Original article

Determination of microbial agents of acne vulgaris and *Propionibacterium acnes* antibiotic resistance in patients referred to dermatology clinics in Kerman, Iran

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Abstract

Introduction and objective: Acne is the most common skin disorder with a very high prevalence. Antibiotics have been used for more than 40 years against *Propionibacterium acnes*, the most common agent of acne. Antibiotic resistance of this bacterium was first reported in 1979, but became a worldwide problem in the recent years. The aim of this study was to determine the prevalence of antibiotic resistance among *P. acnes* isolates from acne patients referred to the dermatology clinics in Kerman, Iran during 2008-2009.

Materials and methods: The samples were obtained from face and trunk lesions of 100 acne patients by a sterile swab and then inserted into a transport medium in which they were inoculated into two blood agar plates. One was incubated aerobically at 37°C for 24h and another one in anaerobic conditions at 37°C for one week. *P. acnes* was isolated, identified and antibiotic susceptibility of each isolate was determined by disk diffusion method.

Results: Viable propionibacteria were detected in 57 patients. Thirty one percent of *P. acnes* isolates were resistant at least to one antibiotic. No resistance was seen to azithromycin and doxycycline. Lowest sensitivity was to clindamycin. Erythromycin resistance was low. No significant difference was detected in antibiotic resistance in the patients with and without treatment history and also no relationship was observed between *P. acnes* resistance and different body sites.

Conclusion: *P. acnes* resistance to current antibiotics is a common problem and selection of appropriate antibiotics in order to decrease treatment failure and sequels of acne is an important issue.

Keywords: *Propionibacterium acnes*; Antibiotic resistance; Acne; Erythromycin

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Introduction

Acne is the most common skin disorder in the world with prevalence about 70-87% [1]. Skin lesions consist of white and black comedones, erythematous papules and pustules, and in severe untreated cases, deep pustules, and multiple nodules and in untreated cases, scarring appear [2]. Lesions are distributed on face, neck, chest, and back [2]. Target of disease is pilosebaceous units and although the 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* [3]. Among them, *P. acnes* is the most important one [4]. In the pathogenesis of acne, the following four factors are important; seborrhea, hypercornification of pilosebaceous duct, colonization of the duct with *P. acnes* and inflammation [5].

The disease usually appears in adolescences, the period in which sexual hormones increases, simultaneously and usually disappears in the second half of the third decade of life [6,7]. Acne can induce stress, anxiety and serious psychiatric problems in patients. It also causes severe problems in the quality of life. This has been confirmed by some questionnaire studies on acne patients which demonstrated that the prevalence and severity of some disorders such as; depression, anxiety, lack of confidence, impaired social contact and suicidal ideation correlate with the severity of acne [6,8-10].

Acne patients usually experience high level of psychiatric and social problems that is similar to the serious chronic diseases such as asthma, epilepsy diabetes and arthritis [2]. Successful and early treatment of acne is very important and delay or failure in treatment causes probably irretrievable sequele.

High level of *P. acnes* resistance to several antibiotics that were nearly used in acne treatment was reported in 1979 from America, England, Australasia and the Far East. Later on, this problem was also reported from many other countries and became a world wide problem [11,12]. Thus, this study was designed to determine *P. acnes* resistance to antibiotics currently used in acne treatment.

Materials and methods

This cross sectional study was carried out on patients referred to the dermatology clinics in Kerman, Iran from March to December 2008. As the frequency of *P. acnes* resistance in average was about 60% in previous studies [2], the sample size was calculated to be 92 patients based on this assumption: $p=0.60$, $\alpha=0.05$ and $d= 10\%$. One hundred patients with moderate to very sever acne were enrolled in this study. Age, sex, residential area, site of acne involvement, site of sampling and treatment history were recorded.

