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Evaluate of Cutibacterium acnes Distribution among Acne Vulgaris Patients

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Abstract
Background: Acne vulgaris is a very common condition of the pilosebaceous unit that is caused by multiple factors and affects roughly 80 % of adolescents, making it one of the most prevalent skin conditions among them.
Aim: The aim of this study to assess distribution of acne vulgaris patients and its severity according to age and gender of patients Isolate and identify of *C. acnes* among acne vulgaris patients.
Materials and methods: skin swabs were collected from 90 patients who had acne vulgaris and attending dermatology private clinics under aseptic conditions for isolating and identifying *Cutibacterium acnes*(C. acnes) using thioglycolate medium and Brain heart infusion -blood agar supplement with and 20 mg/ml of metronidazole and sheep blood. Under anerobic condition at 37 C^0 for 7 days of incubation. Both males and females between the ages of 16 and 30 years old were selected. The growing bacteria were diagnosed based on microscoping and macroscoping examinations of cells and colonies morphology. Moreover, catalase production and hemolysis activity.
Results: In accordance with the findings of the study, 17 and 19-years old made up32.22% of patients, while females made up 54.44 %of the total number of patients suffering from acne. In addition, *C. acnes* was isolated from 13.33 %of patients who suffered from acne, while 32.22 % had mixed cultures. all of the patients who were infected with C. acnes and were younger than 20 years old suffered from severe pustular acne. This was compared to just 75 % of patients who were infected with C. acnes and were older than 20 years old. The finding of this study achieved that younger was more susceptible to opportunistic pathogen C. acnes.

Key words: Acne vulgaris; *Cutibacterium acnes*; Sever acne.

Introduction

Acne vulgaris is a very common condition of the pilosebaceous unit that is caused by multiple factors. Acne vulgaris is a disorder that affects roughly 80 % of adolescents, making it one of the most prevalent skin conditions among this age group. Acne is ranked on a severity scale from mild to severe, with moderate acne falling in the middle(Williams *et al*., 2012). In addition to the cutaneous microbiota and innate immunity, the colonization of the pilosebaceous follicle by *Cutibacterium acnes* play a role in the skin's inflammatory response. The increased production of sebum, along with a change in the composition of the sebum, and hyper-cornification of the pilosebaceous follicle as a result of hyperproliferation and abnormal differentiation of keratinocytes in the upper part of the pilosebaceous follicle are two additional factors that contribute to this chronic inflammatory skin disease. Both of these factors can be traced back to the pilosebaceous follicles (Toyoda & Morohashi, 2001). Environmental variables, hormones, a person's family history, and even stress can all play a role in the severity, incidence, and permanency of acne, but these are not the only elements at play (Elsaie, 2016). The commensal lipophilic Gram-positive bacteria known as Cutibacterium acnes (*C. acnes*), formerly known as Propionibacterium acnes or P. acnes,) is a member of the skin. It is most commonly found in areas of the skin that are abundant in sebaceous glands. It has been considered a commensal bacterium for a very long time and has a role in the maintenance of healthy skin (Teramoto *et al*., 2019). As a result of its role in a number of different illnesses, it has become recognized as an opportunist pathogen. Interactions

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between *C. acnes* and the human host, especially the human skin microbiome, encourage the selection of *C. acnes* strains capable of releasing various virulence factors that boost inflammatory that potentially lead to the development of acne. The aims of this study to assess distribution of acne vulgaris patients and its severity according to age and gender of patients in addition, identifying of *C. acnes* among acne vulgaris patients.

Materials and Methods

The study included patient of ages of (16) and (30) of who had acne vulgrus that diagnosed by dermatologist and who did not take any topical corticosteroids or systemic, isotretinoin, immunosuppressives, chemotherapeutic agents, anti- inflammatory biologic, or oral antimicrobial agents within 30 days prior and without any inflammatory disease such as psoriasis, atopic dermatitis and asthma. It excluded of patients with history of pregnancy, lactation and iron intake. Also, patients who suffering from active malignancy or any cosmetic induced acne. The degree of severity of acne vulgaris including mild, moderate and severe acnes, were determined according to Oberemok & Shalita, (2002) recommended as mentioned in table 1

