibacterium acnes, S. aureus= staphylococciaureus, S. epidermidis= staphylococci epidermidis

## DISCUSSION

Acne is the most common skin disorder in the world with prevalence about 70-87%. Skin lesions consist of white and black comedones, erythematous papules, pustules and in severe untreated cases; deep pustules, multiple nodules and scarring appear. Lesions are distributed on face, neck, chest, and back. Although the acne is not infectious three major organisms have been isolated from the pilosebaceous ducts of acne patients. Among them, *P. acnes* are the most important one (Dreno and Poli 2003; Layton 2010).

**Table 5. Antibioticsusceptibility patternsamong the studied patients (n=38)**

| Antibiotic | Sensitivity | Resistance |
|---|---|---|
| | No. (%) | No. (%) |
| Doxycycline | 29 (76.3%) | 9 (23.7%) |
| *P. acnes* (n=7) | 7 (100%) | 0 (0.0%) |
| *S. aureus*(n=4) | 3 (75.0%) | 1 (25.0%) |
| *S. epidermidis*(n=27) | 19 (70.4%) | 8 (29.6%) |
| Azithromycin | 25 (65.8%) | 13 (34.2%) |
| *P. acnes* (n=7) | 7 (100.0%) | 0 (0.0%) |
| *S. aureus*(n=4) | 3 (75.0%) | 1 (25.0%) |
| *S. epidermidis*(n=27) | 15 (55.6%) | 12 (44.4%) |
| Tetracycline | 23 (60.5%) | 15 (39.5%) |
| *P. acnes* (n=7) | 7 (100%) | 0 (0.0%) |
| *S. aureus*(n=4) | 2 (50.0%) | 2 (50.0%) |
| *S. epidermidis*(n=27) | 14 (51.9%) | 13 (48.1%) |
| Erythromycin | 20 (52.6%) | 18 (47.4%) |
| *P. acnes* (n=7) | 7 (100%) | 0 (0.0%) |
| *S. aureus*(n=4) | 3 (75.0%) | 1 (25.0%) |
| *S. epidermidis*(n=27) | 10 (37.0%) | 17 (63.0%) |
| Clindamycin | 19 (50%) | 19 (50%) |
| *P. acnes* (n=7) | 7 (100%) | 0 (0.0%) |
| *S. aureus*(n=4) | 3 (75.0%) | 1 (25.0%) |
| *S. epidermidis*(n=27) | 9 (33.3%) | 18 (66.7%) |

Our data revealed that the mean age of the acne patients was 21 years and the frequencies of females (55.3%) were higher than males (44.7%). The mean age of the control subjects was 22.1 years and the percentages of females versus males were 60% versus 40%, respectively. The most frequent detected microorganism in patients group was *S. epidermidis* (71.1%), followed by *P. acnes* (18.4%) and then *S. aureus* (10.5%), meanwhile the most frequent detected microorganism in control group was *S. epidermidis* 35%, followed by *P. acnes* 10%, and then *S. aureus* 5%.A similar data were observed by Alexeyev *et al*. (2012). The study group consisted of 38 patients with acne vulgaris, (20 males and 18 females, median age 19 years), from who punch or incisional facial biopsies were obtained.

The control group consisted of 19 subjects (7 males and 12 females) with median age of 28 years. Of 38 acne patients, 18 (47%) were positive for *P. acnes* as measured by IFM, while only 2 (10%) of 19 control subjects were positive for *P. acnes*.

In the same manner, Jahns *et al*. (2012) performed a case–control study aimed at investigating the occurrence and localization of *P. acnes* in facial biopsies in acne. In 14 (37%) samples from patients with acne, *P. acnes* were visualized in large macrocolonies/biofilms in sebaceous follicles compared with only five (13%) control samples. In a similar study performed by Moon *et al*. (2012), 100 acne patients were included in the study with the mean age of the patients of 21.9 years. Fifty-four patients were female (54%) and 46 were male (46%). *S. epidermidis* strains were isolated from the lesions of 36 patients (36%), *P. acnes* strains were isolated from the lesions of 20 patients (20%), and *S. aureus* strains were isolated from the lesions of 8 patients (8%).

