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Antibiotic susceptibility of *Propionibacterium acnes* isolated from patients with acne in a public hospital in Southwest China: prospective cross-sectional study

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ABSTRACT

Objective Antibiotics have been routinely used for several decades against *Propionibacterium acnes* (*P. acnes*), but antibiotic resistance of *P. acnes* is becoming a global problem. Only one related Chinese study is available. The aim of this study was to assess the antibiotic susceptibility of *P. acnes* obtained from patients with acne in Southwest China.

Design This was a prospective cross-sectional study. Cutaneous samples were obtained from acne lesions on the face of 375 patients. Samples were cultured in anaerobic medium to identify the presence of *P. acnes*. Susceptibility tests of isolated *P. acnes* were performed for tetracycline, doxycycline, clindamycin, erythromycin, azithromycin and clarithromycin using the Epsilometer test.

Results *P. acnes* was isolated from 227 patients; 224 isolates (98.7%) were susceptible to doxycycline and 220 (96.9%) were susceptible to tetracycline, followed by clindamycin and clarithromycin in 101 (44.5%) and 102 (44.93%) isolates, respectively. Susceptibility of *P. acnes* was detected for erythromycin in 96 (42.3%) patients, followed by azithromycin in 94 (41.4%). Subjects who received antibiotics (topical and oral) had higher frequencies of antibiotic-resistant *P. acnes* as well as increased antibiotic minimum inhibitory concentrations compared with patients without antibiotic treatment.

Conclusions *P. acnes* was highly sensitive to cyclines (doxycycline and tetracycline). *P. acnes* showed higher resistance rates to macrolides–lincosamides–streptogramins antibiotics (such as erythromycin, azithromycin, clarithromycin and clindamycin). The irrational use of antibiotics for acne treatment is probably a problem in China and elsewhere. These results suggest that dermatologists should be more prudent in prescribing antibiotics for acne.

Strengths and limitations of this study

- The study sample was representative of the population of patients with acne.
- The sample size was small, and the subjects were from a single centre.
- The cross-sectional design prevented the determination of any cause–effect relationship.

INTRODUCTION

Acne is one of the most common skin disorders throughout the world, affecting 67%–95% of adolescents.[1] Actually, acne is clearly a chronic inflammatory skin disease and not primarily an infectious disease. It is characterised by pleomorphic lesions, including comedones, pustules, papules, nodules and cysts.[2] Its pathogenesis is multifactorial and includes abnormal sebum secretion, follicular hyperkeratinisation, *Propionibacterium acnes* (*P. acnes*) hypercolonisation, inflammation and immunity.[3] *P. acnes* is also considered an opportunistic pathogen causing multiple inflammatory diseases (eg, endophthalmitis, endocarditis, osteomyelitis, sarcoidosis, keratitis and the synovitis, acne, pustulosis, hyperostosis, osteomymyelitis (SAPHO) syndrome) as well as inflammatory ailments after surgery or the implantation of foreign devices, including prosthetic aortic valve, hip and shoulder implants.[4]

P. acnes plays a vital role in the pathogenesis of acne by activating the innate and adaptive immunity. Chemotactic factors and proinflammatory cytokines are produced by immune reactions, resulting in local inflammation and potential scarring.[5] Anti-inflammatory and antimicrobial medications are the basis of acne therapy. Therefore, antibiotics are widely used in patients with acne, inhibiting or eradicating the *P. acnes* colonisation, and reducing the production of proinflammatory mediators. Topical and systemic antibiotics are frequently used in the treatment of acne.[6] For the past 30 years, a decrease in the percentage of susceptibility of *P. acnes* strains

to these antibiotics has been reported in many countries, indicating that antibiotic-resistant *P. acnes* among patients with acne is a global problem.[7–14]

With routine and long-term use of antibiotics, the resistance profile of *P. acnes* has been gradually altered, and varies greatly from one region to another. In China, antimicrobial resistance is generally a severe problem, but acne treatment with antibiotics (both topical and oral) is a common practice. Apart from one multicentre cross-sectional observational study,[14] little is known about antibiotic-resistant *P. acnes* among patients with acne in China. Therefore, this study aimed to evaluate the antibiotic susceptibility of *P. acnes* isolated from patients with acne in Southwest China. The resulting findings could help optimise therapeutic strategies for acne in Southwest China.