Table 1: Acne Classification by severity or Inflammatory Lesions

Severity	Papules /Pustules	Nodules
Mild	Few to several	None
Moderate	Several to many	Few to several
Severe	Numerous to extensive	Many

Acnes Vulgaris Patient Samples

The study included Patients acne attending private clinics in the Kirkuk city, Iraq, were involved in this study throughout a 6-month study period from October, 2021 to May, 2022. A dermatologist or a trained nurse was asked to obtain the samples. After decontaminating by wiping the skin 2-3 times with 70% ethyl alcohol, sterile cotton swabs were used to obtain the samples from a lesion. The lesion was opened aseptically in the clinic and swabbed. Swabs were aseptically placed in culture media.

Samples' Culture

Using a vortex mixer, the samples within thioglycolate medium (HiMedia) were homogenized and incubated for 7 days anaerobically using Gas-Pak in anaerobic jar. Subcultures from thioglycolate broth by wire loop were inoculated on BHI-blood agar prepared with some modified following the protocol described by Poudel *et al*., (2021). 2.5% Brain-Heart Infusion and 3% agar per liter of D.W were added. Then, 5% sheep blood and 20 mg/ml of metronidazole were supplemented to cool medium. Inoculated BHI incubated anaerobically condition for 7-14 days (Ganguli *et al*., 1982).

Diagnosis Tests of C. acnes

The phenotypic features of the colonies on BHI-Blood agar were observed. Colony characteristics included dimensions (including width and depth), elevation, and hue (Williams *et al*., 2012). under light microscope of cell forms and grouping of cells after staining by gram stain were examined under oil immersion with a 100X magnificent.

Biochemical Tests

Some important biochemical tests were as carried out at this study to diagnose *C.acnes* including catalase test and hemolysis

Catalase: Freshly prepared hydrogen peroxide (3%, v/v) was flooded on 3 to 4-day-old colonies of *C.acnes* on BHI blood agar plates Production and formation of bubbles (O 2 + water = bubbles) were observed immediately(Puhvel,1968).

Hemolysis: The hemolysis of sheep RBCs response on sheep blood agar, was carried out for the diagnosis in this study (Hoeffler, 1977).

Statistical Analysis: Microsoft EXCEL 2019 and SPSS 23 version software were used for data analysis and the descriptive analysis preformed on frequencies and percentages

Results

Colonial morphology on BHI-blood agar and gram staining of cells under the microscope were used to identity of *C. acnes* isolates with some biochemical testes as conducted by Pruijn *et al*., (2021). Colonies of twelve acne isolates on BHI-blood agar were observed at this study: punctiform, circular, glistening and having hemolysin activity. While under light microscope cells appearances were gram-positive, non-spore former,

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extremely pleomorphic, irregular rods with either curved, clubbed, or pointed ends and have diphtheroid or coryneform shaped such as described by Patrick & McDowell, (2011) (Figure 1).

