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ORIGINAL ARTICLE

Antibiotic Resistance Pattern of *Propionibacterium acnes* Isolated from Patients with Acne Vulgaris at the Dermatology Clinics of Kasr Al-Ainy Teaching Hospital Egypt

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P. acnes (Propionibacterium acnes), Acne Vulgaris, antibiotic resistance, clindamycin, erythromycin, trimethoprim-sulfamethoxazole

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ABSTRACT

Background: *Propionibacterium acnes (P. acnes) has been implicated in the pathogenesis of acne vulgaris since the beginning of the last century. Over several decades, topical and systemic antibiotics have been the main line of treatment for acne vulgaris. However, in the present era of increased antibiotic usage, resistant strains have emerged.* ***Objectives:*** *The aim of this study is to determine the antibiotic resistance pattern of P. acnes isolated from patients with acne vulgaris at the dermatology clinics of Kasr Al-Ainy teaching Hospital.* ***Methodology:*** *Specimens from 100 patients with acne vulgaris were extracted from the pustules, taken by sterile cotton swabs and transported by thioglycolate media. Each swab was inoculated onto two blood agar plates, one incubated aerobically at 37ºC for 24h and the other anaerobically for one week. P. acnes was identified by Gram stain and biochemical tests. Their sensitivity to doxycycline, erythromycin, clindamycin, tetracycline, azithromycin and trimethoprim-sulfamethoxazole was determined on Muller Hinton media by disc diffusion method. Results were interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines.* ***Results:*** *A total of 44 P. acnes isolates were identified from 100 patients with acne vulgaris, out of which 22.7% were resistant to clindamycin, 11.4% were resistant to trimethoprim-sulfamethoxazole and 9% were resistant to erythromycin. Resistance to doxycycline, tetracycline or azithromycin was not detected. Trimethoprim-sulfamethoxazole showed statistically significant difference in the resistance pattern compared to patient's sex ($p = 0.029$) and to receiving previous treatment ($p = 0.018$).* ***Conclusion:*** *P. acnes is prevalent in patients with acne vulgaris (44%) and resistant strains are detected especially in those who have received previous therapy for more than 2 weeks (68%). It is recommended that dermatologists and family physicians follow the guidelines for proper management of acne, with the judicious use of antibiotics, in order to prevent antibiotic resistance.*

INTRODUCTION

Acne vulgaris is a chronic inflammatory disorder of pilosebaceous follicles affecting more than 85% of adolescents and young adults[1]. It is characterized by a variety of eruptions including comedones, erythematous papules, pustules, and sometimes nodules, frequently followed by scarring[2]. Acne is not an infectious disease, but

P. acnes, *Staphylococcus epidermidis* (*S. epidermidis*), and *Staphylococcus aureus* (*S. aureus*), organisms residing on the surface of skin and pilo sebaceous ducts may trigger infection. Pathogenesis of acne may be attributed to inflammation, hyperkeratinisation of the sebaceous duct, high sensitivity to circulating androgens, and bacterial colonization[2]. Widespread and long-term use of antibiotics such as erythromycin, clindamycin, and tetracycline has led to the development of *P. acnes* resistance[3]. The increasing prevalence of antimicrobial resistance in *P. acnes* poses a significant challenge to successful treatment outcomes in acne patients[4].

This study was undertaken to examine the percentage of *P. acnes* within the bacteriological profile isolated from acne vulgaris patients and to ascertain isolated *P. acnes* antibiotic resistance pattern.

METHODOLOGY

A cross-sectional study was undertaken in the Outpatient Clinic of Dermatology Department, Kasr El-Ainy Teaching Hospital, Cairo University; over a period of two years between 2013 and 2014. Inclusion criteria were: (1) 14-25 years of age; and (2) mild-to-severe

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acne vulgaris. Exclusion criteria were: (1) oral or topical antibiotic in the past two weeks; (2) other facial skin diseases. A total of 100 patients with pustular acne vulgaris were included after informed consent was obtained. The study was approved by the ethical Committee of Kasr El-Ainy Teaching Hospital, Cairo University. Detailed history and clinical examination were carried out.