Severity of acne can be classified as in the following: mild acne can be purely commedonal or mild papulopustular, with a few papules and pustules present. Moderate acne is characterized by numerous comedones, few to many pustules, and few small nodules, with no residual scarring. In severe acne papulopustules are numerous, nodules can be detected, inflammation is marked, and scarring is present. Very severe acne can be recognized by sinus tracts, grouped comedones, many deeply located nodules, severe inflammation and scarring [13]. Patients with the following criteria were excluded from the study, payients under 14 years of age, consumed antibiotics in the previous two weeks, presence of other skin diseases on face and trunk and patients with mild acne due to low

probability of positive culture. The samples were taken from skin pustules of 100 acne patients.

The skin was first wiped with 70% ethanol and the material was extracted from skin lesions and took up by sterile swab sticks and inserted into transport anaerobic Thioglycolate medium (Merck, Germany). Indeed we provided a direct smear from this material of each patient.

Samples were transported to the microbiology laboratory immediately. In laboratory, each specimen from transport medium was inoculated in two plates containing Blood agar (Conda, Spania), one of which was incubated in aerobic condition at 37°C for 24h and the other one in anaerobic conditions for one week. After one week if *P. acnes* culture was negative, a subculture grown, material from Thioglycolate medium was prepared again. *P. acnes* colonies were determined by Gram stain and specific tests such as catalase, indole, gelatine and esculine.

Sensitivity of *P. acnes* to antibiotics was determined by culturing in Muller-Hinton media (Conda, Spania) with disk diffusion method and according to the NCCLS guideline [14]. Antibiotic disks were provided from Padtan Teb Company. The antibacterial susceptibility of each isolate was determined (Table 1). Antibiotic resistance to doxycyclin, erythromycin, clindamycin, azithromycin, tetracycline and cotrimoxazole were detected and collected data were analyzed by SPSS software (Version 15) using chi- squared and $p<0.05$ was considered as significant.

Table 1: Zone diameter interpretive chart (as per NCCLS January 2003) (M100-S13 (M2) [14]

Antimicrobial agent	Code	Concentration	Susceptible zone diameter (mm)		
			Resistance	Intermediate	Sensitive
Azithromycin	AZ	15µg	13	14-17	18
Clindamycin	CM	2µg	14	15-20	21
Co-trimoxazole	CT	25µg	10	11-15	16
Doxycycline	DO	30µg	12	13-15	16
Erythromicine	ER	15µg	13	14-22	23
Tetracycline	TE	30µg	14	15-18	19

Results

In this study 100 acne patients (36 male and 64 female) were consecutively enrolled. The mean age of subjects who were 14 to 45 years old was 22.22±5.52. Its range was from. Ninety two patients had facial acne and 21 patients had truncal acne. According to acne grading, 74, 25 and one patients were diagnosed as moderate, sever and very sever acne, respectively. Regarding the histories of the patients, of the 35 patients, 15 patients used topical and 20 patients used systemic antibiotics. The mean duration of treatment was six weeks. Swabs which were taken from face had positive cultures of *P. acnes* in 41 and *Staphylococcus epidermidis* in 32 and *S. aureus* in five samples. 16 *P. acnes* and six *S. epidermis* and one *S. aureus* were isolated from trunk lesions

Table 2 shows the profile of antibiotic *S. aureus* resistance in patients. Sensitivity of the *P. acnes* to clindamycin, cotrimoxazole, erythromycin and tetracycline was 56.9%, 69%, 84.5% and 93.1%, respectively. No resistance to azithromycin and doxycycline was detected in cultures. Four, two and one of *P. acnes*

samples were resistant to 2, 3 and 4 antibiotics, respectively. None of the patients was resistant to all six antibiotics. We didn't find any significant difference in antibiotic resistance between those with and without treatment history and no statistical difference between antibiotic resistance in *P. acnes* isolates from different body sites.

