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Isolation and prevalence of *Cutibacterium acnes* phylotypes among acne patients

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ABSTRACT

Background. *The prevalence, isolation rates, and distribution of* Cutibacterium acnes (C. acnes) *phylotypes among acne patients in the Iraqi population are currently unknown.*

Aim. *To isolate* C. acnes *and determine the distribution of its phylotypes among patients with acne vulgaris, and to compare these findings with those from age- and sex-matched healthy controls.*

Method. *In this cross-sectional study, seventy swab samples were collected from patients with clinically diagnosed acne vulgaris and 70 from age- and sex-matched healthy participants. Samples were cultured under anaerobic conditions. Molecular identification and phylotype differentiation were performed using multiplex polymerase chain reaction (PCR).*

Results. C. acnes *was isolated from 37/70 (52.8%) acne patients and 65/70 (92.8%) healthy controls. All isolates from acne patients belonged to the IA2 phylotype (100%). In contrast, isolates from healthy controls showed greater phylotype diversity, including IA2 (32.3%), IB (43.1%), and type II (24.6%). The difference in phylotype distribution between the two groups was statistically significant (p = 0.0001).*

Conclusion. *Acne lesions demonstrated a marked loss of* C. acnes *phylotype diversity, with complete dominance of the IA2 phylotype, whereas healthy controls harbored a broader range of phylotypes. These findings support the association between phylotype IA2 and acne pathogenesis.*

Keywords: *Cutibacterium acnes,* acne, phylotypes, acne vulgaris

INTRODUCTION

Acne vulgaris is one of the most prevalent dermatological conditions worldwide, affecting approximately 79% to 95% of adolescents and young adults [1,2]. The disease may begin during adrenarche and affects both sexes, although females tend to develop acne more frequently. Clinical manifestations range from mild comedonal lesions to severe inflammatory forms that may result in permanent scarring and psychosocial distress [3].

Cutibacterium acnes (C. acnes) is a Gram-positive, anaerobic bacterium that thrives in the normal human skin microbiota, which helps to maintain and support the natural microbial balance of the skin. However, under certain situations, it can also substantially change its local environment and cause diseases [4]. This bacterium is linked to a wide range of skin diseases, including acne vulgaris.

The etiology and pathogenesis of acne vulgaris are multifactorial and not yet fully understood. Current concepts describe four main pathogenic mechanisms: (1) androgen-induced hyperseborrhea, 2) follicular hyperkeratinization, 3) colonization and proliferation of *C. acnes* and microbial dysbiosis that are associated with its development, and 4) activation of the innate immune response [5].

Despite advances in the genetic characterization and classification of *C. acnes,* the association between this commensal bacterium and acne development remains controversial [6]. Recent molecular studies have

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classified *C. acnes* into at least eight distinct phylotypes (IA1, IA2, IB1, IB2, IB3, IC, II, and III), each exhibiting specific genetic profiles and virulence potential [7]. IA is the most phylotype linked to acne and triggers patients' immune systems, while types II and III are more commonly found in normal skin or deep tissue infections [8].

Further molecular investigations have demonstrated that *C. acnes* strains harbor various putative virulence factors, including lipases, hydrolases, and Christie–Atkins–Munch–Petersen (CAMP) factors, which are more highly expressed in strains isolated from acne patients than in those from healthy individuals [9]. More recently, researchers have reported that in acne skin, the overall abundance of *C. acnes* is similar to that of normal skin; however, there is a loss of C. acnes diversity and a predominance of the virulent IA-1 phylotype [10]. These reports were considered a breakthrough in understanding the pathogenetic role of *C. acnes* in acne development.

Despite these advances, data on the molecular epidemiology and phylotype distribution of *C. acnes* in different geographic regions remain limited. To date, no studies have investigated the distribution of *C. acnes* phylotypes among acne patients in Iraq, and local data are entirely lacking.

Therefore, the present study aimed to isolate *Cutibacterium acnes* from patients with acne vulgaris in Basrah, Iraq, to determine the distribution of its phylotypes using molecular methods, and to compare these findings with those observed in age- and sex-matched healthy controls.

MATERIAL AND METHODS

Study design

A cross-sectional study including 70 patients with clinically diagnosed acne vulgaris and 70 age- and sex-matched healthy volunteers without active acne, recruited from department staff and patient attendees. All participants were recruited at Basrah Teaching Hospital, Iraq, from January to July 2024. The study was approved by the Ethics Committee, and written informed consent was obtained.