***Skin sampling*:**

Samples were collected from pustular acne lesions of 100 patients. The skin was first wiped out with 70% ethanol and the samples were taken using sterile disposable cotton swabs after rupture of the pustule with sterile hypodermic needle. The swabs were inserted into anaerobic transport medium [thioglycolate broth] (Merck, Darmstadt, Germany)[5]. Samples were transported immediately to Medical Microbiology and Immunology Department, Cairo University, to be processed within 24 hours of collection. All specimens were subjected to aerobic and anaerobic culture.

***Bacteriological study*:**

The specimens were inoculated onto blood agar supplemented with hemin and menadione (Thermo Fisher Scientific, UK). Plates were incubated at 37°C under aerobic condition for 24 hrs and under anaerobic condition (AnaeroGen; Oxoid Ltd, Basingstoke, UK) for one week; and examined for growth. After one week if culture was negative for *P. acnes*, a subculture was done from the thioglycolate medium and incubated for another week anaerobically[5].

Anaerobic culture was performed using the Gaspak system, Oxoid AnaeroGen (OXOID Ltd, Wade road, England). The AnaeroGen sachet will absorb the atmospheric oxygen thus reducing the oxygen level in the jar to below 1% within 30 minutes and results in 9-13% carbon dioxide simultaneously. Unlike other gas generating systems, AnaeroGen does not produce hydrogen gas and therefore, does not require a catalyst. Furthermore, no addition of water is needed to activate the reaction[6].

Oxoid anaerobic indicator was used to ensure anaerobiosis. It is a disposable sachet containing a strip of inert material saturated with a resazurin solution. In use, a color change from pink to white indicates redox condition suitable for the growth of most anaerobic organisms. If after incubation the indicator strip has not changed color, the anaerobic culture must be repeated[5].

***Identification*:**

Aerobic bacteria were identified by colony morphology, Gram stain and standard biochemical tests[7]. *P. acnes* strains were identified as small, white to pink, shiny to opaque colonies with entire margins, grown anaerobically, showing Gram positive, non-spore forming, slender, slightly curved rods with positive indole, catalase, and glucose fermentation tests as well as negative bile esculine and urease tests[8].

***Antibiotic susceptibility*:**

Antibiotic susceptibility of *P. acnes* isolates was performed by disc diffusion method using commercially available discs (antibiotic discs; Oxoid, Basingstoke, UK) according to the NCCLS guidelines. Standardized Kirby-Bauer disc diffusion method was performed on Mueller-Hinton agar (LAB™, Lancashire, England). Inocula were adjusted to a 0.5 MacFarland standard from an anaerobic pure culture on blood agar. Mueller-Hinton agar plates were inoculated and incubated at 37°C for 5-7 days anaerobically. Results were interpreted according to the NCCLS guidelines (table 1)[9].

Table 1: Antibiotic discs used to test for *P. acnes* susceptibility[9]

	Concentration	Susceptible zone diameter (mm)		
		Resistant	Intermediate	Sensitive
Clindamycin	2 µg	14	15 - 20	21
Trimethoprim-Sulfamethoxazole	25 µg	10	11 - 15	16
Erythromycin	15 µg	13	14 - 22	23
Tetracycline	30 µg	14	15 - 18	19
Doxycycline	30 µg	12	13 - 15	16
Azithromycin	15 µg	13	14 - 17	18

***Statistical Analysis*:**

The statistical package SPSS version 15 was used to code and enter the data. The data was summarized using descriptive statistics: mean, standard deviation, minimal and maximum values for quantitative variables as well as number and percentage for qualitative values. Statistical differences between groups were tested using Chi Square test for qualitative variables and independent sample t test for quantitative normally distributed variables. P values ≤ 0.05 were considered statistically significant.

RESULTS

The demographic and clinical data of 100 acne patients are shown in table 2. All the patients were between 14 and 25 years of age, with a mean age of 19.6 ± 2.9 years. The majority of patients were between

20 and more years of age (57%), with a predominance of females (72%). The disease duration exceeded 2 years in 57% of patients. Thirty-one percent of patients had history of previous anti-acne treatment. There was no significant difference in the detection of *P. acnes* in relation to the patients' age, sex or duration of the disease. However, there was a statistically significant difference in patients taking previous treatment. *P. acnes* was more prevalent in patients who received previous medical treatment for more than 2 weeks (P = 0.001) (table 2).

