

Cutibacterium Acnes Associated Acne Vulgaris: Bacteriological Characterization, Antibiotic Resistance Profiling and Quantitative Evaluation of Platelet-Rich Plasma Therapy

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How to Cite the Article:

Fahmida Khatoun. "Cutibacterium Acnes Associated Acne Vulgaris: Bacteriological Characterisation, Antibiotic Resistance Profiling and Quantitative Evaluation of Platelet-Rich Plasma Therapy." *Journal of Advanced Biological Sciences*, vol. 02, no. 02, June 2025, pp. 22-27. <https://doi.org/10.66590/jabs2025020205>

Abstract | Background: Cutibacterium acnes drives chronic inflammatory acne vulgaris through Toll-like receptor 2 (TLR2)-mediated NF- κ B activation. Growing substantially in this organism, established treatment options are now limited by antibiotic resistance. Platelet-Rich Plasma (PRP) have been proposed as a non-antibiotic approach, how it affects the acne inflammatory response is not well understood. **Methods:** One hundred patients with clinically diagnosed acne vulgaris was enrolled. C. acnes was isolated by anaerobic culture on Brucella blood agar and confirmed by Gram staining and VITEK 2 ANC identification, antibiotic susceptibility in confirmed isolates were assessed by Kirby-Bauer disc diffusion for clindamycin, minocycline, erythromycin, ciprofloxacin and gentamicin. Using CLSI 2024 breakpoints, the researchers evaluated the cultures. utologous PRP, prepared by single-step centrifugation, was administered intradermally to the 17 patients with PCR-confirmed C. acnes. Documented photographically, the AI Smart Skin Analyzer scored clinical response for acne severity and UV photodamage before and after treatment. Changes within the group was analysed using paired-samples t-tests and Cohen's d. **Results:** PCR confirmed C. acnes in 17 of 100 participants (17%). Minocycline was active against all 17 isolates. Gentamicin resistance was detected in 64.7% of isolates and clindamycin resistance in 52.9%, with several strains showing co-resistance to clindamycin and erythromycin, consistent with possible MLSB resistance. PRP was group together with statistically important improvements in both acne severity scores (mean 10.64%; t (16) = 2.50, p = 0.024, Cohen's d = 0.61) and UV photodamage notches (mean 17.55%; t (16) = 2.79, p = 0.013, Cohen's d = 0.73). **Conclusion:** PRP was associated with measurable clinical improvement in C. acnes-associated acne vulgaris. The resistance data, particularly the prevalence of MLSB co-resistance, support a shift away from empirical macrolide and lincosamide prescribing. Controlled trials with longer follow-up are needed to establish whether PRP's effects are reproducible and durable.

Key Words Cutibacterium Acnes, Platelet-Rich Plasma, Acne Vulgaris, Ai Skin Analyzer, Immunomodulation

INTRODUCTION

Acne vulgaris is one of the most common chronic inflammatory skin conditions in clinical practice, affecting around 85% of adolescents and persisting into adulthood in many cases [1]. Global prevalence exceeds 650 million annually and its psychosocial impact is on a par with that of asthma and diabetes mellitus [2,3].

The condition results from sebaceous gland hypersecretion, follicular hyperkeratinisation and an abnormal immune response to Cutibacterium acnes (C. acnes) within the pilosebaceous unit. C. acnes is a Gram-positive anaerobic rod and the principal commensal of sebaceous follicles. Its cell wall constituents, lipoteichoic acid and peptidoglycan, activate

Toll-like receptor 2 (TLR2) on keratinocytes and monocytes [4], triggering NF- κ B signalling and downstream release of IL-1 β , IL-6, IL-8 and TNF- α . This also suppresses the regulatory responses that would otherwise contain the inflammatory process [5].

Antibiotics have long anchored acne treatment, but sustained prescribing has driven clinically meaningful resistance in *C. acnes* populations. Erythromycin and clindamycin are now unreliable across many regions [6,7], which has led clinicians and researchers toward options that do not rely on antibiotic activity.