In our study, the frequency of *P. acnes* was significantly higher in patients with acne than in healthy control subjects (OR: 4.8, 95% CI: 0.9–27.2; P = 0.043). Also, Jahns *et al*. (2012) found that the occurrence of *P. acnes* in facial samples in acne patients was significantly higher than in control samples (OR: 3.85, 95% CI: 1.22.–12.14; P = 0.021).Regarding antibiotic treatment in our patients, 42.1% of the patients didn't receive any previous antibiotic treatment, 13.2% administered systemic doxycycline, 7.9% administered systemic erythromycin, 13.2% applied topical erythromycin, 10.5% applied topical clindamycin and 13.2% of them had combined treatment. The drawback with regard to the usefulness of long-term treatment with antibiotics is the possible effect on microbial ecology. The normal microflora represents a barrier against colonization by pathogenic bacteria and colonization by already present microorganisms, i.e., colonization resistance. Acne patients who are in general heavily treated with antibiotics may suffer such disturbances (Sullivan *et al*., 2001a). Erythromycin, clindamycin and tetracycline have been shown to strongly suppress the oropharyngeal and gastrointestinal microflora and this may promote an overgrowth of pathogenic bacteria or yeast (Sullivan *et al*., 2005). Topical antibiotics exert selective pressure at the site of application but probably also at other sites of the body by transfer of the product and can easily be transmitted to the patient's close contacts. Oral antibiotics exert selective pressure for resistance development at all body sites where there is a normal microflora. Coagulase-negative *staphylococci* on the skin, *S. aureus*in the nares, streptococci in the oral cavity, enterobacteria in the gut, become resistant. Tetracycline, minocycline, clindamycin and erythromycin resistant coagulase-negative *staphylococci* were identified in acne patients (Dreno *et al*., 2001). Resistance rate of the tested antibiotics were as follow; doxycycline (23.7%), azithromycin (34.2%), tetracycline (39.5%), erythromycin (47.4%), and clindamycin (50%). *S. epidermidis*strains were resistant to doxycycline (29.6%), azithromycin (44.4%), tetracycline (48.1%), erythromycin (63.0%) and clindamycin (66.7%). *S. auras* strains were resistant to doxycycline (25.0%), azithromycin (25.0%), tetracycline (50.0%),

erythromycin (25.0%) and clindamycin (25.0%).Moon *et al*. (2012) reported similar observations that of a total of 36 *S. epidermidis*strains isolated, 25 were resistant to one of the antibiotics (69.4%). *S. epidermidis*strains were resistant to the following antibiotics; minocycline (0.0%), levofloxacin (11.1%), doxycycline (27.3%), tetracycline (30.6%), clindamycin (33.3%) and erythromycin (58.3%). *S. auras* strains were resistant to the following antibiotics; minocycline(0.0%), levofloxacin (0.0%), doxycycline (12.5%), tetracycline (12.5%), erythromycin (25.0%) and clindamycin (25.0%).In our study, tetracycline had lower resistance rate compared to clindamycin or erythromycin. These findings were also in agreement with previous studies (Ross *et al*., 2003; Tan *et al*., 2001). Oral tetracycline is probably less selective as compared to clindamycin or erythromycin. Another possibility would be that tetracycline resistant strains are *in vitro* less fit and have growth rates below those fully sensitive (Coates *et al*., 2002). In addition, biological actions of tetracyclines affecting inflammation, proteolysis, angiogenesis, apoptosis, metal chelation and bone metabolism have been demonstrated. Their therapeutic effects include the following diseases: acne, rosacea, bullous dermatoses, neutrophilic diseases, pyodermagangrenosum, sarcoidosis, aortic aneurysms, cancer metastasis, periodontitis and autoimmune disorders (Sapadin *et al*., 2006). Antibiotic-resistant coagulase negative *staphylococci* were found to colonize skin and nares after topical administration of erythromycin and may transfer their resistant genes to *S. aureus* (Roberts 2002; Levy *et al*., 2003) *S. aureus* strains are often multi-resistant, and include resistance to erythromycin and tetracycline. Acne patients treated with antibiotics are more often colonized by tetracycline resistant *Streptococcus pyogenes* in the oropharynx than acne patients not treated with antibiotics. The normal microbial flora may act as a reservoir for antibiotic resistance genes and under some circumstances there may be a transfer of these genes to pathogenic bacteria during their temporary colonization of the same site (Margolis *et al*., 2005).