Methods patients

This was a prospective study. Patients with acne vulgaris attending the dermatology outpatient clinic of The First Affiliated Hospital of Kunming Medical University were consecutively enrolled between September 2015 and July 2017. Inclusion criteria were: (1) 12–50 years of age; and (2) mild-to-severe acne vulgaris.[15] Exclusion criteria were: (1) oral or topical antibiotic in the past month; (2) oral isotretinoin in the past 2 months and (3) other facial skin diseases. The washout period was the period considered necessary for the disappearance of the efficacy of topical or systemic treatment according to the half-life of drug: 1 month for topical antibiotics and 1 month for systemic treatment (at least 2 months for isotretinoin therapy). All patients matching the criteria during the study period were asked to participate in this study.

Basic clinical information (including age, gender, age of onset and duration of disease) were obtained at the time of patient enrolment or subsequently retrieved from consultation records.

Prior to the initiation of the study, informed consent was obtained from all patients. When patients were <18 years, informed consent was obtained from parents.

Specimen collection, culture and *P. acnes* identification

Acne lesions were squeezed using a comedo extractor, put into a 1.5 mL sterile anaerobic tube (MCT-150-C, Axygen, Corning, Tewkesbury, MA, USA) and sent to the Central Laboratory of Dermatology within half an hour. Six samples were taken from each patient. The samples were inoculated into brucellar blood agar medium supplemented with vitamin K and incubated anaerobically at 35°C for 7 days. *P. acnes* was identified using the VITEK2 system with the 21 348 VITEK 2 Corynebacterium identification card (BioMerieux, Marcy-L'Etoile, France). The pure strains of *P. acnes* were stored at −80°C.

Antibiotic susceptibility testing and MIC determination

Minimum inhibitory concentrations (MICs) were detected by the Epsilometer test (E-test) method using E-trips (AB Biodisk, Solna, Sweden). The E-strip is a plastic strip with the MIC interpretative on one side and a predefined antibiotic in gradient concentration (totally 29 concentrations, ranging 0.016–256 µg/mL) on the other side. A susceptibility test was performed on brucella agar using six E-strips (tetracycline, doxycycline, clindamycin, erythromycin, azithromycin and clarithromycin). All antibiotics were from BioMerieux (Marcy-L'Etoile, France), and incubations were performed at 37°C under anaerobic conditions.[16] The E-test MIC is defined as the point on the scale at which the ellipse of growth inhibition intercepts the strip. Data interpretation was performed according to the recommendations given by the Clinical and Laboratory Standards Institute (CLSI) and the National Committee for Clinical Laboratory Standards as susceptibility and resistance.[17] An MIC below the breakpoint value was defined as susceptibility. Breakpoints for the six antibiotics were tetracycline ≤4 µg/mL, doxycycline ≤4 µg/mL, clindamycin ≤2 µg/mL, erythromycin ≤0.5 µg/mL, azithromycin ≤0.5 µg/mL and clarithromycin ≤0.5 µg/mL.[18]

Relationship between MIC and patients' treatment history

We compared the MICs of different antibiotics between topical use and oral administrations. To further analyse the associations of MIC with various antibiotics and treatment history, the patients were divided into three groups, namely antibiotic (group 1), non-antibiotic (group 2; treatment with other medications or new treatments) and no previous therapy (group 3) groups. Other medications frequently used for treating acne included retinoids, traditional Chinese medicine (TCM) and benzoyl peroxide (BPO). The new treatments included intense pulsed light (IPL), blue and red light, photodynamic therapy (PDT), radiofrequency (RF) and alpha hydroxy acid (AHA).[19–23]

Statistical analysis

SPSS V.22.0 (IBM, Armonk, NY, USA) was used for statistical analysis. Continuous variables were expressed as median (IQR) and compared using the Mann-Whitney U test for two groups or the Kruskal-Wallis and the post hoc rank-sum test for more than two groups. Categorical variables were expressed as frequencies and percentages and compared using the χ^2 test. Two-sided p values<0.05 were considered statistically significant.