PRP is an autologous concentrate of platelets and their released growth factors, among them PDGF, TGF- β 1, VEGF, IGF-1 and EGF. These mediators act on local inflammation and tissue repair and have been proposed to interrupt the NF- κ B-driven cytokine signalling that sustains acne lesions [8,9]. Controlled clinical data remain sparse; most published work consists of small cohort studies with variable preparation methods and the biological basis for any observed effect is not established.

The study was designed to address this across three components: bacteriological isolation and molecular confirmation of *C. acnes* in acne vulgaris patients, antibiotic susceptibility profiling of confirmed isolates and evaluation of PRP's clinical effect by AI-assisted quantitative skin analysis.

MATERIALS AND METHODS

Study Design and Participants

One hundred contributors with clinically diagnose acne vulgaris were enrolled at a specialized dermatology clinic during the period February 2025 to February 2026. Clinical diagnosis was established by direct examination conducted by a consultant dermatologist, who assessed each participant's lesion profile against establish clinical criteria prior to any instrumental measure. The study was conducted at Al-Mass Laboratory, Tikrit, Iraq. Participants ranged in age from 14 to 25 years. Exclusion criteria included current systemic antibiotic therapy, immunosuppressive conditions, pregnancy and concurrent use of topical retinoids.

Isolation, Identification, Preservation and Screening Media

Culture Media and Screening Systems: Brucella blood agar used as the primary solid medium for primary isolation. It prepared by dissolving 28 g of Brucella agar base powder (Oxoid, UK) in 1000 mL of distilled water, autoclaved at 121°C for 15 minutes and supplementing post-sterilisation at 45–50°C with 5% defibrinated human blood, haemin (5 μ g/mL) and vitamin K1 (1 μ g/mL). Brain Heart Infusion (BHI) broth (Oxoid, UK) supplemented with 20% glycerol was used for long-term preservation at –20°C. Thioglycollate broth (Oxoid, UK) served as transport and enrichment medium. Modified indole test medium (peptone 20 g/L, NaCl 0.5 g/L, sodium thioglycollate 0.5 g/L; pH 7.0) prepared in-house and sterilised by autoclaving.

Short-term preservation was performed on BHI agar slants sealed under anaerobiosis at 4°C. For long-term storage, 800 μ L of broth culture was mixed with 200 μ L of sterilised glycerol in cryovials and stored at –20°C.

Sample Collection

Skin specimens was collected from comedonal and inflammatory lesions on the forehead, nose, cheeks, chin, back and chest using sterile swabs moistened with 0.85% normal saline. Sites were prepared with antiseptic; residual alcohol were wiped away with saline before sampling. Swabs were placed immediately into 5 mL thioglycollate broth and transported to the laboratory within two hours, where specimens were vortexed for one minute and then serially diluted and plated onto Brucella blood agar.

Incubation and Colonial Identification

Plates were incubated anaerobically (GasPak jar system with anaerobic indicator strips) at 37°C for seven days. *C. acnes* produces small (1–2 mm), convex, opaque colonies with creamy-white to tan pigmentation and smooth, entire margins. Positive cultures were confirm to species level by the VITEK 2 Compact system (bioMérieux, France) using the ANC identification card.

Biochemical Confirmation

Presumptive isolates underwent four standard anaerobic biochemical tests: catalase activity (3% H₂O₂; positive = effervescence), indole production (Kovács reagent after 48–72 h anaerobic incubation), gelatin liquefaction (anaerobic incubation for up to 14 days) and glucose fermentation with gas detection. *C. acnes* is characteristically catalase-positive, indole-positive, gelatin-positive and glucose-fermenting.

Microscopic Morphology

In a methods section this is the correct and expected structure. Collapsing it would sacrifice clarity for no real gain.

