

## Brief Original Article

# ***In vitro* antimicrobial susceptibility patterns of *Propionibacterium acnes* isolated from patients with acne vulgaris**

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### Abstract

**Introduction:** *Propionibacterium acnes* has been implicated in the development of acne vulgaris. Rampant use of topical and systemic antibiotics for acne vulgaris has led to resistance due to selective pressure. This study aimed to determine antibiotic resistance of *P. acnes*.

**Methodology:** A total of 102 samples were collected from acne lesions and cultured onto sheep's blood agar and brain-heart infusion agar supplemented with 5 g/L glucose and 2 mg/L furazolidone) (BHlg) under aerobic and anaerobic conditions. Species identification was done by conventional methods and the VITEK2 Compact system. The isolates were tested for penicillin, erythromycin, clindamycin, ciprofloxacin, nadifloxacin, and tetracycline by E-test, and minimum inhibitory concentration (MIC) of minocycline was determined by agar dilution on BHlg. MIC results were interpreted as per EUCAST (European Committee on Antimicrobial Susceptibility Testing) and CLSI (Clinical Laboratory Standards Institute) guidelines.

**Results:** *P. acnes* was the most common anaerobe (66%) isolated. Resistance rates using EUCAST and CLSI breakpoints were 10.6% and 6.1%, 7.6% and 0%, 7.8% and 0% for erythromycin, clindamycin, and minocycline, respectively. Tetracycline resistance was observed in 9.2% isolates irrespective of the interpretative criteria used. MIC<sub>50</sub> and MIC<sub>90</sub> values for nadifloxacin (0.25 and 1 µg/mL) were found to be twofold lower than those for ciprofloxacin (0.5 and 1 µg/mL). Similarly, MIC<sub>50</sub> and MIC<sub>90</sub> values for minocycline (0.125 and 0.5 µg/mL) were also two- to threefold lower than those for tetracycline (0.38 and 1 µg/mL).

**Conclusions:** To the best of our knowledge, this is the first study focusing on *P. acnes* resistance from India.

**Key words:** *Propionibacterium acnes*; acne vulgaris; EUCAST; CLSI.

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### Introduction

Acne vulgaris is a chronic inflammatory disorder of pilosebaceous follicles that affects more than 85% of adolescents and young adults [1]. It is characterized by a pleomorphic eruption of comedones, erythematous papules, pustules, and sometimes nodules, frequently followed by scarring [2]. Acne is not an infectious disease, but organisms residing on the surface of skin and pilosebaceous ducts may trigger infection. These organisms include *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*. Pathogenesis of acne is a complex interplay of inflammation, hyperkeratinisation of the sebaceous duct, high sensitivity to circulating androgens, and bacterial colonization [2]. Topical antibiotics such as erythromycin, clindamycin, and tetracycline are routinely used for long-term treatment of acne vulgaris,

which exerts considerable selective pressure for the development of drug resistance [3]. This study was undertaken to examine the bacteriological profile of acne vulgaris and to ascertain its antimicrobial resistance patterns.

### Methodology

A cross-sectional study was undertaken in the outpatient department of dermatology, Safdarjang Hospital, over a period of two years between 2010 and 2012. A total of 102 patients with acne vulgaris were included after informed consent was obtained. The study was approved by ethical committee of Safdarjang hospital (reference 52-11-EC [17/17]). Detailed history and clinical examination was carried out with reference to Pillsbury grading [4].

### Skin sampling

Samples were collected from acne lesions (38 comedones, 18 papules, and 44 pustules) by aseptic techniques using a comedone extractor. In the case of closed comedones (whiteheads), papules, and pustules, the lesion was punctured with a sterile hypodermic needle (25 × 35 mm) using aseptic precautions. All specimens were subjected to aerobic and anaerobic culture.

### Bacteriological study

The specimens were inoculated onto 5% sheep's blood agar, MacConkey agar, and brain-heart infusion agar (HiMedia, Mumbai, India) supplemented with 5 g/L glucose and 2 mg/L furazolidone. Plates were

incubated at 37°C under both aerobic and anaerobic conditions for 2–7 days and examined for growth. Anaerobic culture was performed using the Gaspak system (HiMedia Labs., Mumbai, India).