Inclusion criteria comprised patients aged 10–39 years with clinically diagnosed acne vulgaris of varying severity. Participants who were pregnant at the time of enrollment, had a diagnosed immune system disorder, or had used any topical or systemic antibiotic therapy within the four weeks before sample collection were excluded.

Patients were interviewed utilizing a questionnaire that included parameters such as family history of acne, the patient's skin care regimen, and skin color phototypes. Furthermore, enrolled patients underwent clinical examination by a dermatologist to evaluate the degree and severity of acne.

Sampling

In both groups, the targeted sampling area was cleansed to eliminate any makeup and subsequently wiped gently with a sterile swab containing 70% alcohol. This method may not reduce the bacterial load and potentially impact culture yield, as *C. acnes* is more prevalent within the sebaceous follicles than on the skin surface. In the acne group, samples were obtained from the forehead with a sterile swab from well-defined lesions exhibiting comedones and pustules. The materials were collected with minimal pressure, cultured in thioglycolate broth, and then sent to the laboratory for further analysis. In the control group, samples were collected using sterile swabs from clinically normal, non-lesional forehead skin, following the same sampling procedure. Subsequently, samples were cultivated on brain heart media and Columbia blood agar in an anaerobic environment utilizing Gas-paks (CampyGenTM 2.5 L, ThermoScientific, USA). Suspected colonies underwent Gram staining and were then examined microscopically for colony morphology.

DNA extraction

We performed DNA extraction using a genomic DNA purification kit (Presto Mini gDNA Bacteria Kit, Geneaid China). After measuring the concentration and quality of DNA, samples were stored at -20 °C.

PCR amplification

We confirmed the detection of *C. acnes* using specific primers (PR-246 5'-GCAGGCAGAGTTTGACATCC-3 and PAR25'GCTTCCTCATACCACTGGTCATC-3'). In addition, to determine the phylotypes, we used primers and PCR conditions described in Table 1. For the *C. acnes* specific primers PR-246 and PAR-2, the PCR program included one cycle of 95°C initial denaturation, 35 cycles of 95°C denaturation, 59°C annealing, and 72°C extension, followed by one cycle of final extension at 72°C. The PCR amplification products were visualized by electrophoresis on 1% agarose (Promega, USA). To guarantee the accuracy and validity of the assay, all PCR reaction conditions included positive and negative controls. Moreover, to ensure repeatability, each sample was amplified in duplicate, and all PCR conditions and primers were optimized to avoid false positive and negative results.

DNA sequencing

Although we analyzed all culture-positive samples with PCR, we chose three random PCR results for re-sequencing to ensure the validity of the *C. acnes* isolate. Samples were sent to Macrogen in Korea for sequencing. BLAST (Basic Alignment Search Tool) analysis was carried out on the NCBI database server.

TABLE 1. *Cutibacterium acnes phylotypes primers and PCR conditions [11]*

Primer	Specificity	Gene(s) targeted	Sequence (5' to 3')	Annealing temp (C°)	Amplicon size (bp)
PAMp-1	Type IA_1/IA2/IC	ATPase	GCGTTGACCAAGTCCGCCGA	66	494
PAMp-2			GCAAATTCGCACCGCGGAGC		
PAMp-3	Type IA2 /IB	sodA	CGGAACCATCAACAAACTCGAA	62	145
PAMp-4			GAAGAACTCGTCAATCGCAGCA		
PAMp-5	Type IC	Toxin, Fic family	AGGGCGAGGTCCTCTTCTACCAGCG	66	305
PAMp-6			ACCCTCCAACTGCAACTCTCCGCCT		
PAMp-7	Type II	atpD	TCCATCTGGCCGAATACCAGG	66	351
PAMp-8			TCTTAACGCCGATCCCTCCAT		
PAMp-9	Type III	recA	GCGCCCTCAAGTTCTACTCA	66	225
PAMp-10			CGGATTTGGTGATAATGCCA		

Statistical analysis

Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were presented as frequencies and percentages. The chi-square test was used to compare the isolation rate and phylotype prevalence between the patient and control groups. A p value ≤ 0.05 was considered statistically significant. SPSS (Statistical Package for the Social Sciences), version 23, was used.

RESULTS

Among the 70 acne patients, the mean age ± SD was 16 ± 5 years, and 52 (75%) were females, versus 17 ± 4 years, and 50 (71.4%) were females in the control group (p =0.23 and 0.65, respectively). According to severity, the results indicated that acne was mild in 13 (18.6%) patients, moderate in 29 (41.4%), and severe in 28 (40%) patients (Table 2).

