



# Antimicrobial Susceptibility, Biotypes and Phylotypes of Clinical *Cutibacterium* (Formerly *Propionibacterium*) *acnes* Strains Isolated from Acne Patients: An Observational Study

Nanxue Zhang · Ruoyue Yuan · Kevin Z. Xin · Zhong Lu · Ying Ma

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## ABSTRACT

**Introduction:** The aim of this study was to investigate the distribution of antimicrobial susceptibility, biotypes and phylotypes of clinical *Cutibacterium acnes* (*C. acnes*, formerly *Propionibacterium acnes*) isolates as well as the relationship among demographic factors, *C. acnes* biotypes and phylotypes.

**Methods:** *Cutibacterium acnes* was collected from the skin lesions of acne patients who visited the dermatologic department of Huashan Hospital in Shanghai from October 2016 to March 2017. The agar dilution method was conducted to determine the minimum inhibitory concentrations (MICs) of *C. acnes*, the fermentation test to identify biotypes and then

multiplex touchdown polymerase chain reaction (PCR) to identify phylotypes.

**Results:** Of the 63 *C. acnes* strains we isolated, 18 (28.6%), 31 (49.2%) and 4 (6.3%) strains were resistant to clindamycin, erythromycin and moxifloxacin, respectively; no strains were resistant to tetracycline, minocycline, fusidic acid or  $\beta$ -lactam, while metronidazole was completely resisted; 3 strains showed multidrug resistance (MDR). Biotype III (BIII) was the major biotype (50.8%) followed by BI and BV (both 15.9%), BII (12.7%) and lastly BIV (4.8%). IA<sub>1</sub> was the predominant phylotype (71.4%) followed by IA<sub>2</sub> (19.0%), II (4.8%), IB (3.2%) and IC (1.6%), while III was not detected. Significant differences were observed in the severity of disease: different degrees of acne severity reflected different biotype and phylotype distributions, and the biotype distribution of mild acne was different from that of moderate acne; the phylotype distribution of moderate acne varies from that of severe acne, too. Additionally, there was no significant difference in the distribution of biotypes or phylotypes between resistant and susceptible strains.

**Conclusion:** Erythromycin and clindamycin resistances are the most common in clinical *C. acnes* strains; BIII is the predominant biotype and IA<sub>1</sub> is the major phylotype of *C. acnes*, which are mainly related to disease severity.

Nanxue Zhang and Ruoyue Yuan contributed equally to this work.

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N. Zhang · R. Yuan · Z. Lu (✉) · Y. Ma (✉)  
Department of Dermatology, Huashan Hospital,  
Fudan University, Shanghai, China  
e-mail: luzhong20100806@qq.com

Y. Ma  
e-mail: alle\_ma@163.com

K. Z. Xin  
Johns Hopkins University School of Medicine,  
733 N Broadway, Baltimore, MD, USA

**Keywords:** Acne; Antibiotic resistance; Antimicrobial susceptibility; Biotype; *Cutibacterium acnes*; Phylotype

## INTRODUCTION

Acne is a common chronic inflammatory skin disease in both adolescents and adults that mainly involves epidermis and pilosebaceous units [1]. The pathogenesis of acne is complicated, and the colonization of *Cutibacterium acnes* (*C. acnes*, formerly *Propionibacterium acnes*, which was recently proposed for reclassification as *Cutibacterium acnes* [2]), is considered a crucial factor throughout the whole development of acne. *C. acnes* promotes the abnormal proliferation and differentiation of keratinocytes and increases sebum production. In addition, *C. acnes* can lead to the overexpression of Toll-like receptors (TLRs), protease-activated receptors (PARs) and matrix metalloproteinase (MMP) by keratinocytes, all of which can further activate the innate immune system [3, 4]. As the main therapy of acne, topical and oral antibiotics against *C. acnes* have been used for decades [5]. However, the increasing number of strains of drug-resistant *C. acnes* due to antibiotic abuse in acne treatment has aroused wide concerns [6]. Based on the fact that different strains determine different pathogenicity and resistance, classification of *C. acnes* is of great significance [7]. According to previous studies, we can make use of fermentation tests for biotyping [8] while using multiplex touchdown polymerase chain reaction (PCR) for genotyping [9]. So far, there have been few reports on the biotype and phylotype classification of clinical *C. acnes* isolates, and little is known about the biologic and phylogenetic typing of drug-resistant *C. acnes*. Therefore, in this study, we investigated the antimicrobial susceptibility and biotypes and phylotypes of clinical *C. acnes* isolates as well as the relationship between demographic factors and *C. acnes* biotypes and phylotypes.

