INTERNATIONAL JOURNAL OF CLINICAL, MEDICAL AND HEALTH SCIENCES
ISSN: Applied For
Frequency: Quarterly
Website: http://ijcmhs.com/

Original Research Article

Association of Acne Severity with Antimicrobial Resistance and Biofilm Formation of *Cutibacterium acnes* Among Patients Attending a Tertiary Care Centre: A Cross-Sectional Study

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OPEN ACCESS

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Received: 27-03-2025
Accepted: 30-04-2025
Published: 19-05-2025

ABSTRACT

Background: Acne vulgaris is a common chronic inflammatory disorder of the pilosebaceous unit in which *Cutibacterium acnes* contributes to follicular inflammation. Antimicrobial resistance and biofilm formation may contribute to bacterial persistence and influence therapeutic response. Understanding the relationship between clinical severity and microbiological characteristics may assist in optimizing antimicrobial use in acne management.
Objective: To evaluate the association of acne severity with antimicrobial resistance and biofilm-forming capacity of *C. acnes* among patients with acne vulgaris attending a tertiary care centre.
Methods: A hospital-based cross-sectional observational study was conducted over 12 months among 150 patients with clinically diagnosed acne vulgaris attending the Department of Dermatology and Venereology at a tertiary care centre. Clinical severity was categorized as mild, moderate or severe using a predefined clinical grading system. Appropriate lesion specimens were collected aseptically and processed for anaerobic culture. Confirmed *C. acnes* isolates were subjected to antimicrobial susceptibility testing against clindamycin, erythromycin, azithromycin, doxycycline, tetracycline and minocycline. Biofilm-forming capacity was assessed using a microtiter-plate crystal-violet assay.
Results: Among the 150 patients, 68 (45.3%) yielded *C. acnes*. Mild, moderate and severe acne were present in 43 (28.7%), 71 (47.3%) and 36 (24.0%) patients, respectively. Among the 68 *C. acnes* isolates, resistance was highest to clindamycin [32 (47.1%)], followed by azithromycin [25 (36.8%)] and erythromycin [17 (25.0%)]. Resistance to doxycycline, minocycline and tetracycline was observed in 14 (20.6%), 9 (13.2%) and 8 (11.8%) isolates, respectively. Strong biofilm formation was observed in 34 (50.0%) isolates, moderate formation in 23 (33.8%) and non/weak formation in 11 (16.2%). Resistance to at least one tested antimicrobial was observed in 12 (63.2%) mild, 21 (77.8%) moderate and 19 (86.4%) severe cases; however, the association between acne severity and any antimicrobial resistance was not statistically significant (p=0.213). Previous antibiotic exposure was significantly associated with antimicrobial resistance (p=0.018).
Conclusion: The study demonstrates a substantial burden of antimicrobial resistance and biofilm formation among *C. acnes* isolates obtained from patients with acne vulgaris. Although antimicrobial resistance showed a numerical increase with increasing acne severity, the association was not statistically significant. The findings emphasize the importance of local antimicrobial susceptibility surveillance, rational antibiotic use and consideration of microbiological characteristics when investigating persistent or treatment-resistant acne.

Keywords: Acne vulgaris; *Cutibacterium acnes*; antimicrobial resistance; antibiotic susceptibility; biofilm.

INTRODUCTION

Acne vulgaris is a chronic inflammatory disorder of the pilosebaceous unit and is one of the most frequently encountered dermatological conditions worldwide. The disease is characterized by a combination of follicular hyperkeratinization,

increased sebaceous gland activity, alterations in the cutaneous microbial environment and inflammatory responses. Although *Cutibacterium acnes* is a normal component of the skin microbiota, specific strains and host–microbe interactions have been associated with acne-associated inflammation [1–3].

The microbiological characteristics of *C. acnes* have become increasingly important in the management of acne. Antibiotics, particularly topical and systemic agents, remain useful for selected patients with inflammatory acne. However, repeated or prolonged antibiotic exposure can exert selective pressure and contribute to the emergence of resistant *C. acnes* strains. Macrolide and lincosamide resistance has been reported in several populations, whereas tetracycline-class resistance is generally lower but varies according to geographical region and antimicrobial exposure [4–6].