### Identification

Aerobic and anaerobic bacteria were identified by Gram stain, colony morphology, and standard biochemical tests [5]. *P. acnes* strains were presumptively identified as Gram-positive bacilli grown anaerobically with positive indole, catalase, and nitrate reduction tests. Final identification was confirmed by the automated VITEK2 Compact (Biomérieux, Marcy l'Etoile, France) system.

**Table 1.** Clinical and bacteriological profile of patients.

Profile	No. of patients (%) n = 102
Age at inclusion	
Mean/median	18.74/19
Min/max	11/29
Gender	
Male	63 (63%)
Female	37 (37%)
Grades of acne	
Grade 1	32 (32%)
Grade 2	26 (26%)
Grade 3	30 (30%)
Grade 4	12 (12%)
Family history of acne	
No	56 (56%)
Yes	44 (44%)
Type of lesion sampled	
Comedones	38 (38%)
Pustule	44 (44%)
Papule	18 (18%)
Bacteriological profile	
Aerobes	
<i>Staphylococcus aureus</i>	65 (65%)
<i>Staphylococcus epidermidis</i>	5 (5%)
<i>Klebsiella pneumoniae</i>	4 (4%)
<i>Escherichia coli</i>	2 (2%)
<i>Citrobacter freundii</i>	1 (1%)
<i>Enterobacter aerogenes</i>	1 (1%)
Anaerobes	
<i>Propionibacterium acnes</i>	66 (66%)
<i>Propionibacterium granulosum</i>	2 (2%)
<i>Propionibacterium propionicus</i>	1 (1%)
<i>Clostridium</i> sp.	1 (1%)
Mixed growth	
<i>Propionibacterium acnes</i> + <i>Staphylococcus aureus</i>	39 (39%)
<i>Propionibacterium acnes</i> + <i>Staphylococcus epidermidis</i>	3 (3%)
<i>Clostridium</i> sp. + <i>Enterococcus</i> sp.	1 (1%)
<i>Klebsiella pneumoniae</i> + <i>Staphylococcus aureus</i>	2 (2%)
<i>Escherichia coli</i> + <i>Citrobacter</i> sp.	1 (1%)

**Antibiotic susceptibility**

Antibiotic susceptibility of aerobic isolates was performed on Mueller-Hinton agar (Oxoid Ltd., Basingstoke, England) per Clinical and Laboratory Standards Institute (CLSI) 2011 guidelines [6]. For *P. acnes*, minimum inhibitory concentration (MIC) for erythromycin, clindamycin, ciprofloxacin, and tetracycline were determined by the E-test (AB Biodisk, Solna, Sweden). MICs of minocycline and nadifloxacin (Cipla Industries, Mumbai, India) were determined by agar dilution on BHIg using spot inoculation. Inocula were prepared to a 0.5 MacFarland standard from a 48-hour growth on anaerobic blood agar. MIC results were interpreted per European Committee on Antimicrobial Susceptibility Testing EUCAST [7] and CLSI guidelines [6].

**Results**

The demographic and clinical details of acne patients are shown in Table 1. All the patients were between 11 and 29 years of age, with a mean age of 18.7 years. The majority of patients were between 18 and 19 years of age (27%), with a predominance of males (63%). The patients were also categorized according to Pillsbury grading [4]. Thirty-two percent of the patients belonged to grade 1, while 26%, 30%, and 12%

belonged to grades 2, 3, and 4, respectively. Forty-four percent of patients had a family history of acne, and 14% had history of previous anti-acne treatment.

The bacteriological profiles of the acne patients are shown in Table 1. Of 102 samples processed, 78% samples showed aerobic growth and 70% showed anaerobic growth. *S. aureus* (65; 65%) was the predominant aerobe, followed by *S. epidermidis* (5; 5%) and *Klebsiella pneumoniae* (4; 4%). Among the anaerobes, *Propionibacterium* species were isolated in 69 samples, with *P. acnes* as the predominant species (66; 66%); *Clostridium* sp. was isolated from one patient.