## METHODS

### Patients

Patients > 18 years of age with grade I–IV facial acne according to the Pillsbury scale were included in the present study. Patients who suffered from scar constitution; unable to

cooperate or unwilling to sign informed consent; with severe medical complications and pregnant women were excluded from the study. This study was approved by the Ethics Committee of Huashan Hospital of Fudan University. Informed consent was obtained from all individual participants included in the study.

### Isolation and Identification of *C. acnes*

Samples were isolated from the lesions of acne patients who visited the Dermatologic Department of Huashan Hospital in Shanghai, China, from October 2016 to March 2017. After being sterilized with iodine tincture, the acne lesions (comedones, papules and pustules) were compressed with a comedone extractor. Then the contents were transported immediately with a sample smear and inoculated on Brucella agar (Becton-Dickinson, USA) containing 5% (v/v) lysed defibrinated sheep's blood (Zhuzhai Blood Reagent Supply Station, Shanghai), supplemented with vitamin K<sub>1</sub> (Sigma-Aldrich, USA) and incubated in an anaerobic chamber (Ruskinn Invivo2 400 Hypoxia workstation, 10% CO<sub>2</sub>, 10% H<sub>2</sub>, 80% N<sub>2</sub>) at 37 °C for 48–72 h. The cultured microorganisms were identified as *C. acnes* with an API-20A system (bioMérieux, France). The bacteria were then placed in 40% glycerin broth and stored at –70 °C. The standard *C. acnes* strain (ATCC 6919) was purchased from the Guangdong Institute of Microbiology, China.

### Antimicrobial Agents

Metronidazole, clindamycin, erythromycin, fusidic acid, moxifloxacin, ceftriaxone, cefoxitin, minocycline, tetracycline, ampicillin, sulbactam and imipenem were purchased from the China National Institutes for Food and Drug Control. Ampicillin and sulbactam were mixed at a 2:1 ratio according to recommendations of the Clinical and Laboratory Standards Institute (CLSI).

### Determination of Susceptibility to Antibiotics of *C. acnes*

Agar dilution assays were employed to measure the minimum inhibitory concentrations (MICs)

of clinical *C. acnes* isolates. All the antimicrobials were prepared in concentrations ranging from 0.06 to 128 µg/ml with a two-fold serial dilution. A standard inoculum of  $10^5$  colony-forming units per 1 µl was also prepared and delivered by a multipoint inoculator (Denley A40, Denley Ltd., Billingshurst, Sussex, UK). The MIC, defined as the lowest concentration with no visible bacterial growth, was determined after incubation under anaerobic conditions at 35 °C for 48 h. A standard strain (ATCC 6919) was used as a quality control strain. The breakpoints for the antimicrobial agents were defined based on CLSI 2016 M100-S23 criteria except for fusidic acid, which was based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2016 criteria.

### Statistical Analysis

Data were analyzed using SPSS version 24.0. The distribution of biotypes and phylotypes of *Cutibacterium acnes*, according to different clinical characteristics, and the distribution differences of biotypes and phylotypes between resistant and susceptible strains were analyzed with Fisher's exact test.  $P < 0.05$  was considered statistically significant. For the relationship between disease severity and *C. acnes* biotypes and phylotypes, the Bonferroni test for post hoc comparisons was performed.  $P < 0.0167$  was considered statistically significant.

### Grading of Acne Severity

Acne severity was classified into four grades according to the Pillsbury scale: grade I ( $\leq 30$  eruptions, mainly comedones with a small number of papules and pustules), grade II (31–50 eruptions, comedones with an intermediate amount of papules and pustules), grade III (51–100 extensively distributed eruptions, many papules and pustules with occasional large inflammatory lesions, less than 3 nodules) and grade IV ( $> 100$  eruptions,  $> 3$  nodules or cysts). Grades I and IV were defined as mild and severe acne, respectively, while grades II and III were defined as moderate acne.