Antimicrobial resistance is clinically relevant because it may reduce the effectiveness of commonly prescribed therapies and may contribute to persistent inflammatory disease. Contemporary acne-management guidelines therefore emphasize appropriate antibiotic stewardship, avoidance of unnecessary prolonged antibiotic monotherapy and combination treatment strategies that reduce the risk of resistance [4].

In addition to antimicrobial resistance, biofilm formation has been proposed as a potentially important characteristic of *C. acnes*. Biofilms consist of microbial communities attached to a surface and embedded within an extracellular matrix. In-vitro studies have demonstrated the ability of *C. acnes* to form biofilms and have suggested that biofilm-associated organisms may demonstrate altered susceptibility to antimicrobial agents [7]. Biofilm formation may therefore represent one mechanism contributing to persistence of the organism and treatment difficulties.

The relationship between clinical acne severity, antimicrobial resistance and biofilm formation remains an important area of investigation. Assessing these parameters together may provide a more comprehensive understanding of the microbiological factors associated with acne severity and previous antibiotic exposure.

The present study was undertaken to evaluate the association between acne severity and microbiological characteristics of *C. acnes*, with particular emphasis on antimicrobial resistance and biofilm-forming capacity, among patients attending a tertiary care centre.

MATERIALS AND METHODS

Study design and setting

A hospital-based cross-sectional observational study was conducted over a period of 12 months from jan 2024 dec 2024 in the Department of Dermatology and Venereology at a tertiary care centre.

Study population and sample size

The study included 150 consecutive patients with clinically diagnosed acne vulgaris who attended the dermatology outpatient service during the study period.

Inclusion criteria

Patients with clinically diagnosed acne vulgaris who consented to participate in the study were included.

Exclusion criteria

Patients with conditions likely to interfere with microbiological assessment, those receiving treatment that precluded appropriate specimen collection, and patients from whom an adequate specimen could not be obtained were excluded according to the study protocol.

Clinical assessment

A structured proforma was used to record demographic and clinical characteristics, including age, sex, duration of acne, previous antibiotic exposure, previous topical antibiotic use, previous systemic antibiotic use, truncal involvement and acne scarring.

Acne severity was categorized as mild, moderate or severe according to a predefined clinical grading system. The same grading criteria were applied consistently throughout the study.

Specimen collection and microbiological assessment

Appropriate specimens were collected from representative acne lesions using aseptic precautions. Specimens were transported promptly under conditions suitable for anaerobic bacterial recovery.

Samples were inoculated onto appropriate culture media and incubated under anaerobic conditions according to standard microbiological procedures. Presumptive *C. acnes* isolates were identified using colony morphology and conventional biochemical characteristics. Where available, species confirmation was performed using a validated identification method.

Antimicrobial susceptibility testing

Confirmed *C. acnes* isolates were evaluated for susceptibility to clindamycin, erythromycin, azithromycin, doxycycline, tetracycline and minocycline.

Susceptibility testing was performed using the laboratory-validated method applicable to *C. acnes*. Susceptibility categories were interpreted according to the relevant contemporary breakpoint criteria and laboratory quality-control procedures.

Biofilm formation assay
Biofilm formation was evaluated using a standardized microtiter-plate crystal-violet assay.

Briefly, standardized bacterial suspensions were inoculated into sterile microtiter wells and incubated under appropriate conditions. Following incubation, planktonic organisms were removed and the wells were washed to remove non-adherent cells. Adherent biofilm biomass was stained with crystal violet. After removal of excess stain and appropriate solubilization, optical density was measured using a microplate reader.

Biofilm production was classified as non/weak, moderate or strong according to predefined optical-density thresholds established for the assay.

Statistical analysis
Data were entered into a structured database and analyzed statistically.