Patient and public involvement

Patients were not involved in the study design and implementation. Patients were informed of the study results via WeChat (a social network app in China) or phone calls.

RESULTS

Baseline characteristics and treatment history

Samples were taken from acne lesions of 375 patients with acne (224 women and 151 men). The patients were 12–46 years of age (mean, 22.3 years). Two hundred and twenty-seven strains of *P. acnes* were isolated from the collected

Table 1 Baseline characteristics of the patients with acne

Characteristics	Patients with acne (n=227)
Age (years)	
<25	156 (68.7)
>25	71 (31.3)
Gender	
Male	93 (41.0)
Female	134 (59.0)
Age at onset (years)	
<15	65 (28.6)
15–25	143 (63.0)
>25	19 (8.4)
Duration of disease (years)	
<2	46 (20.3)
>2	181 (79.7)
Disease severity	
Mild	33 (14.5)
Moderate	138 (60.8)
Severe	56 (24.7)
Antibiotic use	93 (41.0)
Topical use	26 (11.5)
Oral administration	67 (29.5)
Non-antibiotic use	53 (23.3)
Without previous therapy	81 (35.7)

Data were expressed as n (%).

samples, while 148 samples yielded no growth and were excluded. Among the 227 patients, 93 were administered antibiotics (group 1), including 67 and 26 and treated orally (group 1a) and topically (group 1b), respectively; 53 cases had no antibiotics but underwent other treatments (group 2), while 81 had no previous therapy (group 3) (table 1). Group 1 represented 63.7% (93/146) of all patients who had previous therapy (groups 1 and 2). The topical antibiotics used included clindamycin (n=19), erythromycin (n=5) and fusidic (n=5). The oral antibiotics employed included azithromycin (n=14), clarithromycin (n=23), minocycline (n=9), doxycycline (n=16), tetracycline (n=2) and roxithromycin (n=10). Other medications included retinoids (n=53), TCM (n=52) and BPO (n=39). New treatments were administered in 41 patients and included IPL, blue light, red light, RF and AHA. The exact treatment history is shown in table 2 and online supplementary table 1.

Antibiotic susceptibility

When comparing the various antibiotic susceptibilities of *P. acnes* isolated from patients with different antibiotic histories (table 3), most *P. acnes* isolates were susceptible to doxycycline and tetracycline. Patients in groups 2 and 3 showed similar results as cases in group 1; that is, *P. acnes* was highly susceptible to doxycycline (p=0.067) and tetracycline (p=0.664). *P. acnes* showed high resistance to other antibiotics, and this was significantly higher in patients in groups 1a and 1b in comparison with groups 2 and 3 (azithromycin, p=0.003; clarithromycin, p<0.001; clindamycin, p=0.001; erythromycin, p<0.001).

Table 2 Treatment history of antibiotics for oral administration (group 1a) and topical use (group 1b)

Profile	Group 1a (n=67)	Group 1b (n=26)
Subjects who received only a kind of antibiotic	19 (28.4)	10 (38.5)
Two kinds of antibiotic	7 (10.4)	3 (11.5)
One type of antibiotic use plus TCM products	15 (22.4)	6 (23.1)
One type of antibiotic use plus BPO	9 (13.4)	2 (7.7)
One type of antibiotic use plus retinoids	8 (11.9)	2 (7.7)
One type of antibiotic use plus physical treatments	3 (4.5)	3 (11.5)
One type of oral antibiotic plus one type of topical antibiotic	6 (9.0)	–

Data were expressed as n (%).
BPO, benzoyl peroxide; TCM, traditional Chinese herbal medicine.