### Fermentation Tests

Determination of the biotype of *C. acnes* was performed based on previously reported procedures [10]. Both basal medium (pH 7.0) and carbohydrate medium (basal medium plus 1 g of carbohydrate per dl) were inoculated with Pasteur pipettes. Of the carbohydrates used, deoxyribose, ribose and xylose were sterilized by filtration and added to autoclaved basal media. Incubation was performed at 37 °C for 7 days. The pH values of the cultures and uninoculated media were measured. Then, the "sugar-specific pH" (SpH) for each carbohydrate was calculated by the equation  $SpH = (A - B) - (C - D)$ , where *A*, *B*, *C* and *D* are the pH of the carbohydrate medium, inoculated carbohydrate medium, basal medium and inoculated basal medium, respectively.  $SpH \geq 0.35$  was interpreted as fermentation positive and  $SpH < 0.35$  as fermentation negative. Ribose (+), erythritol (+) and sorbitol (+) strains were defined as biotype I (BI); ribose (+), erythritol (+) and sorbitol (–) strains as biotype II (BII); ribose (+), erythritol (–) and sorbitol (+) strains as biotype III (BIII); ribose (+), erythritol (–) and sorbitol (–) strains as biotype IV (BIV); ribose (–), erythritol (–) and sorbitol (–) strains as biotype V (BV).

### Multiplex Touchdown PCR

Multiplex touchdown PCR was employed according to the report of Barnard et al. [11], which can identify phylotypes of *C. acnes* isolates in a single reaction based on its pattern of reaction with six primer sets that target the 16S rRNA gene (all isolates), ATPase (types IA<sub>1</sub>, IA<sub>2</sub> and IC), sodA (types IA<sub>2</sub> and IB), atpD (type II), recA (type III) housekeeping genes and a Fic family toxin gene (type IC). Bacterial genomic DNA was prepared with TaKaRa Taq. PCR amplification was carried out using longGene L96G. The samples contained 10 × PCR buffer 2 µl, primers set mix 2 µl, dNTP mix 2 µl, rTaq 0.3 µl, DNA templates 2 µl and ddH<sub>2</sub>O 11.7 µl in a total volume of 20 µl. The samples were initially heated at 94 °C for 1 min followed by 14 cycles consisting of 94 °C for 30 s, 66 °C

(decreasing incrementally by 0.3 °C per cycle) for 30 s and 72 °C for 1 min, followed by 11 cycles at 94 °C for 30 s, 62 °C for 30 s and 72 °C for 1 min, culminating with a final cycle at 72 °C for 10 min. A negative control (PCR water) and six positive control samples representing all phylotypes were included in all experiments. The PCR products were analyzed by electrophoresis on 1.5% (wt/vol) agarose gels.

## RESULTS

### Culture and Identification of *C. acnes*

Samples were collected from 100 patients with acne, and 69 *Cutibacterium* strains were identified. Of these strains, *C. acnes* was found in 63 patients and *Cutibacterium granulosum* in 6 patients.

### MICs of *C. acnes*

The MIC range of clindamycin and erythromycin for *C. acnes* was  $\leq 0.06$  to  $> 128$   $\mu\text{g}/\text{ml}$

ml. Among the isolated *C. acnes* strains, 58.7% (37/63) were susceptible to clindamycin and 28.6% (18/63) were resistant to clindamycin; 50.8% (32/63) were susceptible to erythromycin and 49.2% (31/63) were resistant to erythromycin. The MIC range of moxifloxacin was  $\leq 0.06$  to  $> 2$   $\mu\text{g}/\text{ml}$ , and 6.3% (4/63) were resistant to moxifloxacin. In addition, three *C. acnes* strains were simultaneously resistant to clindamycin, erythromycin and moxifloxacin, hence exhibiting multidrug resistance (MDR). The MIC range was 0.125–1  $\mu\text{g}/\text{ml}$  for tetracycline and 0.06–1  $\mu\text{g}/\text{ml}$  for minocycline. No strains were resistant to tetracycline or minocycline. The MIC range was 0.25–2  $\mu\text{g}/\text{ml}$  for fusidic acid. Fusidic acid showed good antimicrobial susceptibility against most *C. acnes* strains (93.7% were susceptible and 6.3% showed moderate sensitivity), and resistance was not observed. In vitro,  $\beta$ -lactam antibiotics demonstrated favorable antibiotic activities, while metronidazole was completely resisted (Table 1).