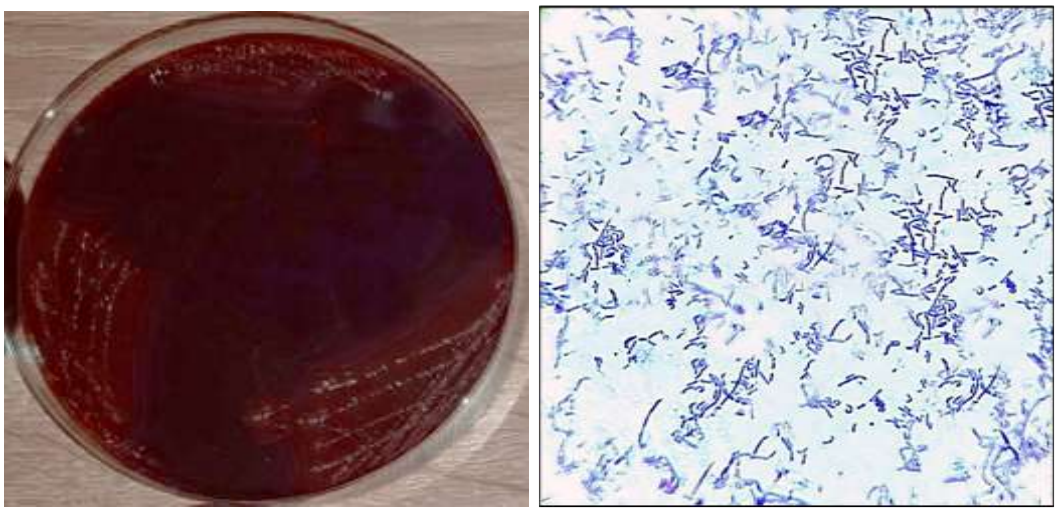

Figure 1: Macroscopic and microscopic examination. (A): colonies appearance of *C. acnes* isolates on BHI-blood agar was glistening, circular and heamolysis producing. (B): *C. acnes* cells are gram positive non spore forming pleomorphic, branched and unbranched rods not filamentous. Cells occurs in singly, in pairs or short chains.

The study demonstrated that Cutibacterium acnes was isolated from 13.33% of acne patients. While, 32.22% were mixed cultures of bacteria Table 2.

Table 2: Culture results of Acnes Samples on BHI-blood agar

Culture results	No.	%
Cutibacterium acnes	12	13.33
Mixed Culture	29	32.22
Negative	49	54.44
Total	90	100

Many different types of bacteria, both aerobic and facultative anaerobic, were shown to be responsible for the acne infection in the current investigation. One-third of the isolates (13.33 %) were found to be *C. acnes*.

Distribution of Patients with C. *acnes* Infection According to Type of Acne

The study showed that most patients (83.33%) who infected with *C. acnes* were suffering from severe pustular acne and the lowest with mild and moderate pustular acne as shown in Table 3.

Table 3: Distribution of Patients with *C. acnes* Infection According to Type of Acne.

Type of acne	No.	%
Mild pustular acne	**1**	**8.33**
Moderate pustular acne	**1**	**8.33**
Severe pustular acne	**10**	**83.33**
Total	**12**	**100**

The study showed that all patients infected with *C. acnes* under 20 years old were suffering from severe pustular acne compared with 75% of patients infected with *C. acnes* whose age ≥ 20 years, (Table 4).

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Table 4: Distribution of patients with C. *acnes* infection within type of acne and age groups.

Age groups	Mild pustular acne		Moderate pustular acne		Severe pustular acne		Total	
	No.	%	No.	%	No.	%	No.	%
<20	0	0	0	0	4	100	4	100
≥20	1	12.5	1	12.5	6	75	8	100

The study showed that all patients infected with *C. acnes* males were suffered from severe pustular acne compared with 75% of female's patients, Table 5.

Table 5: Distribution of Patients with C. *acnes* Infection within Type of Acne and Genders

Gender	Mild pustular acne		moderate pustular acne		Severe pustular acne		Total	
	No.	%	No.	%	No.	%	No.	%
Female	1	9.09	1	9.09	9	81.82	11	100
Male	0	0	0	0	1	100	1	100