P. acnes MIC differences of subjects in relation to various previous therapies

There were no obvious differences in MIC medians between groups 1a and 1b (oral and topical antibiotic groups) as shown in table 4. Compared with group 1, groups 2 and 3 showed lower levels of *P. acnes* MIC. Moreover, *P. acnes* MICs in group 2 were similar to those of group 3 (table 5).

DISCUSSION

In China, the common topical drugs for acne treatment include adapalene, BPO, clindamycin gel and fusidic acid cream. Adapalene and BPO easily cause skin irritation, for example, redness and burning, when used for the first time. Besides, due to the tense relationship between doctors and patients in China, many doctors prioritise clindamycin gel or fusidic acid cream, which show no obvious irritation in acne treatment. The aim of this study was to assess whether topical antibiotics would lead to antibiotic resistance. The results showed that both topical and oral antibiotics caused drug resistance, suggesting that while prescribing topical antibiotics for acne, dermatologists run the risk of promoting drug resistance.

As shown above, *P. acnes* was highly sensitive to cyclines (doxycycline and tetracycline). Meanwhile, *P. acnes* showed higher resistance rates to macrolides–lincosamides–streptogramins (MLS) antibiotics (such as erythromycin, azithromycin, clarithromycin and clindamycin).

Table 3 *P. acnes* susceptibility to antibiotics in each group

Antibiotics, n (%)	Group 1 (n=93)			Group 2 (n=53)	Group 3 (n=81)	P value
	Group 1a (n=67)	Group 1b (n=26)	Total (n=93)			
Azithromycin	18 (26.9)	8 (30.8)	26 (28.0)*†	27 (50.9)	41 (50.6)	0.003
Clarithromycin	17 (25.4)	9 (34.6)	26 (28.0)‡	30 (56.6)	46 (56.8)	<0.001
Clindamycin	21 (31.3)	7 (26.9)	28 (30.1)*†	29 (54.7)	44 (54.3)	0.001
Erythromycin	18 (26.9)	7 (26.9)	25 (26.9)*‡	27 (50.9)	44 (54.3)	<0.001
Doxycycline	65 (97.0)	25 (96.2)	90 (96.8)	53 (100)	81 (100)	0.067
Tetracycline	65 (97.0)	24 (92.3)	89 (95.7)	52 (98.1)	79 (97.5)	0.664

Data were expressed as n (%).
*P<0.05 versus group 2.
†P<0.05 versus group 3.
‡P<0.001 versus group 3.

P. acnes predominantly inhabits the pilosebaceous unit region. *P. acnes* induces the production of interleukin 1α and modulates the proliferation and differentiation of keratinocytes, as well as comedo formation.[24] *P. acnes* promotes the secretion of proinflammatory mediators by human keratinocytes, sebocytes and peripheral blood mononuclear cells via immune reactions.[25–27] Moreover, *P. acnes* have been involved in lipogenesis, thus exacerbating acne inflammation.[22 28] Such evidence suggests that *P. acnes* may play significant roles in the pathogenesis of acne.

Indeed, antibiotics targeting *P. acnes* have been a major approach of acne treatment for over half a century and are thought to work largely by inhibiting *P. acnes* colonisation, hence limiting inflammatory reactions. Currently, it is estimated that about 60% of all antibiotics prescribed by dermatologists target acne vulgaris.[29] Nearly 8% of all antibiotics prescribed are thought to be for dermatological indications in the UK.[30] In the USA, dermatologists prescribe almost 5% of all antibiotics, although they only account for ≤1% of the physician population.[31] Crucially, topical antibiotics are often used in the treatment of mild-to-moderate acne, and oral antibiotics tend to be used for this purpose as well.[6 32] Based on our experience, antibiotics are widely used in our region for acne treatment. Topical and oral antibiotics are conventionally used in the treatment of acne as the first choice. Of subjects who had received acne treatment, nearly 64% had previous antibiotic therapy, even sometimes with two kinds of antibiotics used simultaneously. It is noteworthy that the data regarding *P. acnes* susceptibility to antibiotics for the topical and oral antibiotic groups were close, highlighting that antibiotic resistance is as serious with topical antibiotics as oral ones. Therefore, dermatologists should be cautious when prescribing antibiotics, regardless of the method of administration.