**Table 1** Susceptibility and resistance rates (%) of antibiotics against *Cutibacterium acnes*

Antibiotics	Breakpoints ( $\mu\text{g}/\text{ml}$ )		Number	%R	%I	%S	MIC <sub>50</sub> ( $\mu\text{g}/\text{ml}$ )	MIC <sub>90</sub> ( $\mu\text{g}/\text{ml}$ )	MIC range ( $\mu\text{g}/\text{ml}$ )
	S	R							
Ampicillin	$\leq 0.5$	$\geq 2$	63	0	0	100	$\leq 0.06$	0.25	0.06 to 0.25
Ampicillin/sulbactam	$\leq 8$	$\geq 32$	63	0	0	100	$\leq 0.06$	0.125	0.06 to 0.25
Ceftriaxone	$\leq 16$	$\geq 64$	63	0	0	100	$\leq 0.06$	0.125	0.06 to 0.25
Cefoxitin	$\leq 16$	$\geq 64$	63	0	0	100	0.25	0.5	0.125 to 2
Imipenem	$\leq 4$	$\geq 16$	63	0	0	100	$\leq 0.06$	$\leq 0.06$	0.06 to 0.06
Moxifloxacin	$\leq 0.5$	$\geq 2$	63	4.8	0	95.2	0.25	$\leq 0.25$	0.06 to 2
Clindamycin	$\leq 2$	$\geq 8$	63	28.6	12.7	58.7	0.5	$> 128$	0.06 to $> 128$
Erythromycin	$\leq 0.5$	$\geq 8$	63	49.2	0	50.8	$> 128$	$> 128$	0.06 to $> 128$
Fusidic acid	$\leq 1$	$\geq 4$	63	0	6.3	93.7	0.5	1	0.25 to 2
Minocycline	$\leq 4$	$\geq 16$	63	0	0	100	$\leq 0.06$	0.125	0.06 to 1
Tetracycline	$\leq 4$	$\geq 16$	63	0	0	100	0.25	0.25	0.125 to 1
Metronidazole	$\leq 8$	$\geq 32$	63	100	0	0	$> 128$	$> 128$	$> 128$ to $> 128$

R resistance, I intermediate, S susceptibility

### Clinical Characteristics and Biotypes of *C. acnes*

All patients were grouped according to family history, age, gender, disease duration, disease severity and previous treatment. Of these patients, 57.1% were male and 42.9% female. The mean age was  $22.4 \pm 4.5$  years, and the mean course of disease was  $13.1 \pm 1.1$  months. According to the Pillsbury grading system, grade I was found in 5 patients, grade II in 16, grade III in 23 and grade IV in 19. The numbers of patients treated with antibiotics or retinoids were: oral antibiotics ( $n = 31$ ), topical antibiotics ( $n = 28$ ), oral isotretinoin ( $n = 14$ ) and topical retinoids ( $n = 29$ ). BIII was the major biotype (50.8%; 32/63), BI and BV each accounted for 15.9% (10/63), BII accounted for 12.7% (8/63), and BIV accounted for only 4.8% (3/63). The distribution of BIII had little correlation with all but only one disease demographic factor: disease severity. The biotype distribution was significantly different among four degrees of acne severity ( $P = 0.048 < 0.05$ ), while Bonferroni correction showed that there were no significant statistical differences in six pairwise comparisons ( $P > 0.0083$ ). When we classified acne severity as three degrees including mild, moderate and severe, Fisher's exact test showed that the biotype distribution of *C. acnes* was significantly different among the three disease degrees ( $P = 0.031 < 0.05$ ), and Bonferroni correction suggested that statistical difference was only found between mild and moderate acne ( $P = 0.016 < 0.0167$ ). The standard *C. acnes* strain (ATCC 6919) was biotyped as BIII (Table 2).