**Antibiotic susceptibility**

Among 65 *S. aureus* strains isolated, highest resistance was seen with penicillin (45; 70.2%), followed by erythromycin (38; 59%), clindamycin (25; 39.7%), gentamicin (20; 30.3%), tetracycline (38; 25%), ciprofloxacin (11; 17.9%), and oxacillin (13%; 20.4%). *P. acnes* also showed resistance to penicillin (4; 6.7%), erythromycin (7; 10.6%), clindamycin (4; 6.1%), tetracycline (6; 9.2%), and ciprofloxacin (2; 3%). No resistance was observed to minocycline (0; 0%).

**Table 2.** Clinical breakpoints of *P. acnes* using EUCAST (2003) and CLSI (2007) guidelines.

Antimicrobial	EUCAST clinical breakpoint	CLSI clinical breakpoint
Penicillin	> 0.5 µg/mL	≥ 2 µg/mL
Clindamycin	≥ 0.25 µg/mL	≥ 8 µg/mL
Erythromycin	≥ 0.5 µg/mL	≥ 2 µg/mL
Tetracycline	≥ 2 µg/mL	≥ 16 µg/mL
Minocycline	ND	≥ 16 mcg/mL
Nadifloxacin	ND	ND
Ciprofloxacin	ND	≥ 8 mcg/mL

EUCAST: European Committee on Antimicrobial Susceptibility Testing; CLSI: Clinical and Laboratory Standards Institute; ND: not defined.

**Table 3.** Minimum inhibitory concentrations (MICs) of antibiotics against 66 *P. acnes* isolates.

Antimicrobia l	≤ 0.0 3	0.0 47	0.0 6	0.1 25	0.2 5	0.3 8	0.5	1	2	4	8	16	32	64	128	≥ 256	512	MI C <sub>50</sub>	MIC <sub>90</sub>	EUCAST (% R)	CLSI (% R)
Penicillin	38	0	24	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0.0 3	0.06	ND	4 (6.1%)
Clindamycin	0	28	4	27	2	0	2	2	0	1	0	0	0	0	0	0	0	0.1 25	0.25	7 (10.6%)	0
Erythromycin	30	0	28	3	0	0	1	0	0	0	0	0	0	0	0	0	4	0.0 6	0.25	5 (7.6%)	4 (6.1%)
Tetracycline	0	0	2	11	17	25	3	2	0	0	0	0	5	1	0	0	0	0.3 8	1.0	6 (9.2%)	6 (9.2%)
Minocycline	0	0	29	15	11	0	6	0	1	4	0	0	0	0	0	0	0	0.1 25	0.5	ND	0
Ciprofloxacin	0	0	4	10	11	17	11	5	4	2	0	0	0	2	0	0	0	0.5	2.0	ND	2 (3.0%)
Nadifloxacin	0	0	16	17	19	-	10	3	1	0	0	0	0	0	0	0	0	0.2 5	1.0	ND	ND

EUCAST: European Committee on Antimicrobial Susceptibility Testing; CLSI: Clinical and Laboratory Standards Institute; R: resistant; ND: not defined.

The clinical breakpoints for *P. acnes* proposed by CLSI and EUCAST are shown in Table 2. Clinical breakpoints have not been proposed for minocycline and ciprofloxacin by EUCAST. No breakpoint values are available for nadifloxacin in either EUCAST or CLSI. MIC<sub>50</sub>, MIC<sub>90</sub>, and MIC range of all the antibiotics are shown in Table 3. All the antibiotics showed bimodal distribution of resistance against *P. acnes*.

The penicillins showed strong activity against *P. acnes* (MIC range: 0.03–32 µg/mL), and the majority of the isolates (38/66; 58%) had MICs lower than the lowest antibiotic concentration on the E-test strip. Based on EUCAST guidelines, only 6.7% of *P. acnes* strains were resistant to penicillin. Topical antibiotics such as clindamycin and erythromycin were active against the majority of *P. acnes* strains, and resistance was observed in 10.6% and 7.6% (EUCAST), and 0% and 6.1% (CLSI) of strains, respectively. No inducible clindamycin resistance was observed in these strains.