The addition of an antibiotic to an ecologically stable environment can result in a destabilization of that environment. Other organisms may be selected and may occupy the cutaneous niche, e.g., *staphylococci*. These results may also indicate that once a patient becomes colonized with resistant bacteria, this is stabilized within the population and may persist a long time, regardless of the treatment. The cost of resistance may be ameliorated by compensatory mutations in previously low-fitness resistant clones ensuring their unimpaired fitness for survival and causing the stabilization of the resistant strains (Nagaev *et al*., 2001). The breakpoint divides organisms into susceptible (sensitive) and resistant groups based on their *in vitro* susceptibility to antibiotics and their clinical efficacy. Criteria for susceptibility and resistance breakpoints tend to be lower in European than in American studies which results in higher resistance rates in Europe than in United States (European Committee on Antimicrobial Susceptibility Testing (EUCAST), 2003; Goldstein *et al*. (2006). Regarding *P. acnes* susceptibility and resistance rate in our study, all *P. acnes* isolated from our samples were sensitive to antibiotics. Similar to *P. acnes* collected from the skin of acne patients, antibiotic resistance was less frequently encountered among isolates from various infections (Ross *et al*., 2003). In agreement to our data, Oprica (2006) found that all the *P. acnes* isolates were found to be uniformly susceptible to bactericidal antibiotics used for the treatment. These data are also in agreement with a study performed on clinical isolates of *P. acnes* collected from site of infections (Mory *et al*., 2005). We have tested *P. acnes* susceptibility to azithromycin, which may be a promising treatment of acne vulgaris and serious infections caused by *P. acnes*. None of the tested isolates was found to be resistant against azithromycin, but clinicians should be aware that propionibacteria may become resistant in the future against this antimicrobial agent. In several studies, many non-traditional antibiotics showed good *in vitro* activity against clinical isolates of propionibacteria like linezolid, daptomycin, vancomycin and retapamulin (Goldstein *et al*., 2003; Wilcox, 2005; Bonora *et al*., 2006; Gales *et al*., 2006; Goldstein *et al*., 2006; Rybak 2006). The international variations in resistance rates emphasize the importance of selective pressure exerted by improperly used anti-microbial agents in general medicine. However, sales data cannot be equated with antibiotic exposure without consideration of the patient compliance, low compliance to treatment or self medication, these all are very important contributors to the resistance problem (Albricht *et al*., 2004). It is still an open question how much of the antibiotic efficacy in acne is due to the anti-bacterial or to the anti-inflammatory effect. A low dose of doxycycline was shown to be clinically effective even though the number of bacteria on the skin did not change. Tetracycline combined with a topical treatment is a good clinical option and efforts should be made in preventing the development of resistance and the accumulation of resistant bacteria (Skidmore *et al*., 2003; Dreno *et al*., 2004). Epidemiological studies are necessary in order to track patterns of antibiotic resistance and these data may help in designing guidelines for acne treatment. Further studies are necessary to compare the *in vivo* and *in vitro* fitness of wild type and resistant *P. acnes*.

## REFERENCES


Albricht, W.C., Monnet, D.L. and Harbarth, S. 2004. Antibiotic selection pressure and resistance in Streptococcus pneumoniae and Streptococcus pyogenes. *Emerg Infect Dis*; 10: 514-7.

Alexeyev, O.A. and Jahns, A.C. 2012. Sampling and detection of skin Propionibacterium acnes: Current status. Anaerobe 18:479-483.

Alexeyeva, O.A., Lundskoga, B., Gancevicieneb, R., Palmerc, R.H., McDowelld, A., Patrickd, S., Zouboulise, C., Golovlevaf, I. 2012.Pattern of tissue invasion by Propionibacterium acnes in acne vulgaris. *J Dermatol Sci.,* 67:61–76.