### Clinical Characteristics and Phylotypes of *C. acnes*

IA<sub>1</sub> was the predominant phylotype (71.4%, 45/63), followed by IA<sub>2</sub> (19.0%, 12/63), II (4.8%, 3/63), IB (3.2%, 2/63) and IC (1.6%, 1/63), while III was not detected. In accordance with the biotype distribution, disease severity was the only disease demographic factor that had correlation with the distribution of IA<sub>1</sub>. The phylotype distribution was significantly different

among the four degrees of acne severity ( $P = 0.033 < 0.05$ ), while Bonferroni correction showed that there were no significant statistical differences in six pairwise comparisons ( $P > 0.0083$ ). When acne severity was classified as three degrees including mild, moderate and severe, Fisher's exact test showed that the phylotype distribution of *C. acnes* was significantly different among the three disease degrees ( $P = 0.015 < 0.05$ ), and Bonferroni correction suggested that statistical difference was only found between moderate and severe acne ( $P = 0.011 < 0.0167$ ). The standard *C. acnes* strain (ATCC 6919) was determined as IA<sub>1</sub> (Table 3).

### Distribution of Biotypes of Antibiotic-Resistant and -Susceptible *C. acnes*

The overwhelming majority of *C. acnes* biotypes were found to have antibiotic-resistant and -susceptible subtypes. The only exception was biotype I, which lacked a moxifloxacin-resistant subtype. In addition, among the three MDR strains, BIII, BII and BV were found, respectively. Consequently, BIII was the dominant biotype in both antibiotic-resistant and -susceptible *C. acnes* strains, and there was no significant difference in the distribution of biotypes between resistant and susceptible strains ( $P > 0.05$ ) (Table 4).

### Distribution of Phylotypes of Antibiotic-Resistant and -Susceptible *C. acnes*

All phylotypes were found to have antibiotic-susceptible subtypes except the undetected phylotype III. Clindamycin- and moxifloxacin-resistant subtypes were only observed in phylotype IA<sub>1</sub> and IA<sub>2</sub>, while erythromycin-resistant subtypes also in the phylotype IB and II division. Moreover, the three MDR *C. acnes* isolates were identified to be two IA<sub>1</sub> and one IA<sub>2</sub> strains. There was no significant difference in the distribution of phylotypes between resistant and susceptible strains ( $P > 0.05$ ) (Table 5).



**Table 2** Distribution of biotypes of *Cutibacterium acnes* according to different clinical characteristics

	Distribution of <i>C. acnes</i> biotype					<i>P</i> value
	BI	BII	BIII	BIV	BV	
Family history						
Yes ( <i>n</i> = 39)	6	5	18	2	8	0.814
No ( <i>n</i> = 24)	4	3	14	1	2	
Age (years)						
< 25 ( <i>n</i> = 44)	6	7	22	2	7	0.814
≥ 25 ( <i>n</i> = 19)	4	1	10	1	3	
Gender						
F ( <i>n</i> = 27)	7	4	10	1	5	0.247
M ( <i>n</i> = 36)	3	4	22	2	5	
Disease duration						
< 2 years ( <i>n</i> = 50)	8	5	25	2	10	0.270
≥ 2 years ( <i>n</i> = 13)	2	3	7	1	0	
Disease severity						
I ( <i>n</i> = 5)	1	2	0	0	2	0.048*
II ( <i>n</i> = 16)	5	0	7	2	2	
III ( <i>n</i> = 23)	3	2	14	1	3	0.016**
IV ( <i>n</i> = 19)	1	4	11	0	3	
Previous treatment						
Oral antibiotics ( <i>n</i> = 31)	5	5	17	1	3	0.998
Topical antibiotics ( <i>n</i> = 28)	3	5	15	2	3	
Oral isotretinoin ( <i>n</i> = 14)	1	2	7	1	3	
Topical retinoids ( <i>n</i> = 29)	3	5	16	2	3	
Total	10 (15.9%)	8 (12.7%)	32 (50.8%)	3 (4.8%)	10 (15.9%)	