Discussion

Catalase production of twelve isolates were positive. *C. acnes* is not an obligate anaerobe, the incidence of oxygen is not terminal *C. acnes*. Anaerobiosis is typically essential for isolation and growth (Cove *et al*., 1983; McLaughlin *et al*., 2019) This is confirmed by the fact that the organism can be grown aerobically when a very heavy inoculum is used. However, aerobic growth is always slower and poorer than after anaerobic incubation (Puhvel, 1968; Sowmiya *et al*., 2015). *C. acnes* has been shown to survive for long periods of time in human tissues with a low oxidative potential. Anaerobic bacteria are characterized by their inability to grow on solid media in the presence of atmospheric oxygen. Conversely, *C. acnes* is considered an aerotolerant anaerobe because it possesses enzymatic systems able to detoxify oxygen, allowing it to be sustained on the surface of the skin (Gajdács *et al*., 2017; Naghdi & Ghane, 2017). Ninety people who were thought to have acne were analyzed in this study. Forty-one samples showed viable bacterial or other organism growth consistent with the results of two earlier investigations Ghodsi *et al*., (2009); Tan, (2008), which found that 48% of the patients investigated had positive acne culture results, this conclusion was confirmed by the present investigation. Positive culture has been estimated to be somewhere between 58% and 62%, according to field surveys by Zandi *et al*., (2011). Al-Zoman & Al-Asmari, (2008) stated that even lower prevalence rates, with only 11.88 % of individuals suffering from acnes. It's worth noting that technological mistakes are just one potential because of our area's pervasive unfavorable culture. Potentially other causes of culture failure include infections caused by viruses that are resistant to the standard methods of isolation employed here (Alshamrani *et al*., 2019). Furthermore, it is probable that the *C. acnes* is not viable in those particular skin samples, which therefore impeds growth in the culture medium (Cavalcanti *et al*., 2011). Also C. acnes colonization Levels on the skin differ from person-to-person and from the area of the body sampled (McDowell *et al*., 2005). the neck, forehead, and shoulder showing some of the highest concentrations of the bacterium compared to other sites such as the abdomen, hip, knee, and chest (Patel *et al*., 2008). The findings here corroborate those of similar research. The prevalence of C. acnes infection was 23%, according to a research conducted in India by Hassanzadeh et al., (2008). The most common bacterium responsible for acne infections is *C. acnes* (Nakatsuji *et al*., 2010;Y. Wang *et al*., 2018). The skin's microenvironment (pH) is optimal for growth of *C.acnes* that support this bacterium to produces a variety of extracellular products (lipases, proteases, and chemotactic factors) responsible for the initiation and maintenance of the inflammatory response. It plays a major role in the pathogenesis of acne and is an important opportunistic pathogen causing superficial skin infection. Isolation of *C. acnes* was achieved in a sample size of 12 participants for this investigation (13.33 %). This is similar to the findings of two earlier investigations (Bojar & Holland, 2004 ; H. Gollnick, 2003b), which also found that C. acnes is present in a high proportion of the samples they analyzed. Among the multiple commensal microorganisms present in the healthy skin flora, *C. acnes* is a ubiquitous gram-positive anaerobic bacterium that predominan tly resides deep within the sebaceous follicle in contact with keratinocytes. Conversely, at the skin surface

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Cutibacterium are less represented (<2% of all bacteria) in favor of Staphylococci, especially *Staphylococcus epidermidis* (*S. epidermidis*), which dominate with >27% of the total bacteria population (Dréno *et al*., 2018). Findings from other research corroborate with current results, demonstrating that pateints with severe acne are disproportionately represented among acne sufferers (Williams *et al*., 2012; Nast *et al*., 2016). In addition, Ramos-e-Silva & Carneiro, (2009) revealed that those infected with C. acnes were more likely to experience severe pustular acne. While, Alkhawaja *et al*., (2020) mentioned that 27.0% of the patients had mild pustular acne, 57.0% had moderate pustular acne, and 16.0% had severe pustular acne. Although phylotype IA1 was the most represented in both populations, a recent pilot observational study looked at the potential link between acne severity and a specific C. acnes subtype or subpopulation in the lesions, and found a significant distribution of *C. acnes* in patients with severe acne infection(Paugam *et al*., 2017).Another study utilizing the same SLST methodology similarly achieved that phylotype IA1 was more common in severe acne than in mild acne (with 60 %, 57.1 % and 63.3 % of strains in the severe, moderate and mild acne groups, respectively). It was hypothesized that phylotype IA2, which is extremely resistant to clindamycin, was more commonly linked with moderate to severe acne (Nakase *et al*., 2021). Altogether, these contradictory results emphasized the fact that the severity of acne may be attributable to host and environmental variables in addition to a particular C. acnes strain, which might result in varying degrees of innate immune activation in severe acne (Paugam *et al*., 2017). About 62.5% of this study sample experienced severe pustular acne between the ages of 15-20, according to Alkhawaja *et al*., (2020). Some researchers have hypothesized that the stressors associated with applying to high school and universities and going through puberty may be to blame for this (Chiu *et al*., 2003; Yosipovitch *et al*., 2007). In addition, the decline in sebaceous gland activity after puberty accounts for the diminishing acne severity with age (Heng & Chew, 2020).

Conclusions

Most patients who infected with *C. acnes* were suffered from severe pustular acne and the lowest with mild and moderate pustular acne and all patients infected with *C. acnes* under 20 years of old were suffering from severe pustular. More studies are required to evaluate the role of virulence factors of C. acnes in severity of acne vulgaris patients.

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