Antibiotic Susceptibility Testing

Modified for anaerobic bacteria, the researchers determined antibiotic susceptibility by the Kirby–Bauer disc diffusion method, this was performed on Brucella blood agar in accordance with CLSI M11 (10th ed., 2024) breakpoints. The inocula was prepared from 48–72-h anaerobic cultures in sterile saline, they were adjusted to 0.5 McFarland turbidity ($\approx 1 \times 10^8$ CFU/mL). Inoculating the plates by rotating three-directional swabbing, antibiotic discs (Himedia, India) was applied aseptically and incubated anaerobically at 37°C for 48 hours. The diameter of the inhibition zones were measured with an electronic calliper, they were classified as Susceptible (S), Intermediate (I), or Resistant (R) per CLSI 2024 criteria. The antibiotics tested includes Clindamycin (CD, 2 μ g), minocycline (MI, 30 μ g), erythromycin (E, 15 μ g), ciprofloxacin (CIP, 10 μ g) and gentamicin (Gen, 10 μ g).

PRP Preparation and Injection Procedure

Autologous venous blood was collected into commercially available PRP tubes containing Acid-Citrate-Dextrose (ACD) anticoagulant and a gel separator; 8–10 mL of PRP were prepared from this blood, tubes were centrifuged for 10 minutes according to the manufacturer's protocol. Centrifuging for 10 minutes, the whole blood separated into a platelet-poor plasma layer, a platelet-rich plasma fraction above the gel interface and a red cell pellet below. The PRP fraction (4–5 mL per patient) were aspirated under aseptic conditions for immediate intradermal injection.

Cleansed and disinfected prior to injection, the practitioner prepared to treat the face. Using a 4 mm needle, PRP was administered intradermally within active acne lesions, it was also distributed across the face according to lesion distribution. The number of sessions range from one to two, this was determined by disease severity. No topical medications or systemic antibiotics was permitted during the 7–10-day follow-up period.

Haematological Assessment

Drawn concurrently with PRP collection before and after the treatment session, the laboratory technicians performed the Complete Blood Count (CBC) using EDTA-anticoagulated blood. The Complete Blood Count (CBC) from the patient samples were analyzed using an automated cell counter; the parameters of the test includes White Blood Cell Count (WBC) with differential, Red Blood Cell Count (RBC), haemoglobin concentration, haematocrit and Platelet Count (PLT). Clotting plain-tube samples for 10 minutes, serum was obtained followed by centrifugation at 2000–3500 rpm for 5–10 minutes.

AI Smart Skin Analyzer Assessments

All 17 PRP-treated participants underwent quantitative skin evaluation at baseline and final follow-up using the AI Smart Skin Analyzer, a standardised digital imaging system scoring multiple skin parameters on a 0–100 scale, where higher values indicate better skin health. Two parameters were recorded: the Acne parameter, which reflects severity and distribution of acne involvement and the UV Damage parameter, which reflects extent of surface photodamage.

Per-participant improvement percentage was calculated as: Improvement (%) = $\frac{[(\text{post-treatment score} - \text{Pre-treatment score}) / \text{Pre-treatment score}] \times 100}{1}$. Positive values denote improvement; negative values denote worsening.

RESULTS

Bacteriological Isolation and Cultural Characteristics

Of the 100 participants, anaerobic culture on Brucella blood agar yielded isolates with colonial morphology consistent with *C. acnes* in 17 cases (17%). Growth

appeared consistently after seven days under anaerobic conditions. Colonies were small to medium-sized, convex, opaque and creamy-white to tan with smooth, entire margins. Growth was markedly better on Brucella blood agar supplemented with haemin and vitamin K1 than on unsupplemented blood agar, as expected given the organism's fastidious nutritional requirements. The sentence" PCR confirmation was performed in our previously published study and those confirmed isolates were included in the present analysis. Therefore, the current study utilized the previously published PCR results. Gram staining of confirmed isolates showed uniform crystal violet-iodine retention after decolorization, producing the characteristic Gram-positive purple coloration. Cells appeared as small pleomorphic rods and diphtheroid forms, arranged singly, in pairs and in short chains.