TABLE 2. *Demographic and clinical characteristics of acne patients and controls*

Variable	Acne patients (n=70)	Controls (n=70)	p-value
Age (years), mean ± SD	16±5	17±4	0.23
Sex, n (%)			
Female	52 (75%)	50 (71.4%)	0.65
Male	18 (25%)	20 (28.6%)	0.66
Acne severity			
Mild	13 (18.6%)	—	—
Moderate	29 (41.4%)		
Severe	28 (40%)		

The morphological characteristics of colonies were monitored for all samples after 5-7 days. The *C. acnes* colonies appeared as white, circular, glistening colonies on Columbia blood agar (Figure 1). Gram staining showed gram-positive curved rods, diphtheroid, or coryneform shapes (Figure 2).

FIGURE 1. *The morphological characteristics of* C. acnes *colonies with a circular, white, glistening appearance*

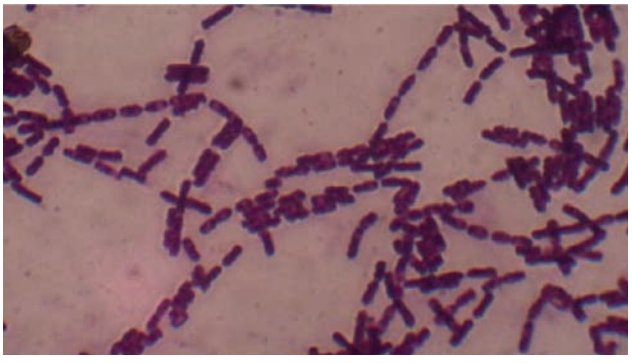

FIGURE 2. *Gram staining showed Gram-positive curved rods, a coryneform shape of* C. acnes

Molecular detection of *C. acne*

The results showed that 37 samples out of 70 (52.8%) acne patient samples and 65 out of 70 (92.8%) patients in the control group were positive for *C. acnes*-specific primers in PCR detection (Figure 3). The difference was statistically significant between the two groups (p = 0.001)

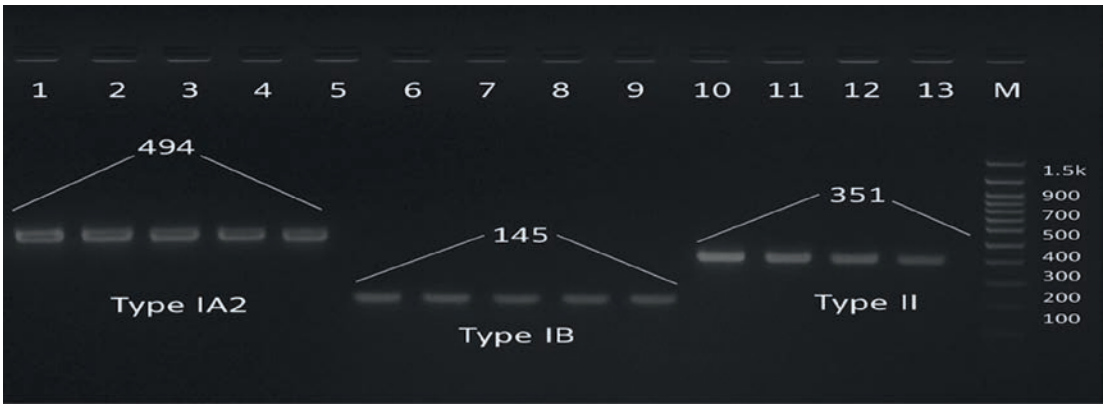

FIGURE 3. *1% Agarose gel electrophoresis for Cutibacterium acnes specific primers after staining with (RedSafe dye). Lane 1-10 positive samples. Lane 16 ladder L100-1500; the size of the product is 334 bp*

DNA sequences

We chose random PCR samples for sequencing and BLAST analysis to make sure that the primers were specific to *C. acnes.* The results confirmed the specificity of the *C. acnes*-specific PCR and allowed us to pursue our research accordingly.