Table 2: General characteristics of patients with *P. acnes*

	No.	%	*P* value
Age (years)			
< 20	20 / 43	46.5%	0.660
≥ 20	24 / 57	42.1%	
Sex			
Female	34 / 72	47.2%	0.298
Male	10 / 28	35.7%	
Duration of disease (years)			
< 2	21 / 43	48.8%	0.397
> 2	23 / 57	40.4%	
Previous treatment			
Yes	21 / 31	67.7%	**0.001**
No	23 / 69	33.3%	

The bacteriological profiles of the acne patients are shown in Table 3. Of 100 samples processed, 91% samples showed aerobic growth and 47% showed anaerobic growth. *S. epidermidis* (61; 61%) was the predominant aerobe, followed by *S. aureus* (28; 28%) and Candida (2; 2%). Among the anaerobes, *Propionibacterium* species were isolated from 47 samples, with *P. acnes* as the predominant species (44; 44%); *Propionibacterium granulosum* (indole test: negative) was detected in 3% of samples (table 3). Out of the 44 *P. acnes* isolates, only one was isolated in pure form and 43 were isolated in mixed form (table 4).

Table 3: Isolated microorganisms from the studied patients

	%
Aerobic	
S. aureus	28
S. epidermidis	61
Candida	2
Anaerobic	
P. acnes	44
P. granulosum	3

Table 4: Percentage of *P. acnes* isolated from patients suffering from acne vulgaris

	%
Mixed growth with *P. acnes*	43
Pure *P. acnes*	1
No growth	5
Bacteria other than *P. acnes*	51

***Antibiotic susceptibility pattern*:**

10 *P. acnes* strains showed resistance to clindamycin (22.7%), 5 strains were resistant to trimethoprim-sulfamethoxazole (11.4%) and 4 strains were resistant to erythromycin (9.1%). Three isolates showed resistance to both clindamycin and erythromycin. No resistance was observed to azithromycin (0%), doxycycline (0%) nor tetracycline (0%) (table 5).

Table 5. Antibiotic susceptibility pattern of isolated *P. acnes*

Antibiotic	*P. acnes* susceptibility pattern (no. = 44)		
	Susceptible No. (%)	Intermediate No. (%)	Resistant No. (%)
Clindamycin	31 (70.5%)	3 (6.8%)	10 (22.7%)
Trimethoprim- Sulfamethoxazole	35 (79.5%)	4 (9.1%)	5 (11.4%)
Erythromycin	37 (84.1%)	3 (6.8%)	4 (9.1%)
Tetracycline	43 (97.7%)	1 (2.3%)	0 (0%)
Doxycycline	44 (100%)	0 (0%)	0 (0%)
Azithromycin	44 (100%)	0 (0%)	0 (0%)

There were no statistically significant differences in the antibiotic resistance pattern of isolated *P. acne* in relation to patients' age, sex, disease duration and previous treatment. However, there was a statistically significant difference in the resistance pattern of trimethoprim-sulfamethoxazole when compared to sex of patients (P = 0.029) and history of taking previous treatment (P = 0.018) (table 6) (Figures 1, 2).

Table 6: Prevalence of antibiotic resistance to *P. acnes* according to age, sex, disease duration and previous treatment

	Clindamycin	Trimethoprim – Sulfamethoxazole	Erythromycin	Tetracycline	Doxycycline	Azithromycin
Age (years)						
< 20	4 / 20 (20%)	1 / 20 (5%)	2 / 20 (10%)	0 / 20(0%)	0 / 20(0%)	0/20(0%)
≥ 20	6 / 24 (25%)	4 / 24(16.7%)	2 / 24(8.3%)	0 / 24(0%)	0 / 24(0%)	0 / 24(0%)
p value	0.817	0.478	0.261	0.000	0.000	0.000
Sex						
Female	10 / 34 (29.4%)	2 / 34(5.9%)	3/34(8.8%)	0/34(0%)	0/34(0%)	0 / 34(0%)
Male	0 / 10 (0%)	3 /10(30%)	1 / 10(10%)	0 / 10(0%)	0 / 10(0%)	0 / 10(0%)
P value	0.066	**0.029**	0.623	0.000	0.000	0.000
Duration of disease (years)						
< 2	4 / 21 (19%)	3/21(14.3%)	2 / 21(9.5%)	0 / 21(0%)	0 / 21(0%)	0 / 21(0%)
> 2	6 / 23(26.1%)	2/ 23(8.7%)	2 / 23(8.7%)	0 / 23(0%)	0 / 23(0%)	0 / 23(0%)
P value	0.713	0.832	0.230	0.000	0.000	0.000
Previous treatment						
Yes	6 / 21(28.6%)	4 / 21(19%)	4 / 21(19%)	0 / 21(0%)	0 / 21(0%)	0 / 21(0%)
No	4 / 23(17.4%)	1 / 23(4.3%)	0 / 23(0%)	0 / 23(0%)	0 / 23(0%)	0 / 23(0%)
P value	0.187	**0.018**	0.061	0.000	0.000	0.000