Antibiotic Susceptibility Profile

Antimicrobial susceptibility result for all 17 PCR-confirmed *C. acnes* isolates is shown in Table 2. Minocycline was active against every isolate, with inhibition zone diameters of 50–60 mm and no resistance in any strain.

Gentamicin produced the smallest zones overall (10–18 mm) and the highest resistance rate at 64.7% (11/17 isolates). Clindamycin resistance was recorded in 52.9% of isolates (9/17), with three strains show no inhibition at all. Erythromycin resistance was present in 17.6% (3/17), with another four isolates at intermediate susceptibility. Ciprofloxacin resistance was also 17.6% (3/17); the remaining strains produced inhibition zones of 34–46 mm (Table 1).

Isolates 15 and 81 were resistant to both clindamycin and erythromycin, consistent with the Macrolide Lincosamide Streptogramin B (MLS_B) phenotype arising from point mutations in the 23S rRNA gene (Figure 1).

Table 1: Antibiotic Susceptibility Patterns of *C. Acnes* Isolates (n = 17) by Kirby–Bauer Disc Diffusion

Sample	Gen.	Cipro.	Clind.	Min.	Er.
1	R	S	S	S	S
2	R	S	R	S	S
5	R	S	R	S	S
9	R	R	S	S	I
10	I	S	S	S	S
15	R	S	R	S	R
17	R	R	R	S	I
18	S	S	S	S	S
23	S	I	R	S	S
35	R	S	R	S	S
42	R	S	S	S	S
56	R	R	R	S	S
67	S	S	S	S	R
73	S	S	S	S	S
80	R	S	R	S	I
81	R	S	R	S	R
95	S	S	S	S	I
Resistance %	64.7%	17.6%	52.9%	0%	17.6%

S: Susceptible I: Intermediate, R: Resistant



Figure 1: Inhibition Zone Diameters (mm) for *Cutibacterium Acnes* Isolates Determined by Kirby-Bauer Disc Diffusion

Haematological Safety of PRP Procedure

Pre- and post-treatment CBC parameters remained within normal reference ranges across all 17 participants assessed. Mean WBC was $7.18 \pm 1.81 \times 10^3/\mu\text{L}$ before treatment and $7.25 \pm 1.08 \times 10^3/\mu\text{L}$ after, with no individual exceeding the upper normal limit. Lymphocyte and granulocyte percentages showed no clinically meaningful redistribution (pre: $32.35 \pm 3.86\%$ and $60.48 \pm 4.54\%$; post: $32.18 \pm 5.07\%$ and $61.45 \pm 5.44\%$, respectively). Red cell parameters were stable: RBC 4.43 vs. $4.38 \times 10^6/\mu\text{L}$, haemoglobin 13.04 vs. 12.91 g/dL and haematocrit 38.86% vs. 38.31% .

Platelet count was the only parameter to change appreciably, falling from $284.50 \pm 63.11 \times 10^3/\mu\text{L}$ to $268.10 \pm 68.87 \times 10^3/\mu\text{L}$, a mean net reduction of $16.4 \times 10^3/\mu\text{L}$ consistent with platelet extraction during PRP preparation. All post-procedure counts remained within the normal range.

Clinical Response: Photographic Documentation

Post-treatment photographic assessment demonstrated clinical improvement across participants. The predominant changes comprised:

- Reduction in the number and severity of active inflammatory, erythematous and pustular lesions
- Attenuation of facial erythema
- Diminution of post-inflammatory hyperpigmentation
- improvement in skin texture and tone

Serial images at pre-treatment, intermediate and final time points showed progressive lesion resolution. Residual post-inflammatory marks and mild scarring persisted in participants with more severe baseline involvement; the overall clinical response was favorable (Figure 2).