As shown above, most *P. acnes* isolates were susceptible to doxycycline and tetracycline in all three patient groups. This phenomenon may be related to the widespread use of macrolide antibiotics (especially for respiratory system infections) and inducible resistance, suggesting that the antibacterial spectrum of macrolides antibiotics was gradually narrowed. Meanwhile, tetracycline and doxycycline are mainly used for acne, skin infections and sexually transmitted diseases, with a narrower range of use, resulting in lower resistance rate.

Antibiotic resistance is a global issue and 'antimicrobial resistance is a ticking time bomb for the UK and for the world'.[33] Overuse and misuse of antibiotics play an important role in the development of antibiotic resistance.[34] Therefore, an adequate and reasonable use of antibiotics would decrease antibiotic resistance. In fact, antibiotics are widely used in dermatology despite limited information on their usefulness for acne. Currently,

Table 4 Minimum inhibitory concentration (MIC) differences of various antibiotics for oral administration (group 1a) and topical use (group 1b) against *Propionibacterium acne* isolates

Antibiotics	Group 1a (n=67)	Group 1b (n=26)	P value
Azithromycin	0.032 (0.016–0.205)	0.032 (0.018–0.117)	0.652
Clarithromycin	0.047 (0.02–0.125)	0.047 (0.016–0.125)	0.912
Clindamycin	0.047 (0.023–0.125)	0.047 (0.023–0.5)	0.705
Erythromycin	0.032 (0.021–0.102)	0.032 (0.016–0.125)	0.950
Doxycycline	0.125 (0.047–0.38)	0.158 (0.06–0.283)	0.786
Tetracycline	0.25 (0.094–0.38)	0.315 (0.094–0.5)	0.266

The values were MIC (μg/mL) and were expressed as median (IQR).

Table 5 Minimum inhibitory concentration (MIC) differences of three groups

Antibiotics	Group 1 (n=93)	Group 2 (n=53)	Group 3 (n=81)	P value
Azithromycin	0.032 (0.016–0.19)	0.023 (0.016–0.032)*	0.023 (0.016–0.032)*	0.050
Clarithromycin	0.047 (0.016–0.125)	0.023 (0.016–0.032)†	0.023 (0.016–0.032)†	0.074
Clindamycin	0.047 (0.023–0.125)	0.023 (0.016–0.032)†	0.023 (0.016–0.043)†	0.021
Erythromycin	0.032 (0.02–0.11)	0.016 (0.016–0.047)*	0.020 (0.016–0.032)*	0.053
Doxycycline	0.125 (0.047–0.38)	0.047 (0.032–0.125)*	0.032 (0.032–0.125)*	<0.001
Tetracycline	0.250 (0.094–0.38)	0.064 (0.047–0.25)†	0.064 (0.047–0.25)†	<0.001

The values were MIC (μg/mL) and were expressed as median (IQR).
*P<0.05 versus group 1.
†P<0.001 versus group 1.

individuals with acne usually take prolonged courses (3–6 months) of a single antibiotic, leading to exposure at different concentrations and potential resistance.[35 36] It is necessary to realise that, indeed, antibiotics alleviate acne symptoms to some extent, but resistance, cross resistance and topical antibiotic failure are consequences of antibiotic use in treating acne.[6 12 37] It was reported that combination with retinoids or BPO therapy improves antibiotic resistance compared with antibiotics as single therapy.[38–41] Therefore, the Global Alliance to Improve Outcome in Acne Group recommended nine easy-to-follow points for limiting antimicrobial resistance of *P. acnes*: (1) combination of topical retinoid plus antimicrobial as first-line therapy; (2) antibiotics should not be used as monotherapy; (3) avoid the combination of oral and topical antibiotics; (4) concurrent use of BPO-containing products; (5) limit antibiotic use to short periods; discontinue when there is only slight improvement or no further improvement; (6) oral antibiotics should reasonably be used for 3 months; (7) do not switch antibiotics without adequate justification; (8) avoid antibiotics as maintenance therapy and (9) use topical retinoids for maintenance therapy, with BPO added when necessary.[6]