Cutibacterium acnes phylotypes differentiation

Based on the phylotype PCR, we found that all the *C. acnes* isolates obtained from the acne patient group were IA-2 phylotypes, representing 100% of the isolates. Figures 4 and 5 subsequently show positive samples for the ATPase and sodA target genes, which differentiate between IA1/IA2/IC phylotypes. SodA genes' phylotype-specific primers represent IA2/IB (as shown in Table 2). The sizes of the ATPase and SodA genes are 494 and 145 bp, respectively. In contrast, in the comparative healthy control group, more diverse phylotypes (types IA2 in 32.3%, IB in 43.1%, and II in 24.6% of the isolates) were detected based on target primers, as shown in Figure 6 and Table 3.

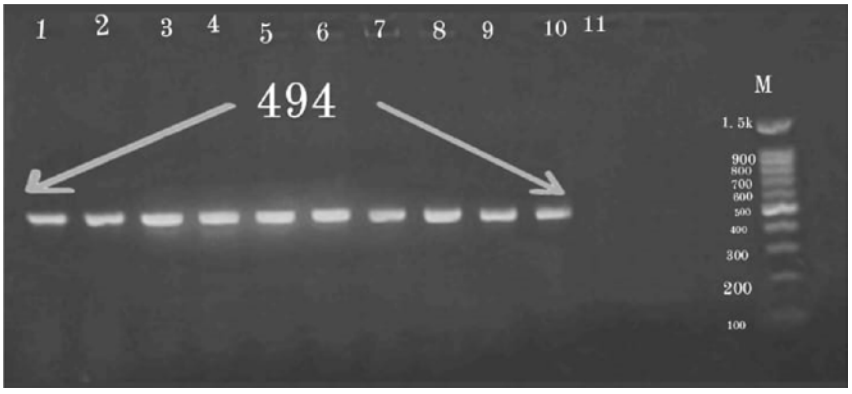

FIGURE 4. *Agarose gel electrophoresis for ATPase gene PCR products after staining with (RedSafe dye). Samples (lane 1-10), lane 15 ladder L100-1500; the size of the product is 494bp*

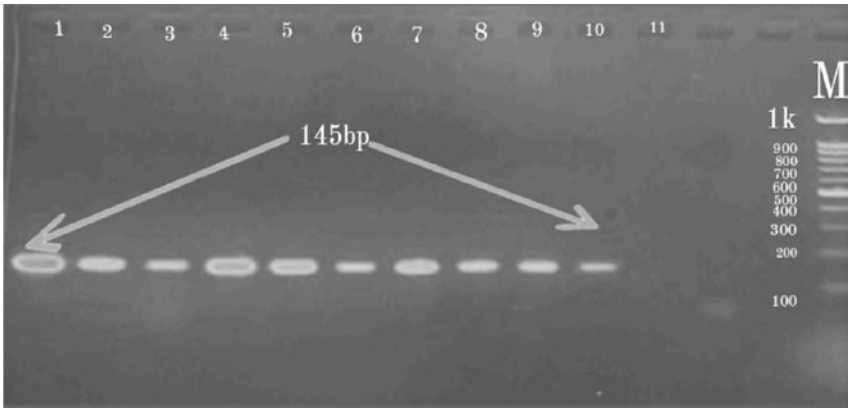

FIGURE 5. *Agarose gel electrophoresis for sodA gene PCR products from 1-10 lanes, staining with (RedSafe dye) ladder lane 14(Accuris SmartCheck™ DNA Ladders) L100 - 1500 bp, all isolates of C acnes were positive for ATPase genes are also positive for sodA genes, indicating the IA-2 phylotypes, the size of the product is 145bp*

TABLE 3. *Cutibacterium acnes isolation rates and phylotype distribution among acne patients and controls*

Variable	Acne patients (n=70)	Controls (n=70)	p-value
Culture positivity	37 / 70 (52.8%)	65 / 70 (92.8%)	0.001
IA2	37 (100%) isolates	21 (32.3%) isolates	0.001
IB	0 (0%) isolates	28 (43.1%) isolates	0.001
II	0 (0%) isolates	16 (24.6%) isolates	0.001
Total	37 (100%)	65 (100%)	0.001

DISCUSSION

In the present study, *Cutibacterium acnes* was isolated from 52.8% of acne patients and 92.8% of healthy controls, and a low rate of culture positivity was observed among the acne group. This finding is consistent with previous reports, which demonstrate wide variability in *C. acnes* isolation rates, ranging from 40% to 90%, depending on sampling techniques, lesion type, and microbiological methods used [12,13]. The lower culture rate observed in the acne group may be partly explained by the likelihood of prior antimicrobial exposure in some patients, even beyond the defined exclusion period, as well as by sampling from inflammatory lesions, where local environmental conditions may reduce bacterial recoverability or culturability

Using multiplex PCR, the most abundant *C. acnes* phylotype among acne cases was the 1A2 phylotype (100% of cases), while types IA1, IB, IC, II, and III were not detected, whereas a diverse and wide variety of phylotypes of *C. acnes* was demonstrated among comparative healthy controls, including types IA2, IB, and II. To our knowledge, these results provide the first molecular characteristics and phylotype clones of *C. acnes,* with a predominance of phylotype IA2 among Iraqi patients with acne.