AI Smart Skin Analyzer Outcomes

Pre- and Post-Treatment Scores: Individual pre- and post-treatment scores for the Acne and UV Damage parameters, together with calculated improvement percentages, are presented in Table 2.



Figure 2: Real Photo Taken Before and After PRP Treatment

Table 2: Pre- and Post-Treatment AI Smart Skin Analyzer Scores and Improvement Percentages (n = 17)

Participant	Acne Pre	Acne Post	Acne Δ (%)	UV Pre	UV Post	UV Δ (%)
1	83	95	+14.46	54	82	+51.85
2	56	73	+30.36	83	89	+7.23
3	88	84	-4.55	70	92	+31.43
4	51	57	+11.76	54	70	+38.59
5	63	83	+31.75	92	91	-1.09
6	78	82	+5.13	90	96	+6.67
7	70	82	+17.14	86	88	+1.18
8	96	100	+4.17	81	81	0.00
9	73	72	-1.37	91	87	-4.40
10	96	81	-15.63	55	73	+32.73
11	68	96	+41.18	55	90	+63.64
12	96	100	+4.17	89	91	+2.25
13	82	81	-1.22	56	58	+3.57
14	56	75	+33.93	85	74	-12.94
15	96	100	+4.17	93	93	0.00
16	96	95	-1.04	87	88	+1.15
17	51	57	+11.76	54	77	+42.59

Δ (%) = [(Post – Pre) / Pre] $\times$ 100, UV = Ultraviolet damage index, Higher scores denote better skin health (less severe pathology)

Analysis of the Acne Parameter

Descriptive Statistics: Among all 17 participants, pre-treatment Acne scores ranged from 51 to 96 ($M = 76.41$, $SD = 16.88$). Post-treatment scores ranged from 57 to 100 ($M = 83.12$, $SD = 13.63$). The mean raw change was $\Delta = +6.71$ points, corresponding to a mean improvement of 10.95%.

Individual Response Profiles

Twelve of 17 participants (70.6%) showed a positive change in Acne score post-treatment. The largest gains were recorded in participants 11 (+41.18%), 14 (+33.93%) and 5 (+31.75%). Five participants (29.4%) showed a marginal decline; the most notable decrease was in participant 10 (-15.63%), likely reflecting inter-individual variability in treatment response or transient worsening at treatment initiation.

Inferential Statistics

A paired-samples t-test yielded $t(16) = 2.50$, $p = 0.024$ (two-tailed), confirming a statistically significant improvement at the $\alpha = 0.05$ level. Effect size was Cohen's $d = 0.61$, denoting a moderate effect.

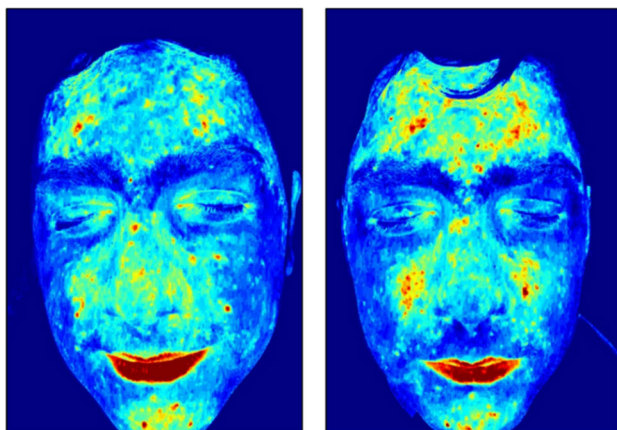


Figure 3: Pre- and Post-PRP Treatment Clinical Images in Colour-Mapped (pseudo-thermal) Format Demonstrating Reduction in Facial Inflammation

Analysis of the UV Damage Parameter

Descriptive Statistics: Pre-treatment UV scores ranged from 54 to 93 ($M = 73.87$, $SD = 17.09$). Post-treatment scores ranged from 58 to 96 ($M = 83.40$, $SD = 10.74$). The mean raw change was $\Delta = +9.53$ points, equating to a mean improvement of 17.55%.