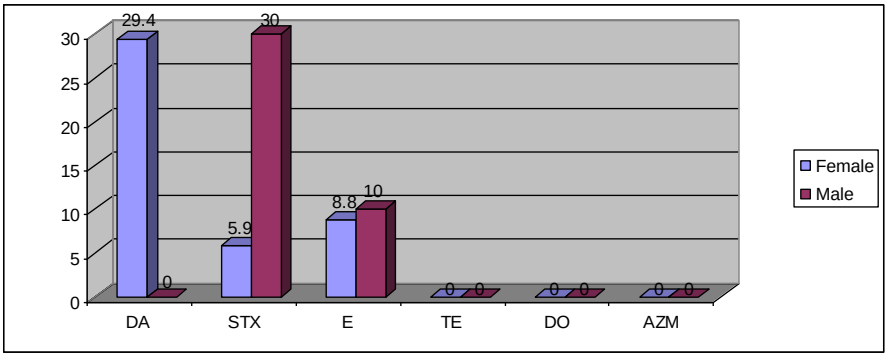

Fig. 1: Antibiotic resistance pattern in relation to sex

DA: Clindamycin; SXT: Trimethoprim-sulfamethoxazole; E; Erythromycin; TE: Tetracycline; DO: Doxycycline, AZM: Azithromycin.

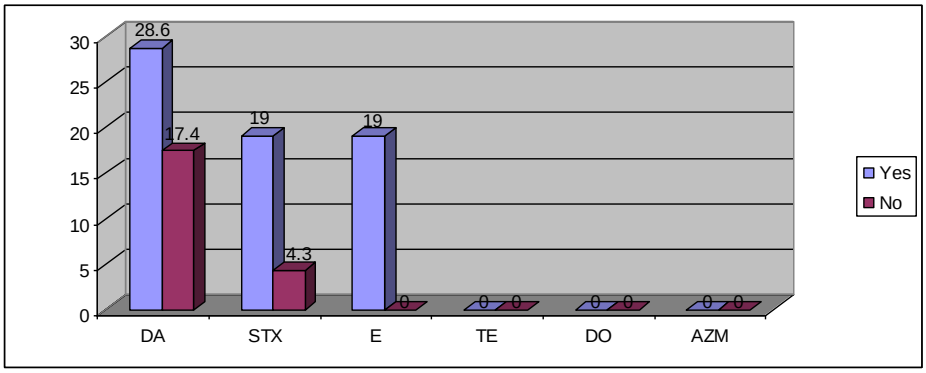

Fig. 2: Antibiotic resistance pattern in relation to previous treatment

DA: Clindamycin; SXT: Trimethoprim-sulfamethoxazole; E; Erythromycin; TE: Tetracycline; DO: Doxycycline, AZM: Azithromycin.

DISCUSSION

Cutaneous *P. acnes* has been implicated in acne, although their role in inflammation is still poorly understood. There is widespread resistance in *P. acnes* due to over use of topical and systemic antibiotics for treatment of acne vulgaris[10]. The difference in the *in vitro* antibiotic susceptibility patterns of *P. acnes* among different countries is not clear; however it may be attributed to the different antibiotic prescribing habits and/or different ethnicity of the patients as well as genetic mutations[11]. Different studies have used various interpretative criteria to estimate the resistance among *P. acnes* isolates to different anti-acne drugs. The available data on antibiotic resistance among these strains are deficient; also, no standard criterion is available for determining the resistance among anti-acne drugs in *P. acnes*. Virtually, drug susceptibility is not prescribed, owing to the slow growth of the bacteria and the cost and complexity of testing methods. However, it is important to get resistance information so that correct therapeutic decisions can be made, particularly in resistant cases not responding to routine therapy[2].