\*Significant at 0.05 level, by Fisher's exact test

\*\*Comparison of *C. acnes* biotype distribution between mild and moderate acne by Bonferroni correction, significant at 0.0167 level

## DISCUSSION

*Cutibacterium acnes* is an anaerobic gram-positive bacillus and exists as a skin commensal in the human skin microbiota [12]. As the predominant bacterium in sebaceous sites, *C. acnes* is confirmed as the most important acne-inducing pathogen. Antibiotic therapy against *C.*

*acnes* has remained the major acne therapy for years. However, antibiotic abuse has led to increased levels of resistance, decreased *C. acnes* sensitivity to treatment and ultimately treatment failure [13].

The first resistant *C. acnes* strain was reported by Leyden in 1976 [14], and the resistance rate of *C. acnes* has increased from 20% in 1978 to

**Table 3** Distribution of phlotypes of *Cutibacterium acnes* according to different clinical characteristics

Clinical characteristics	Distribution of <i>C. acnes</i> phlotypes						P value
	IA <sub>1</sub>	IA <sub>2</sub>	IB	IC	II	III	
Family history							
Yes ( <i>n</i> = 39)	25	9	2	0	3	0	0.187 (NS)
No ( <i>n</i> = 24)	20	3	0	1	0	0	
Age (years)							
< 25 ( <i>n</i> = 44)	31	9	1	1	2	0	0.922 (NS)
≥ 25 ( <i>n</i> = 19)	14	3	1	0	1	0	
Gender							
F ( <i>n</i> = 27)	17	8	0	1	1	0	0.150 (NS)
M ( <i>n</i> = 36)	28	4	2	0	2	0	
Disease duration							
< 2 years ( <i>n</i> = 50)	25	9	2	1	3	0	0.945 (NS)
≥ 2 years ( <i>n</i> = 13)	10	3	0	0	0	0	
Disease degree							
I ( <i>n</i> = 5)	3	1	0	1	0	0	0.033*
II ( <i>n</i> = 16)	8	7	1	0	0	0	
III ( <i>n</i> = 23)	20	2	0	0	1	0	0.011**
IV ( <i>n</i> = 19)	14	2	1	0	2	0	
Previous treatment							
Oral antibiotics ( <i>n</i> = 31)	21	7	1	0	2	0	1.000 (NS)
Topical antibiotics ( <i>n</i> = 28)	18	6	1	0	3	0	
Oral isotretinoin ( <i>n</i> = 14)	10	3	0	0	1	0	
Topical retinoids ( <i>n</i> = 29)	19	6	1	0	3	0	
Total	45 (71.4%)	12 (19.0%)	2 (3.2%)	1 (1.6%)	3 (4.8%)	0	

NS not significant

\*Significant at 0.05 level, by Fisher's exact test

\*\*Comparison of *C. acnes* phylotype distribution between moderate and severe acne by Bonferroni correction, significant at 0.0167 level

62% in 1996 [15]. Many countries have reported that > 50% of *C. acnes* strains are resistant to antibiotics, especially to macrolides [6]. In general, resistance to erythromycin and clindamycin are the most common, while tetracycline remains relatively susceptible. Studies show high resistance rates of erythromycin and

clindamycin in Europe, such as > 70% in France and even 91% in Spain, while tetracycline resistance ranges from 0% to 26.4% [16, 17]. Likewise, there is great variability among resistance rates in different Asian countries. A Japanese study by Nakase et al. [18] reported 23.2% resistance to erythromycin, 18.8% to

**Table 4** Distribution of biotypes of antibiotic-resistant and -susceptible *Cutibacterium acnes*

	Distribution of <i>C. acnes</i> biotype					<i>P</i> value
	I	II	III	IV	V	
Erythromycin						
R ( <i>n</i> = 31)	2	6	15	2	6	0.163 (NS)
S ( <i>n</i> = 32)	8	2	17	1	4	
Clindamycin						
R ( <i>n</i> = 18)	1	3	10	1	3	0.914 (NS)
I ( <i>n</i> = 8)	1	1	4	–	2	
S ( <i>n</i> = 37)	8	4	18	2	5	
Moxifloxacin						
R ( <i>n</i> = 4)	–	1	2	–	1	0.750 (NS)
S ( <i>n</i> = 59)	10	7	30	3	9	
MDR						
<i>n</i> = 3	–	1	1	–	1	