Categorical variables were expressed as frequencies and percentages. Continuous variables were summarized using mean and standard deviation where appropriate.

Associations between categorical variables were evaluated using the chi-square test or Fisher's exact test depending on expected cell frequencies. A two-sided p-value <0.05 was considered statistically significant.

For analysis of antimicrobial resistance, “any antimicrobial resistance” was defined as resistance to at least one of the six tested antibiotics.

RESULTS

Demographic and clinical characteristics
A total of 150 patients with acne vulgaris were included. The mean age of the study population was 23.4 ± 5.1 years. There were 77 (51.3%) male and 73 (48.7%) female participants.

Moderate acne was the most frequent severity category, accounting for 71 (47.3%) patients, followed by mild acne in 43 (28.7%) and severe acne in 36 (24.0%).

Previous antibiotic exposure was reported in 59 (39.3%) patients. Previous topical antibiotic use was reported in 44 (29.3%), while 30 (20.0%) had a history of systemic antibiotic use. Truncal involvement was present in 51 (34.0%) patients, and acne scarring was documented in 45 (30.0%).

Table 1. Demographic and clinical characteristics of the study population

Variable	Category	n	%
Sex	Male	77	51.3
	Female	73	48.7
Acne severity	Mild	43	28.7
	Moderate	71	47.3
	Severe	36	24.0
Previous antibiotic exposure	Yes	59	39.3
	No	91	60.7
Previous topical antibiotic use	Yes	44	29.3
	No	106	70.7
Previous systemic antibiotic use	Yes	30	20.0
	No	120	80.0
Truncal involvement	Yes	51	34.0
	No	99	66.0
Acne scarring	Yes	45	30.0
	No	105	70.0

Distribution of acne severity
Of the 150 participants, 43 (28.7%) had mild acne, 71 (47.3%) had moderate acne and 36 (24.0%) had severe acne.

Table 2. Distribution of acne severity

Acne severity	n	%
Mild	43	28.7
Moderate	71	47.3
Severe	36	24.0

Total	**150**	**100.0**

C. acnes culture status according to acne severity

C. acnes was isolated from 68 of the 150 participants, giving an overall culture positivity rate of 45.3%.

Among mild cases, 19 of 43 (44.2%) were culture positive. Among moderate cases, 27 of 71 (38.0%) were culture positive, while 22 of 36 (61.1%) severe cases yielded *C. acnes*.

The association between acne severity and *C. acnes* culture status was not statistically significant (χ^2=5.168, p=0.075).

Table 3. *C. acnes* culture status according to acne severity

Acne severity	Total	Culture positive, n (%)	Culture negative, n (%)
Mild	43	19 (44.2)	24 (55.8)
Moderate	71	27 (38.0)	44 (62.0)
Severe	36	22 (61.1)	14 (38.9)
Total	**150**	**68 (45.3)**	**82 (54.7)**

Antimicrobial resistance among *C. acnes* isolates

Among the 68 culture-positive isolates, clindamycin resistance was the most frequently observed resistance phenotype, occurring in 32 (47.1%) isolates. Azithromycin resistance was observed in 25 (36.8%) isolates, while erythromycin resistance was observed in 17 (25.0%).

Doxycycline resistance was identified in 14 (20.6%) isolates, minocycline resistance in 9 (13.2%) and tetracycline resistance in 8 (11.8%).

Table 4. Antimicrobial resistance among *C. acnes* isolates

Antibiotic	Isolates tested	Resistant, n	Resistance (%)
Clindamycin	68	32	47.1
Erythromycin	68	17	25.0
Azithromycin	68	25	36.8
Doxycycline	68	14	20.6
Tetracycline	68	8	11.8
Minocycline	68	9	13.2

Biofilm-forming phenotype

Of the 68 *C. acnes* isolates, 34 (50.0%) demonstrated strong biofilm formation, 23 (33.8%) demonstrated moderate biofilm formation and 11 (16.2%) were classified as non/weak biofilm producers.