Our study showed a high prevalence of acne in female patients (72%), which was in agreement with the studies done by Hassan *et al.* (73%)[12], Moon *et al.* (68%)[13], Zandi *et al.* (64%)[5] and Zhu T *et al.* (59%)[14]. Fourty three percent of our patients had a disease duration of less than 2 years while 57% had a disease course of more than 2 years. In agreement, Moon *et al.*[13]reported 38% and 60% respectively and Zhu T *et al.*[14]reported 20% and 80% respectively. In contrast, Sardana *et al.*[15] reported 52% and 46% respectively. In the present study, 69% of patients did not receive any anti-acne drugs. Similarly, this was reported by Moon *et al.* (73%)[13], Zandi *et al.* (65%)[5]and Zhu T *et al.* (64%)[15]. In contrast, Mendoza *et al.*[4] reported that 75% of patients received previous anti-acne treatment.

Out of the 100 patients diagnosed with acne vulgaris, *P. acnes* represented 44% of isolates when cultured anaerobically. In agreement with our results, *P. acnes* was detected in 41%, 35%, 35% and 32% of the samples of the following studies Zandi *et al.*[5], Hassanzadeh *et al.*[16], Hassan *et al.*[12] as well as Dhillon and Varshney[17] respectively. Other studies reported a higher percentage of isolated *P. acnes* as Schafer *et al.*[18](96%) as well as Abdel Fattah and Darwish[19] (88%). This may be due to the greater number of patients included in their study as compared to our study. In contrast, Moon *et al.*[13] reported a lower percentage (20%) which may be attributed to the short duration of the study.

In this study, out of the 44 *P. acnes* isolates, only one was isolated in pure form (mixed growth was detected in 43%). *S. epidermidis* (61%) and *S. aureus* (28%) were the most prevalent bacteria detected aerobically. In agreement with this results, Hassanzadeh *et al.*[16] reported that *S. epidermidis* were detected in 53% of isolates. Moreover, Nishijima *et al.*[20] reported that *S. epidermidis* was detected in more than 50% of their isolates and Hassan *et al.*[12] reported the detection of *S. epidermidis* in 44% of the isolates. In contrast, other studies reported that the most frequent bacterium isolated from patients with acne was *S. aureus*. Dhillon and Varshney[17] detected *S. aureus* in 41% and *S. epidermidis* in 20%. Toyoda and Morohashi[21] detected *S. aureus* in 48% and *S. epidermidis* in 23%. This difference in microbial profile in our study could be explained by the variations in geographical location, host factors, and antibiotic usage.

Moreover, we studied the significance of *P. acnes* detection in relation to the patients' age, sex, disease duration and history of previous treatment. The only statistically significant relation we found was the increased incidence of *P. acnes* (68%) in patients receiving previous treatment for more than 2 weeks (p=0.001). Similarly, Tan *et al.*[22] and Schafer *et al.*[18] reported a prevalence of 75% and 70% respectively. Thus, confirming that *P. acnes* is one of the most common causes of resistant acnes lesions[18]. However, other studies as Abdel Fattah and Darwish[19] reported a lower frequency (33%).

Regarding the susceptibility pattern, we found that 22.7% of the isolated *P. acnes* were resistant to clindamycin, 11.4% were resistant to trimethoprim-sulfamethoxazole and 9% were resistant to erythromycin. Several studies in different countries reported clindamycin resistance: In India, 90% by Sardana *et al.*[15], in Egypt, 85% by Hassan *et al.*[12] and 65% by Abdel Fattah and Darwish[19], in Hong Kong, 53.5% by Luk *et al.*[11], in Iran, 50% by Zandi *et al.*[5], in Mexico, 35% by Gonzalez *et al.*[23], in Korea, 26.7% by Moon *et al.*[13] and in Japan, 10.3% by Ishida *et al.*[24]. Our percentage of clindamycin resistance was lower than most studies, probably due to the lower number of isolated *P. acnes* (44%). In addition, different geographical regions may affect the distribution of bacteria involved in acne vulgaris[12]. However, in agreement with all these studies, we found that clindamycin resistance was higher than erythromycin resistance within the isolated *P. acnes*. This is possible due to the fact that clindamycin is the most commonly prescribed antibiotic for patients with acne, in most communities including ours[12].