Fisher's exact test, significant at 0.05 level

R resistant, I intermediate, S susceptible, MDR multidrug-resistant, NS not significant

clindamycin, 4.3% to doxycycline and 0% to minocycline. However, in Hong Kong, a recent study found clindamycin resistance the most common (53.5%), followed by erythromycin (20.9%) and tetracyclines (16.3%) [19]. The different resistance rates between countries may be associated with different prescribing patterns and usage limitations. Current research also found that the presence of *C. acnes* resistance was related to age, duration and severity of disease [18, 19]. The relationship between resistance and history of antibiotic use remains controversial. Previous antibiotic treatment does not seem to be a premise for the presence of resistance because resistant *C. acnes* have also been found in patients without antibiotic therapy, which might be due to contact-mediated spread of resistant *C. acnes* [17, 20].

Our results suggest that erythromycin resistance (49.2%) is the most prevalent in clinical

**Table 5** Distribution of phlotypes of antibiotic-resistant and -susceptible *Cutibacterium acnes*

	Distribution of <i>C. acnes</i> phlotype						<i>P</i> value
	IA <sub>1</sub>	IA <sub>2</sub>	IB	IC	II	III	
Erythromycin							
R ( <i>n</i> = 31)	22	6	1	–	2	–	1.000 (NS)
S ( <i>n</i> = 32)	23	6	1	1	1	–	
Clindamycin							
R ( <i>n</i> = 18)	14	4	–	–	–	–	0.640 (NS)
I ( <i>n</i> = 8)	5	1	1	–	1	–	
S ( <i>n</i> = 37)	26	7	1	1	2	–	
Moxifloxacin							
R ( <i>n</i> = 4)	3	1	–	–	–	–	1.000 (NS)
S ( <i>n</i> = 59)	42	11	2	1	3	–	
MDR							
<i>n</i> = 3	2	1	–	–	–	–	

Fisher's exact test, significant at 0.05 level

R resistant, I intermediate, S susceptible, MDR multidrug-resistant, NS not significant

*C. acnes* isolates, followed by clindamycin (28.6%), which is consistent with the overall trend throughout the world. As the two most frequently prescribed topical antibiotics, erythromycin and clindamycin have been used in acne treatment for decades. However, it has been reported that the efficacy of topical erythromycin reduced from 70% in 1975 to 25% in 2000 with widespread usage [21], which led to a decreased therapeutic effect and its infrequent use in acne treatment now [22]. Cyclines are mostly used for moderate to severe acne and remain effective against *C. acnes*, which is demonstrated in this study since no strains of minocycline- or tetracycline-resistant *C. acnes* were observed. Quinolones are used for acne in some regions [23], but it is not recommended to use these potent antibiotics in acne because they should be reserved for serious conditions such as infections by methicillin-resistant



*Staphylococcus aureus* [24]. Fusidic acid is an antibiotic targeting gram-positive cocci and has been proven to have antibacterial and immunomodulatory effects [25]. In spite of the rare emergence of resistance and good antimicrobial susceptibility of fusidic acid, the application of fusidic acid in acne treatment is considered overused and is still disputed [26]. Moreover, it should be noted that three moxifloxacin-resistant *C. acnes* strains also showed resistance to both erythromycin and clindamycin, indicating multidrug resistance (MDR), which is defined as non-susceptibility to at least one agent in three or more antimicrobial categories [27]. Current research indicates that overexpression of efflux pumps may be an important mechanism of MDR [28]. However, the role of the efflux pump in multidrug-resistant *C. acnes* remains unknown. On the whole, dermatologists should strictly follow the application principle and specification of antibiotics in treating acne to prevent the rise of drug-resistant strains.