Bek-Thomsen, M., Lomholt, H.B. and Kilian, M. 2008. Acne is not associated with yet-uncultured bacteria. *J. Clin. Microbiol.,* 46:3355-3360.

Bonora, M.G., Ligozzi, M., Luzzani, A. Emergence of linezolid resistance in Enterococcus faecium not dependent on linezolid treatment. *Eur J ClinMicrobiol Infect Dis*; in press.

Bruggemann, H. 2005. Insights in the pathogenic potential of *Propionibacterium acnes* from its complete, genome. *SeminCutan Med Surg*., 24:67-72.

Coates, P., Vyakrnam, S. and Eady, E.A. 2002. Prevalence of antibiotic-resistant propionibacteria on the skin of acne patients: 10-year surveillance data and snapshot distribution study. *Br. J. Dermatol*, 146: 840-8.

Dessinioti, C. and Katsambas, A.D. 2010. The role of *Propionibacterium acnes* in acne pathogenesis: facts and controversies. ClinDermatol 28:2-10.

Dreno, B., Bettoli, V., Ochsendorf, F. 2004.European recommendations on the use of oral antibiotics for acne. *Eur. J. Dermatol*, 14: 391-9.

Dreno, B., Poli, F. 2003. Epidemiology of acne. Dermatology 206: 7-10.

Dreno, B., Reynaud, A. and Moyse, D. 2001. Erythromycin-resistance of cutaneous bacterial flora in acne. *Eur. J. Dermatol,* 11: 549-53.

EUCAST, 2003.European Committee on Antimicrobial Susceptibility Testing. Bussiness Meeting 13th European Congress of Clinical Microbiology and infectious Diseases, Glasgow, UK.

Farrar, M.D. 2004. Ingham E: Acne: inflammation. *ClinDermatol,* 22:380-384.

Fitz-Gibbon, S., Tomida, S., Chiu, B.H., Nguyen, L., Du, C., Liu, M., Elashoff, D., Erfe, M.C., Loncaric, A. and Kim, J. *et al*. 2013. Propionibacterium acnes Strain Populations in the Human Skin Microbiome Associated with Acne. *J Invest Dermatol*., 21:10-38.

Gales, A.C., Sader, H.S. and Andrade, S.S. 2006. Emergence of linezolid-resistant Staphylococcus aureus during treatment of pulmonary infection in a patient with cystic fibrosis. *Int J Antimicrob Agents in press*.

Ghodsi, S.Z., Orawa, H. and Zouboulis, C.C. 2009. Prevalence, severity, and severity risk factors of acne in high school pupils: a community based study. *J Invest Dermatol*, 129:2136-2144.

Goldstein, E.J., Citron, D.M. and Merriam, C.V. 2006. Comparative in vitro activities of retapamulin (SB-275833) against 141 clinical isolates of Propionibacterium spp., including 117 P. acnes isolates. Antimicrob Agents Chemother; 50: 379-81.

Goldstein, E.J., Citron, D.M., Merriam, C.V. 2003. In vitro activities of daptomycin, vancomycin, quinupristin-dalfopristin, linezolid, and five other antimicrobials against 307 gram-positive anaerobic and 31 Corynebacterium clinical isolates. *Antimicrob Agents Chemother,* 47: 337-41.

Hanna, S., Sharma, J. and Klotz, J. 2003. Acne vulgaris: More than skin deep. *Dermatol Online J.,* 9:8.

Jahns, A.C., Lundskog, B., Ganceviciene, R., Golovleva, I., Zouboulis, C.C., McDowell, A., Patrick, S., Alexeyev, O.A. 2012.An increased incidence of Propionibacterium acnes biofilms in acne vulgaris: a case-control study.*Br. J. Dermatol,* 167(1):50-8.

James, W.D. 2005. Clinical practice: Acne. *N Engl J Med.,* 352:1463–1472.

Layton, A.M. 2010. Disorders of the sebaceous glands. In: Burns T, Breathnach S, Cox N, Griffiths C, (eds), Rook's textbook of dermatology. 8th ed. Oxford, *Blackwell Science*, 42; 1-89.