Table 2: Percentage of *P. acnes* resistance isolated from different body sites of acne patients

Antibiotics	Face			Trunk			Total		
	S*	I**	R***	S	I	R	S	I	R
Clindamycin	61	31.7	7.3	47.1	35.3	17.6	56.9	32.8	10.3
Cotrimoxazole	70.7	7.3	22	64.7	5.9	29.4	69	6.9	24.1
Erythromycin	85.4	2.4	12.2	82.4	5.9	11.8	84.5	3.4	12.1
Tetracycline	95.1	0	4.9	88.2	5.9	5.9	93.1	1.7	5.2
Doxycycline	100	0	0	94.1	5.9	0	98.3	1.7	0
Azithromycin	100	0	0	94.1	5.9	0	98.3	1.7	0

* Sensitive, ** Intermediate, *** Resistance

Discussion

Acne is a chronic inflammatory disease of pilosebaceous units of face, neck, chest and back. Among different organisms recovered from pilosebaceous units, *P. acnes* is the most important one in the pathogenesis [4]. Antibiotics have been used more than 40 years to treat acne vulgaris. *P. acnes* antibiotic resistance was reported in USA in 1979 and then in other countries in the world [12,13]. Before prescribing an oral antibiotic for acne, history of topical and systemic antibiotic usage must be taken.

In the present study, resistance rate to one antibiotic was 31% that is lower in comparison to Coates *et al*. [15] study who found 64% of *P. acnes* resistant strains in 1997. In a 10-year study in Leeds they have found that resistant rate at least to one antibiotic was 34.5%, 64% and 55.5% in 1991, 1997 and 2000, respectively. In spite of high usage of antibiotic in our country, we did not find high antibiotic resistance in our patients. In this study, it was revealed that the sensitivity of *P. acnes* isolates of face and trunk were to azithromycin, doxycycline and tetracycline was higher than clindamycin and cotrimoxazole. Azithromycin has been prescribed to treat acnea in recent years, so antibiotic resistance to this antibiotic has not yet developed. However, clindamycin has extensively been used as a topical solution in these patients, and this is why we found highest antibiotic resistance to this antibiotic in *P. acnes* samples isolated from our patients.

Resistance to cotrimoxazole may be due to its high usage in sinusitis. Bacterial sensitivity to erythromycin was 84.5%. Tan *et al*. [16] study in 2007 revealed that the *P. acnes* has the highest to lowest resistance to the following antibiotics: erythromycin, clindamycin, cotrimoxazole and tetracycline. Lowest resistance was found to. Oprica *et al*. [17] in 2004 recovered resistant *P. acnes* strains in 37% of patients treated with antibiotics while resistance rate was 13% in non-antibiotic treated group.

Nord and Oprica [18] study in 2006 and Oprica *et al*. [17] study in 2004 have been shown the highest bacterial resistance rate to erythromycin, clindamycin and tetracycline. Ross *et al*. [19] in 2003 showed that 50% of *P. acnes* isolates were resistant to clindamycin and 20% to tetracycline in 664 acne patients. In their

study in five European countries, the prevalence of resistant isolates to at least one antibiotic was lowest in Hungary (51%) and highest in Spain (94%). Coates *et al*. [15] study in 1991 to 2001 revealed that erythromycin resistance was the most common and tetracycline resistance was the lowest.

We didn't find high antibiotic resistance to erythromycin and this is due to less prescription of erythromycin in our country. In Hasanzadeh *et al*. [20] study in 2008 it revealed that clindamycin and erythromycin were the least effective antibiotics for *P. acnes* and rifampicin the most effective one. Kaminsky [21] in 2003 had found erythromycin resistance to be the most common and to tetracycline to be the least common. Del Rosso *et al*. [22] in 2009 also observed that the efficacy of oral tetracycline and erythromycin markedly decreased and they prescribed mostly doxycycline and minocycline to treat acne.

We didn't find any meaningful relationship between antibiotic resistance and antibiotic treatment history and this isn't consistent with Ross *et al*. [19] and Nord and Oprica [18] studies. They had found greater antibiotic resistance in patients who had treatment history that may be due to shorter period of antibiotic therapy. Several reports have shown that antibiotic resistant of *P. acnes* increased with the duration of treatment.

Conclusion

Antibiotic resistance of *P. acnes* is a significant problem. Fortunately no resistance to azithromycin and doxcycline was observed in our study and resistance to tetracycline was low. Sensitivity to erythromycin was higher in our study than other studies and no significant difference was found between patients with treatment histories and those without. These findings may be due to our sample size or less consume of antibiotic and genetic factors in our province.

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