As early as 1972, Johnson and Cummins [29] classified *C. acnes* into serotypes I and II according to the findings from the agglutination test. In addition, they found serotype I had galactose as a cell wall sugar, while serotype II lacked galactose. Then, in 1979, Kishishita et al. [10] compared the biochemical and serologic characteristics of 128 *C. acnes* strains from healthy volunteers with 3 standard strains (ATCC 6919, 11827 and 11828). They classified *C. acnes* into five biotypes (BI to BV) according to the fermentation tests of ribose, erythritol and sorbitol. Several studies [8, 30] have indicated that BIII is the predominant biotype of *C. acnes*, probably due to its higher lipase activity than other biotypes. Higaki et al. [8] investigated the relationship between the severity of acne and *C. acnes* biotype, finding that BIII was the major biotype of *C. acnes* in all levels of acne severity but most commonly isolated from severe acne lesions. However, the sample sizes in these studies are generally small, which is inadequate for accurate conclusions. In our study, among the 63 isolated *C. acnes* strains, BIII was the most common biotype (50.8%), consistent with previous studies. BIII was also the dominant biotype in severe (57.9%) and

moderate acne (53.8%). It is speculated that *C. acnes* of BIII has some specific characteristics, such as production of free fatty acids, which may contribute to its growth and proliferation in severe acne lesions [8]. Additionally, our results suggested that different degrees of acne severity reflected different biotype distributions, and the biotype distribution of mild acne was different from that of moderate acne.

Multilocus sequence typing (MLST) of *C. acnes* currently represents the clear gold standard for accurate phylotype identification, but its expensive, time-consuming and technically undemanding characteristics restrict the application. Consequently, Barnard et al. [11] developed multiplex touchdown PCR as a rapid, precise, high-throughput typing method for phylogenetic classification. It can identify the six highly distinct evolutionary phylotypes of *C. acnes* in a single reaction, known as IA<sub>1</sub>, IA<sub>2</sub>, IB, IC, II and III, which display differences in inflammatory properties, production of virulence determinants and association with various conditions [31–33]. We utilized this technique, and our study revealed that IA<sub>1</sub> was the predominant phylotype of skin *C. acnes* isolates, consistent with former researches [34–36]. Neuraminidase and dermatan-sulfate binding adhesins DsA1 and DsA2 were assumed to be virulence factors of type IA division on account of its exclusive association with acne and the recurrent nature of the disease [9, 37].

Furthermore, we studied the biotype and phylotype distribution of resistant and susceptible *C. acnes*. Biotype BIII and phylotype IA<sub>1</sub> took the lead in both groups. BIII occupied the main biotype status of antibiotic-susceptible and -resistant *C. acnes*, including erythromycin (53.1% vs. 48.4%), clindamycin (48.6% vs. 55.6%) and moxifloxacin (50.9% vs. 50.0%). IA<sub>1</sub> is also the major phylotype of isolated *C. acnes* strains susceptible and resistant to erythromycin (71.9% vs. 71.0%), clindamycin (70.3% vs. 77.8%) and moxifloxacin (71.2% vs. 75.0%). The three MDR strains were classified into the biotype BII, BIII and BV group each and two strains into IA<sub>1</sub> as well as one strain into the IA<sub>2</sub> group of phylotypes. Among the different approaches utilized by *C. acnes* to confer resistance to antibiotics, specific point mutations in the rRNA operon

represent a major mechanism. McDowell et al. [35] conducted MLST analysis of 40 antibiotic-resistant *C. acnes* strains and found 95% strains had at least one specific point mutation in the rRNA operon. Besides, 85% of the resistant isolates belonged to the type IA<sub>1</sub> group. Nevertheless, it is difficult to draw any definitive conclusions on the significance of these data until a wider prospective study is conducted and a greater number of isolates are analyzed. In our study, there was no significant difference in the distribution of biotypes or phylotypes between susceptible and resistant *C. acnes*.

## CONCLUSIONS

Increases in *C. acnes* resistance have become a crucial issue in the treatment of acne. Resistance to erythromycin and clindamycin is common, while cyclines still have good antimicrobial susceptibility. BIII group and IA1 group are the major biotype and phylotype of *C. acnes*, respectively, and the predominant types in moderate and severe acne as well. Different degrees of acne severity also reflect different biotype and phylotype distributions. Our findings may provide evidence for clinical studies about drug resistance and help elucidate the biologic and phylogenetic characteristics of *C. acnes*.

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**Data Availability.** The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

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