Table 5. Biofilm-forming phenotype of *C. acnes* isolates

Biofilm category	n	%
Non/weak	11	16.2
Moderate	23	33.8
Strong	34	50.0
Total	**68**	**100.0**

Any antimicrobial resistance according to acne severity

Resistance to at least one of the six tested antimicrobial agents was observed in 52 (76.5%) of the 68 isolates.

The proportion of isolates demonstrating any antimicrobial resistance was 63.2% among mild cases, 77.8% among moderate cases and 86.4% among severe cases.

Although the proportion of resistance increased with increasing acne severity, the association was not statistically significant (χ^2=3.094, p=0.213).

Table 6. Any antimicrobial resistance according to acne severity

Acne severity	*C. acnes* isolates	Any resistance, n (%)	No resistance, n (%)
Mild	19	12 (63.2)	7 (36.8)
Moderate	27	21 (77.8)	6 (22.2)
Severe	22	19 (86.4)	3 (13.6)
Total	**68**	**52 (76.5)**	**16 (23.5)**

Biofilm formation and antimicrobial resistance
Any antimicrobial resistance was identified in 7 (63.6%) non/weak biofilm producers, 15 (65.2%) moderate biofilm producers and 30 (88.2%) strong biofilm producers.

Although resistance was numerically more frequent among strong biofilm producers, the association between biofilm category and any antimicrobial resistance was not statistically significant (χ^2=5.241, p=0.073).

Table 7. Biofilm phenotype according to antimicrobial resistance

Biofilm category	Isolates	Any resistance, n (%)	No resistance, n (%)
Non/weak	11	7 (63.6)	4 (36.4)
Moderate	23	15 (65.2)	8 (34.8)
Strong	34	30 (88.2)	4 (11.8)
Total	**68**	**52 (76.5)**	**16 (23.5)**

Previous antibiotic exposure and antimicrobial resistance
Previous antibiotic exposure was significantly associated with the presence of antimicrobial resistance among *C. acnes* isolates (p=0.018).

This finding indicates that prior exposure to antibiotics may be an important factor associated with recovery of resistant *C. acnes* strains. The effect estimate should be reported with its 95% confidence interval after calculation from the original 2×2 dataset.

DISCUSSION
The present study evaluated the association of clinical acne severity with *C. acnes* recovery, antimicrobial resistance and biofilm formation among patients attending a tertiary care centre. The study provides an integrated clinical and microbiological assessment of acne, with particular emphasis on characteristics that may influence persistence and treatment response.

C. acnes was recovered from 68 (45.3%) of the 150 patients. Culture positivity was highest among patients with severe acne (61.1%), compared with 44.2% among mild and 38.0% among moderate cases. However, the association between clinical severity and culture status was not statistically significant. This suggests that recovery of *C. acnes* alone may not adequately explain differences in clinical severity and supports the concept that acne pathogenesis involves multiple interacting host and microbial factors.

Antimicrobial resistance was common among the isolates examined. Clindamycin demonstrated the highest resistance rate (47.1%), followed by azithromycin (36.8%) and erythromycin (25.0%). Resistance to doxycycline, minocycline and tetracycline was comparatively lower.

The predominance of clindamycin and macrolide resistance is consistent with the broader literature describing increased resistance to frequently used topical and systemic antibiotic classes in *C. acnes*. Resistance rates, however, differ considerably among geographical regions and populations. A recent systematic review and meta-analysis demonstrated substantial variation in resistance rates among *C. acnes* isolates, emphasizing the importance of local surveillance rather than relying solely on international estimates [5,6].

The findings also have clinical relevance because clindamycin and macrolides have historically been used extensively in acne management. Current acne guidelines emphasize antimicrobial stewardship and recommend limiting unnecessary antibiotic exposure [4]. The relatively high resistance observed in the present study reinforces the importance of appropriate antibiotic selection and avoidance of prolonged antimicrobial therapy without clinical justification.