In addition, 3 out of the 44 *P. acnes* isolates (7%) had cross resistance to both clindamycin and erythromycin. Another Egyptian study also reported cross-resistance to clindamycin and erythromycin in 20 out of 98 patients (20%)[19]. Moreover, a study from Greece similarly reported cross-resistance between the two antibiotics in 23 out of 79 patients (29%)[25]. The cross resistance to clindamycin and erythromycin found

in our study could be attributed to the use of both antibiotics as topical and systemic solutions for acne patients[19]. The underlying mechanism of erythromycin and clindamycin cross- resistance was elucidated by Ross *et al*. who identified four phenotypes with cross susceptibility to macrolide, lincosamide and streptogramin B (MLS) antibiotics. Genetic mutations occur mainly in 23S rRNA, and strains that possess the *erm* (X) resistance gene are highly resistant to MLS antibiotics[10].

In this study, 11.4% of isolated *P. acnes* were resistant to trimethoprim-sulfamethoxazole. This may be attributed to its high usage in sinusitis. Similarly, the following studies reported resistance to trimethoprim-sulfamethoxazole: 26.3% by Schafer *et al.*[18], 15% by Ross *et al.*[10] and 8% by Oprica *et al.*[26]. On the other hand, Gonzalez *et al.* reported 68% trimethoprim-sulfamethoxazole resistance[23]. This higher percentage could be attributed to the overuse of this antibiotic by physicians, as well as the fact that it is usually sold in Mexico and other countries as an over-the- counter medication for other infections[23].

Regarding tetracycline and doxycycline susceptibility, all *P. acnes* isolates in this study were highly susceptible to both antibiotics. Our results are also consistent with those of Giannopoulos *et al.* who reported no resistance to tetracycline in Greece[25], Schafer *et al.* in Chile[18], Ross *et al.* in Italy and Hungary[10] and Doğan *et al*.in Turkey[27]. This is possibly attributed to the fact that topical cyclines are not usually prescribed for patients with acne vulgaris which in turn decreases the emergence of *P. acnes* cycline-resistant strains[19]. The European guidelines for acne recommend tetracycline as a first line treatment in acne patients[28]. Tetracycline and doxycycline resistance patterns were detected worldwide. In Egypt (18% & 6% respectively)[19], in Hong Kong (16.3% for both)[11], in Mexico (14% & 20% respectively)[23], in Singapore (11.5 % for both)[21] in France (9.5% for both)[29], in Japan (2% for both)[23] and in southwest China (3% & 1% respectively)[14]. In contrast, a higher tetracycline resistance pattern was detected in Iran (35%)[5] and in India (30%)[15], due to its high usage in these countries.

In the present study, all isolates were susceptible to azithromycin. The Egyptian study by Abdel Fattah and Darwish reported a low percentage of azithromycin resistance (5%) and suggested azithromycin as an important line for acne treatment in Egyptian patients[18]. Similarly, no azithromycin resistance was reported in Iran[5]. However,in contrast to our findings, higher resistance to azithromycin was reported: In China 59 % by Zhu T *et al.*[14], in Mexico 82% by Gonzalez *et al.*[23], and in India 100% by Sardana *et al.* suggesting it is probably attributed to a cross resistance mechanism similar to that existing between clindamycin and erythromycin, and recommended that further studies are required to exclude the possibility of cross-resistance between this macrolide antibiotic and lincosamides (clindamycin)[15].

When trying to correlate the prevalence of resistant *P. acnes* isolates with age, disease duration and previous treatment, no statistically significant results were found. A significant difference was found when correlating previous acne treatment with trimethoprim-sulfamethoxazole resistance (p=0.018). In agreement, Schafer *et al.*[18], Abdel Fattah and Darwish[19], Nord and Oprica[30] and Ross *et al.*[10] reported that resistant *P. acnes* were more prevalent in patients who received previous acne treatment. No resistant *P. acnes* isolates were detected in patients who did not receive previous treatment.

Owing to the fact that acne often requires long-term treatment, there is a concern that *P. acnes* resistance may be associated with the development of resistance in other organisms such as *S. aureus* and *S. epidermidis*. Thus, it is important that antibiotics be used judiciously, particularly in such a common condition as acne vulgaris[13].

CONCLUSION

We concluded that highest resistance rates were detected with clindamycin, followed by trimethoprim-sulfamethoxazole and erythromycin (22.7%, 11.4% and 9% respectively), while all *P. acnes* isolates were susceptible to azithromycin and doxycycline and almost all of them (98%) were susceptible to tetracycline. This can change the strategy for treatment of acne.

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- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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