Levy, R.M. Huang, E.Y. and Roling, D. *et al*. 2003.Effect of antibiotics on the oropharyngeal flora in patients with acne. *Arch Dermatol.*, 139: 467-71.

Margolis, D.J., Bowe, W.P., Hoffstad, O. *et al*. 2005. Antibiotic treatment of acne may be associated with upper respiratory tract infections. *Arch Dermatol*., 141: 1132-6.

Moon, S.H., Roh, H.S., Kim, W.H., Kim, J.F., Ko, J.Y., Ro, Y.S. Antibiotic resistance of microbial strains isolated from Korean acne patients. *J Dermato.,* l2012; 39: 833–837.

Mory, F., Fougnot, S. and Rabaud, C.*et al*. 2005. In vitro activities of cefotaxime, vancomycin, quinupristin/dalfopristin, linezolid and other antibiotics alone and in combination against Propionibacterium acnes isolates from central nervous system infections. *J AntimicrobChemother*., 55: 265-8.

Nagaev, I., Bjorkman, J., Andersson, D.I. *et al*. 2001. Biological cost and compensatory evolution in fusidic acid-resistant Staphylococcus aureus. *MolMicrobiol.,* 40: 433- 9.

Oprica, C. 2006. Characterization of antibiotic resistant *Propionibacterium Acnes* from acne vulgaris and other diseases.Karolinska University Press, Stockholm; 6-22.

Performance standards for antimicrobial disc susceptibility test: Eighth Editor national committee for clinical laboratory standards. 2003; 23(1).

Roberts, M.C. 2002. Resistance to tetracycline, macrolide-lincosamide-streptogramin, trimethoprim, and sulfonamide drug classes. *MolBiotechnol*., 20: 261-83.

Ross, J.I., Snelling, A.M. and Carnegie, E. *et al*. 2003. Antibiotic-resistant acne: lessons from Europe. *Br. J. Dermatol.,* 148: 467-78.

Ross, J.I., Snelling, A.M., Eady, E.A., Cove, J.H., Cunliffe, W.J., Leyden, J.J., Collignon, P., Dreno, B., Reynaud, A., Fluhr, J., Oshima, S. 2001. Phenotypic and genotypic characterization of antibiotic-resistant Propionibacterium acnes isolated from acne patients attending dermatology clinics in Europe, the U.S.A., Japan and Australia. *Br. J. Dermatol.,* 144: 339-346.

Rybak, M.J. 2006. The efficacy and safety of daptomycin: first in a new class of antibiotics for Gram-positive bacteria. *ClinMicrobiol Infect*; 1: 24-32.

Sapadin, A.N., Fleischmajer, R. 2006. Tetracyclines: Non-antibiotic properties and their clinical implications. *J. Am. AcadDermatol,* 54: 258-65.

Skidmore, R., Kovach, R., Walker, C. *et al*. 2003. Effects of subantimicrobial-dose doxycycline in the treatment of moderate acne. *Arch Dermatol.*, 139: 459-64.

Sullivan, Å.,Edlund, C., Nord, C.E. 2001a. Effect of antimicrobial agents on the ecological balance of human microflora. Lancet Infect Dis; 1: 101-14.

Sullivan, Å., Edlund, C., Nord, C.E. 2005. Interaction between antimicrobial agents and the oropharyngeal and intestinal microflora. In: Bryskier A. Antimicrobial Agents. Antibacterials and Antifungals. ASM Press, Washington.

Szabo, K. and Kemeny, L. 2011. Studying the genetic predisposing factors in the pathogenesis of acne vulgaris. *Hum Immunol*, 72:766-773.

Tan, H.H., Goh, C.L., Yeo, M.G. *et al*. 2001.Antibiotic sensitivity of Propionibacterium acnes isolates from patients with acne vulgaris in a tertiary dermatological referral centre in Singapore. *Ann Acad Med Singapore*, 30: 22-5.

Wilcox, M.H. 2006. Update on linezolid: the first oxazolidinone antibiotic. Expert Opin Pharmacother 2005; 6: 2315-26.

Zouboulis, C.C. 2009.Propionibacterium acnes and sebaceous lipogenesis: a love-hate relationship? *J Invest Dermatol,* 129:2093-2096.

*******