The majority of the strains were sensitive to ciprofloxacin (MIC < 0.5 µg/mL). Low-level resistance was seen in 11 strains (MIC: 0.5–4 µg/mL), and two strains (3.0%) showed high-level resistance (MIC > 8 µg/mL) (CLSI). Nadifloxacin, a topical fluoroquinolone, demonstrated high activity against *P.*

*acnes* (MICs ≤ 0.5 µg/mL) for the majority of the isolates (62/66; 94%) except three strains with reduced susceptibilities (MIC > 1 µg/mL) (same strains that were resistant to ciprofloxacin) and one strain with MIC > 2 µg/mL. MIC<sub>50</sub> and MIC<sub>90</sub> values for nadifloxacin (0.25 and 1 µg/mL, respectively) were twofold lower than those of ciprofloxacin (0.5 and 2 µg/mL, respectively). As no breakpoint values have been proposed by both CLSI and EUCAST for nadifloxacin, their resistance values could not be determined. Tetracycline showed similar resistance when interpreted by EUCAST and CLSI (9.2%); no resistance was observed with minocycline when interpreted using CLSI guidelines. MIC<sub>50</sub> and MIC<sub>90</sub> (0.125 and 0.25 µg/mL, respectively) of minocycline were also two- to threefold lower than those of tetracycline (0.38 and 1 µg/mL, respectively).

Multidrug resistance (resistance to three or more classes of drugs) was observed in 9/66 (13.63%) of the strains.

**Table 4.** Minimum inhibitory concentration (MIC) range and percentage of resistance of *P. acnes* isolated from countries across the world.

Country, year (no. of isolates)	Ref guidelines	MIC (µg/mL)											
		Erythromycin		Clindamycin		Tetracycline		Minocycline		Ciprofloxacin		Nadifloxacin	
		Range	% R	Range	% R	Range	% R	Range	% R	Range	% R	Range	% R
Japan, 2014 (n = 69)	CLSI	0.06–256	23.2	0.06–256	18.8	-	4.3	0.06–8	0	0.25–32	4.3	0.25–16	-
Colombia, 2013 (n = 100)	CLSI	-	35	-	15	-	8	-	1	-	-	-	-
Chile, 2013 (n = 80)	CLSI	0.125–8	12.5	0.125–8	7.5	0.25–2	0	-	-	-	-	-	-
Japan, 2006-07 (n = 50)	CLSI	0.063–256	10.4	0.063–256	8.3	-	-	0.125–0.5	0	0.5–2	0	0.125–1	-
Japan, 2008 (n = 43)	CLSI	0.063–256	20.9	0.063v256	18.9	-	-	0.25–16	2.3	0.125–8	0	0.063–4	-
Mexico, 2010 (n = 49)	CLSI	0.03–256	46	0.03–256	36	0.5–256	14	0.125–8	0	0.125–16	4	-	-
Japan, 2008 (n = 48)	CLSI	0.063–256	10.4	0.031–256	8.3	0.5–4	4	0.125–4	0	0.5–2	0	0.125–1	-
Chile, 2006 (n = 53)	CLSI	0.03–32	3.8	0.03–32	1.9	0.03–8	1.9	0.03–1	0	-	-	0.03–0.12	-
Korea, 2011 (n = 31)	EUCAST	0.016–0.125	0	0.016–0.25	3.2	0.094–0.38	0	0.023–0.5	0	-	-	-	-
Hong Kong, 2011 (n = 86)	EUCAST	0.03–128	20.9	0.06–12	53.5	0.5–32	16.3	0.12–8	16.3	-	-	-	-
Europe, USA, Japan, Australia, 2001 (n = 73)	Not Specified	0.03v512	-	0.03–64	-	0.125-64	-	0.06-16	-	-	-	0.06-0.25	-
Current study, India (n = 66)	EUCAST	0.03–512	7.6	0.047–4	10.6	0.06-64	9.2	0.06-4	-	0.06-64	-	0.06-8	-
	CLSI	-	6.1	-	0	-	9.2	-	0	-	3	-	-

R: resistant; CLSI: Clinical and Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing.

## Discussion

Cutaneous *Propionibacterium* has been implicated in acne, although their role in inflammation is still poorly understood. There is widespread resistance in *P. acnes* due to overuse of topical and systemic antibiotics for treatment of acne vulgaris [3,8-16], as shown in Table 4. Various studies have used different interpretative criteria to estimate the resistance among *P. acnes* strains to different anti-acne drugs. There is a paucity of data on antibiotic resistance among these isolates in the literature; also, no standard interpretative criterion is available for estimating the resistance among anti-acne drugs in *P. acnes*. Normally, drug susceptibility is not requisitioned, 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. At the same time, if facilities that are equipped to perform anaerobic culture are available, the samples should be sent there to get the exact sensitivity pattern for the particular patient, because based on the findings of the study, a fair amount of resistance was observed in the causative organisms.