Individual Response Profiles

Eleven of 17 participants (73.3%) showed UV score improvement; one participant showed no change. The largest gains were recorded in participants 11 (+63.64%), 1 (+51.85%), 17 (+42.59%) and 4 (+38.59%). Three participants (20.0%) recorded a marginal decline, most notably participant 14 (-12.94%) and participant 9 (-4.40%).

Inferential Statistics

A paired-samples t-test yielded $t(16) = 2.79$, $p = .013$, with Cohen's $d = 0.72$, indicating a moderate-to-large and statistically significant treatment effect (Figure 3).

Comparative Analysis of Acne and UV Damage Outcomes

UV Damage improvement (mean 17.55%) exceed that of the Acne parameter (mean 10.64%) and the UV Damage response was more consistent across participants (73.3% vs. 70.6% with positive change). Both endpoints reach statistical significance at the $\alpha = .05$ threshold. Participant 11 showed the highest increase in both parameters simultaneously (+41.18% acne; +63.64% UV), suggesting that individual factors including baseline skin condition and treatment adherence may substantially influence overall response.

DISCUSSION

This study Characterising *C. acnes* in acne vulgaris patients in Iraq, three components was evaluated: bacteriological isolation, antibiotic susceptibility profiling and quantitative clinical assessment of PRP therapy. The susceptibility data of the clinical isolates are stable with global surveillance finding, minocycline were

fully active across all isolates. Supporting its continued use as a first-line systemic agent for moderate-to-severe acne in this population, the researchers strongly endorsed the drug.

The clindamycin resistance rate of 52.9% and erythromycin resistance rate of 17.6%, with confirmed MLSB co-resistance in isolates 15 and 81, is consistent with resistance patterns reported by Ross *et al.* [6] and Dreno *et al.* [7], across European and Middle Eastern populations, these rates confirm previously established trends. Gentamicin resistance at 64.7% was the highest document in the panel; given the aminoglycoside's theoretical activity against Gram-positive organisms, the data suggest empirical gentamicin use should be avoided in *C. acnes*-associated conditions. The resistance profile as a whole supports a move toward antibiotic stewardship and non-antibiotic options such as PRP.

AI Smart Skin Analyser assessment showed statistically significant improvements in acne severity ($p = 0.024$, $d = 0.61$) and UV photodamage scores ($p = 0.013$, $d = 0.73$), consistent with published clinical evidence for PRP in inflammatory acne. Nofal *et al.* [8], report reductions in inflammatory lesion counts and erythema indices after intralesional PRP, assign the effect to platelet degranulation and local enrichment of PDGF, TGF- $\beta 1$ and VEGF. The larger improvement in UV Damage scores than in Acne scores may reflect difference in the underlying mechanisms: UV photodamage is driven by oxidative collagen degradation and melanogenic disruption, processes that the angiogenic and matrix-remodelling mediators in PRP may address more directly than the NF- κB -centred inflammatory cascade underlying acne lesions [10].

CONCLUSIONS

Antibiotic susceptibility profiling demonstrated full minocycline efficacy alongside clinically significant clindamycin and gentamicin resistance, including MLSB co-resistance, supporting a move away from empirical macrolide and lincosamide prescribing. PRP therapy produced statistically significant and clinically meaningful improvements in both acne severity and UV photodamage as quantified by AI Smart Skin Analyser assessment. These findings collectively position PRP as a pleiotropic, non-antibiotic immunomodulatory therapy for *C. acnes*-mediated acne vulgaris, warranting evaluation in larger randomised controlled trials with serial molecular endpoints.

Declarations

Data Availability Statement

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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