The observed predominance of phylotype IA2 and reduced phylotype diversity in acne patients is aligned with the current concepts in acne pathogenesis, which propose that for acne to develop, there is microbiome dysbiosis of normal commensals characterized by a loss of *C. acnes* phylotype diversity and a predominance of phylotypes IA1 and IA2, rather than simple bacterial overgrowth, whereus, phylotypes II and III are more frequently associated with normal healthy skin [14,15]. In a study, using multilocus sequence typing confirmed that certain clones of phylotype IA were strongly associated with acne, while phylotype II was linked to healthy skin [16]. These acne-associated phylotypes can express virulent factors more easily than other phylotypes found in healthy people, and are associated with severe inflammatory acne phenotype [17,18]. Additionally, experimental studies have shown that phylotype IA strains can stimulate the innate immune system by prompting the secretion of pro-inflammatory cytokines from monocytes and keratinocytes, thereby contributing to acne-associated inflammation [19]. When combined, these literature-based findings give a reasonable biological background for the phylotype distribution seen in the current study, although causal mechanisms were not directly addressed.

Several limitations should be acknowledged. First, the cross-sectional and single-center design limits the generalizability of the findings. Second, the relatively low culture positivity rate among acne patients may have influenced the observed phylotype distribution. Third, environmental and behavioral factors that were not systematically assessed – such as the hot and humid climate of southern Iraq and the use of inexpensive, potentially comedogenic or irritating cosmetic products – may have contributed to interindividual variability in bacterial recovery and microbiota composition.

However, our findings may be considered as a further step in understanding the microbiological aspects of acne pathogenesis and contribute to a more nuanced understanding of *C. acnes* taxonomy and phylogenetic relationships, potentially paving the way for targeted therapeutic strategies against pathogenic phylotypes.

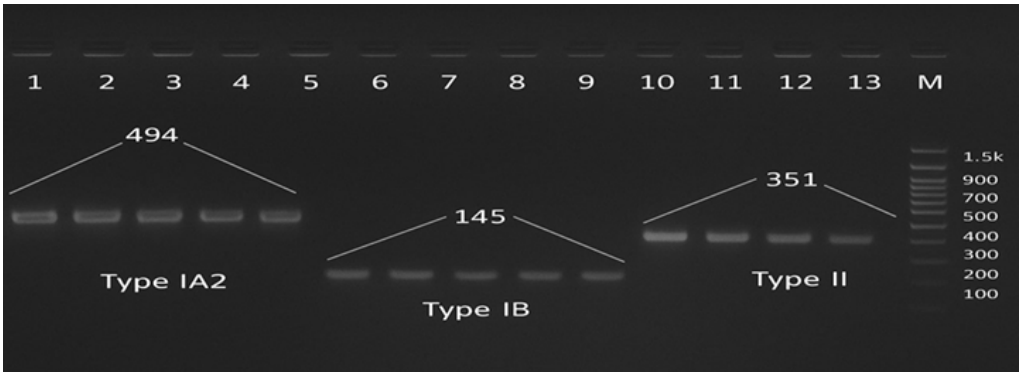

FIGURE 6. *Phylotypes distribution among healthy control samples. Lane 1-5 represent IA-2 phylotypes, the product size 494 bp, the lane 6-10 represent phylotypes IB, product size 145 bp, the lane 11-15 represents phylotypes type II, the product size 351 bp. LadderL100-1500*

CONCLUSION

Acne vulgaris is a chronic skin condition influenced by the interaction between *C. acnes* phylotypes and various demographic factors. While phylotypes IA2 are the most common clade associated with acne patients compared to diverse phylotype isolates in healthy controls, our findings contribute to understanding the microbial imbalance in acne and may assist clinicians in considering phylotype-targeted therapies and designing future microbiome-based acne interventions.

Conflict of interest

The authors declare no conflicts of interest.

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