Our results confirm that *P. acnes* (66%) and *S. aureus* (65%) predominated the bacterial flora in acne vulgaris patients. This is in contrast to a study done in France by Dreno *et al.*, where among 16 different organisms isolated from acne patients, *S. epidermidis* (95%) and *P. acnes* (90%) were predominant, followed by *S. capitis* (47.5%), *Micrococcus* (47.5%), and *P. granulosum* (32.5%) [17]. Another study done in Iran by Zandi *et al.* showed a predominance of *P. acnes* (57%), followed by *S. epidermidis* (32%) and *S. aureus* (5%) [18]. This difference in microbial profile in our study could be explained by the variations in geographical location, host factors, and antibiotic usage, as has been described previously.

In the present study, it was observed that resistance among *P. acnes* to anti-acne drugs was lower than that among *S. aureus*. The resistance among *S. aureus* was two- to sevenfold higher than *P. acnes*, with *S. aureus* versus *P. acnes* for: penicillin (70.2% versus 6.1%), ciprofloxacin (17.9% versus 3%), erythromycin (59% versus 7.6%), clindamycin (39.7% versus 10.6%), and tetracycline (25% versus 9.2%). These findings stress that anti-acne antibiotics continue to maintain activity against *P. acnes*. Lower resistance may be due to the fact that the majority of patients do not undergo treatment of acne in India.

Previously, researchers have used either EUCAST or CLSI guidelines to analyze the resistance profile of *P. acnes*. In present study, resistance rates of erythromycin and clindamycin were higher when analyzed according to EUCAST guidelines (7.6% and 10.6%, respectively), as compared to CLSI (6% and 0%, respectively). Tetracycline resistance was observed in 9.2% of isolates, irrespective of the interpretative criteria used. MIC breakpoints for interpretation are lower in EUCAST guidelines than in CLSI guidelines. From a clinical and epidemiological point of view, EUCAST guidelines are better for analyzing the resistance data than are CLSI guidelines. CLSI guidelines have been used by researchers for drugs where EUCAST data is not available.

In the present study, it was observed that the highest number of isolates (38) showed minimum MIC values of 0.03 µg/mL to penicillin, followed by erythromycin and clindamycin. The MIC range of penicillin was 0.03–0.06 µg/mL, although only four strains had MIC > 32 µg/mL. Penicillin is not a very good anti-acne antibiotic; however, as it is not available in topical preparations, and has various adverse effects, namely penicillin allergy and anaphylaxis. Among topical antibiotics, erythromycin (0.03–512 µg/mL) and clindamycin (0.047–4 µg/mL) showed very low resistance.

MIC<sub>50</sub> and MIC<sub>90</sub> values of nadifloxacin (0.25 and 1 µg/mL) were found to be twofold lower than those of ciprofloxacin (0.5 and 2 µg/mL); thus, nadifloxacin emerged as a better drug for management of acne patients. MIC<sub>50</sub> and MIC<sub>90</sub> of minocycline (0.125 and 0.5 µg/mL) was also found to be two- to threefold lower than those of tetracycline (0.38 and 1 µg/mL). Our findings are in sync with studies across the world showing no resistance to *P. acnes* against minocycline.

We searched for relevant studies indexed in the PubMed, Medline, and Google databases for articles with words "*Propionibacterium*" and "*Propionibacterium acnes* India". Based on available literature and to the best of our knowledge, this is the first study from India focusing on the bacteriology of acne vulgaris and *P. acnes* resistance.

## Conclusions

Antibiotic resistance in acne vulgaris has gradually risen over the years, making it difficult to treat these patients. This is corroborated by evidence of reduced clinical response to antibiotic therapy, potential increase in pathogenicity of *P. acnes*, and transfer of resistance to more pathogenic organisms. Effective strategies to combat antibiotic resistance in acne are



required and include judicious and limited duration of antibiotic usage and the use of topical retinoids in lieu of antibiotics.

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