According to the aforementioned guidelines, the present observational study indicated that there is irrationality in antibiotic use for many patients with acne. For instance, antibiotics were used as monotherapy and concurrent use of oral and topical antibiotics was also reported. Clindamycin was the most common topical antibiotic received by the patients included in this study. Indeed, clindamycin monotherapy is on the low end of the acne efficacy spectrum, and evidence suggests that clindamycin shares similar effects as the vehicle.[42] Macrolides (roxythromycin, azithromycin and clarithromycin) are more frequently used than cyclines (minocycline, doxycycline and tetracycline). Actually, MLS antibiotics show higher resistance rates compared with cyclines.

With increased use of various antibiotics, the emergence of antibiotic resistance in *P. acnes* has gradually become a global problem. In the 1970s, *P. acnes* resistance to topical antibiotics was first reported in the USA. Since then, numerous studies about the antibiotic resistance of *P. acnes* and MICs of frequently used antibiotics have confirmed high resistance levels and higher MICs. In Spain, the prevalence of resistant strains to one antibiotic has been reported to be 94%.[37] In addition, a study in the UK showed definite increases of antibiotic resistant *P. acnes* strains from 34.5% in 1991 to 64% in 1997.[43] Recently, a Japanese study showed that *P. acnes* resistance to antibiotics increases with acne severity.[13] Another study provided evidence of associations of the development of antibiotic-resistant *P. acnes* with long duration of antibiotic treatment, long course of acne and elevated age.[12] In Korea, patients with a treatment history of topical or oral antibiotics show higher MICs to doxycycline compared with those without antibiotic administration.[44] Data from the only previous study in China about *P. acnes* resistance showed that macrolides and lincomycin face a serious resistance state.[14] The present cross-sectional study suggests that the use of antibiotics (topically and orally) may increase the odds of antibiotic resistance in *P. acnes*, elevating the MICs of antibiotics, especially MLS antibiotics, and promoting antibiotic-resistant strains. Importantly, we showed that other medicines or new treatments without antibiotics did not promote antibiotic resistance or alter MICs, with similar results to no treatment history for acne. Therefore, alternatives to antibiotics in the treatment of acne may not alter the antibiotic susceptibility of *P. acnes*.[44]

The present study was not without limitations. The sample size was relatively small, and all subjects were from a single centre. In addition, the cross-sectional design prevented the determination of any cause–effect relationship. Additional multicentre studies are necessary to examine adequately the issue of antibiotic resistance in *P. acnes*. Macrolides, lincomycin and tetracycline antibiotics can affect the rRNA subunit in bacteria. However, we could not generate relevant data; amplification and sequencing of relevant gene fragments involved in bacterial resistance should be performed in the future.

CONCLUSIONS

Overall, antibiotics have been used for the acne treatment for several decades, and the antibiotic resistance of *P. acnes* is a result of antibiotic use in the treatment of acne. *P. acnes* was highly sensitive to cyclines (doxycycline and

tetracycline). *P. acnes* showed higher resistance rates to MLS antibiotics (such as erythromycin, azithromycin, clarithromycin and clindamycin). The irrational use of antibiotics for acne treatment is probably a problem in China and elsewhere. These results suggest that dermatologists should be more cautious in prescribing antibiotics for acne. It is time to examine combination and alternative therapy to antibiotics. New devices (IPL/RF/PDT) are now widely accepted by patients with acne for safety, convenience and effectiveness. Future studies should examine such alternatives.

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Contributors WZ and QW carried out the studies, participated in collecting data and drafted the manuscript. DS, JL and YL performed the statistical analysis and participated in its design. TZ, LH and WW helped to draft the manuscript. All authors read and approved the final manuscript.

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Competing interests Not required.

Patient consent Obtained.

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Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.278cr0g. Encoding: 1=topical use; 2=oral administration; 3=no antibiotic use; 4=without previous therapy.

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