Biofilm formation was another important finding. Half of the isolates were classified as strong biofilm producers, while approximately one-third demonstrated moderate biofilm formation. Biofilm-associated growth has been investigated as a potential mechanism for persistence of *C. acnes*. Coenye et al. demonstrated that *P. acnes* can form biofilms and reported an association between biofilm formation and altered antimicrobial susceptibility [7].

In the present study, antimicrobial resistance was more frequent among strong biofilm producers than among non/weak or moderate biofilm producers. However, the association did not reach statistical significance (p=0.073). This finding should therefore be interpreted as a numerical pattern rather than evidence of a statistically established relationship.

The lack of statistical significance may reflect the relatively small number of culture-positive isolates available for subgroup analysis. Only 68 isolates were available for antimicrobial resistance and biofilm analyses, reducing the statistical power to detect moderate associations.

Previous antibiotic exposure showed a statistically significant association with antimicrobial resistance (p=0.018). This finding is biologically plausible because prior antimicrobial exposure may exert selective pressure favouring resistant

organisms. However, the relationship may also be influenced by treatment history, disease severity, duration of disease and other clinical factors.

A recent Indian study investigating *C. acnes* resistance and biofilm formation similarly highlights the relevance of evaluating these microbiological characteristics in patients with acne [8]. Differences in sample size, patient selection, antimicrobial testing methodology and resistance definitions should nevertheless be considered when comparing findings between studies.

The relationship between antimicrobial resistance and biofilm formation deserves further investigation. Biofilm-associated organisms may have altered antimicrobial susceptibility because of reduced metabolic activity, extracellular matrix effects and limited penetration of antimicrobial agents. Nevertheless, an in-vitro biofilm assay cannot fully reproduce the complex microenvironment of human pilosebaceous follicles.

The present study also demonstrates that increasing acne severity was accompanied by a numerical increase in the proportion of isolates showing resistance to at least one antimicrobial agent, from 63.2% in mild disease to 86.4% in severe disease. However, the difference was not statistically significant. Therefore, the findings do not establish an independent association between acne severity and antimicrobial resistance.

Strengths of the Study

1. The study combines clinical dermatological assessment with microbiological investigation.
2. A defined cohort of 150 patients was evaluated over a 12-month period.
3. Multiple clinically relevant antimicrobial agents were assessed.
4. Both antimicrobial resistance and biofilm-forming capacity were evaluated in *C. acnes* isolates.
5. Previous antibiotic exposure was included as an important clinical variable.
6. The study examines microbiological findings in relation to clinical acne severity rather than reporting resistance patterns alone.

LIMITATIONS

1. The study was conducted at a single tertiary care centre, which may limit generalizability to other populations.
2. The cross-sectional design prevents determination of temporal or causal relationships.
3. Only 68 *C. acnes* isolates were available for antimicrobial resistance and biofilm analyses, limiting statistical power for subgroup comparisons.
4. Culture recovery may be influenced by lesion selection, specimen collection, transport and anaerobic culture conditions.
5. In-vitro biofilm formation may not completely reflect biofilm behavior within human pilosebaceous follicles.
6. Previous antibiotic exposure was based on clinical history and may be affected by recall or documentation limitations.
7. Larger multicentre studies with longitudinal follow-up are required to determine whether antimicrobial resistance and biofilm formation predict persistence, recurrence or treatment failure.

CONCLUSION

The present study demonstrates that antimicrobial resistance and biofilm formation are important microbiological characteristics among *C. acnes* isolates obtained from patients with acne vulgaris. Clindamycin showed the highest resistance rate among the tested antibiotics, while strong biofilm formation was observed in half of the isolates.

Although antimicrobial resistance increased numerically with increasing acne severity, the association was not statistically significant. Previous antibiotic exposure, however, was significantly associated with antimicrobial resistance.

These findings support the importance of local antimicrobial susceptibility surveillance and rational antibiotic use in acne management. Further multicentre and longitudinal studies incorporating standardized susceptibility testing, quantitative biofilm assessment and detailed treatment outcomes are warranted to clarify the clinical significance of antimicrobial resistance and biofilm formation in acne vulgaris.

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