

# **Isolation and Characterization of *Probionibacterium acne* from Acne Vulgaris Patients**

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## **Abstract :**

In this study, 110 skin swabs were obtained from patients suffering from acne vulgaris from consultation unit in Merjan teaching Hospital, and allergy specialized center in Al-Hilla from both sexes .

It was found that acne vulgaris was higher in female (62.7%) and more prevalence in (16-20) years patients and lower in older especially after 30 years age where there is only one case.

Three isolates of anaerobic gram positive *Probionibacterium acnes* were identified and diagnosis by culture and biochemical tests, all bacterial strains possessed capsule and produce lipase and protease enzymes and contained the third colonization factor antigen (CFA-III) while only one strain produce a narrow zone of  $\beta$ -hemolysis .

The effect of some antibiotics on *Probionibacterium acnes* was investigated, and the results showed that all isolates were sensitive to Tetracycline ,Deoxycycline, Chloromphenecol, Neomycine, and Kephalexin, while they were resistant to Erythromycin, Fusidic acid, Vencomycin, Lincomycin and Gentamycin.

Also retinoic acid effect on bacterial growth was studied. It was stated that lactic at high concentration 20 $\mu$ g/ml could cause inhibition of *Probionibacterium acnes* growth. Also temperature over 50 C $^{\circ}$  would kill the bacteria.

## **Introduction :**

Acne is a chronic inflammatory disease of the pilosebaceous unit, primarily of the face, nose, upper back, chest, shoulders and upper arms. It is one of the commonest dermatoses affecting the teenage population (Sehgar & Jain, 1995).

On the face it occurs most frequently on the cheeks, and in lesser degree on the nose, forehead and chin (Andrews, 1990).

Acne starting usually between the ages of 12 and 14 years, tending to be earlier in females. The peak age for severity in females is 16-17 and in males 17-19 years (Lucky, *et al.*, 1991).

Acne frequently occurs in individuals with high sebum production and bacteria is observed at acne regions. Products generated by ingestion and degradation of sebum by *Propionibacterium acnes* are considered to cause inflammation around pores, and aggravate the symptoms (Ingham, *et al.*, 1992).

Endocrine, keratinization, and bacterial factors are considered to be the major factors in acne development. An increase in testosterone after puberty is considered to increase sebum productivity of sebaceous gland. Free fatty acid produced by *P. acnes* derived lipase, induced impairment of keratinization and sebum accumulated in the hair follicular duct (Downing, *et al.*, 1986).

When untreated, acne usually lasts for several years until it spontaneously remits. Adolescent acne (acne vulgaris) has a strong tendency to be hereditary and is less likely to be seen in Asians and dark skinned people. The pilosebaceous follicle is the target organ in acne, explaining the distribution of acne primarily on the face, chest and areas with the greatest concentration of pilosebaceous glands.

## **Patients and Methods:**

One hundred and ten samples were collected from acne patients who attended the consultation unit of Merjan teaching Hospital in Babylon province during the period of November 2004 to April 2005; their ages ranged from 13-30 years old.

The patients were suffering from severe-moderate inflammatory acne. After an incubation period, rod shaped bacteria was stained with Gram stain then with Albert

stain, then tested with biochemical test including ; Catalase, Indole production, Nitrate reduction, Esculin hydrolysis, then to detect some virulence factors and adhesions such as capsule, by negative stain (Cruickshank, *et al.*, 1975), haemoagglutination test, production of hemolysin, siderophore production and colonization factor antigens.

The bacterial isolates were also tested for production of some enzymes especially lipase and protease, also the effect of Retinoic acid and many other antibiotics were used to test the sensitivity of the bacteria (Maccfeiden *et al.*, 1995).

### **1.Sidrophore production assay :**

M9 agar was used for assay which is supplemented with 0.25 gm glucose after sterilization, 200µm of dipyrrolyl is added ( Nassif *et al.*,1989) .

### **2. Retinoic acid assay :**

- A. Nutrient broth is prepared and distributed in tubes and the retinoic acid is added to each tube at various volume to gain the final concentration ( 10, 20, 40, 80 and 100 mg/ml).
- B. Positive control is prepared by using Nutrient broth from Retinoic acid.
- C. The tubes in item 1 and 2 are inoculated with 0.5 ml of bacterial suspension and then incubated anaerobically for 24 hour at 37C°.
- D. After incubation, the absorbency was read at wave length 520 nm.
- E. Draw standard curve.

### **3.Assay for colonization factor antigens :**

Assay for colonization factors was used depending on (Smyth, 1982) as follow :

#### **A.CFA/I :**

- I.Inoculate bacterial strain on tryptic soy agar which is incubated in 37C° for 24 h.
- II.RBCs suspension was prepared from group A with phosphate buffer saline (repeated three times) the 3% (v/v) of RBCs was prepared.
- III. Bacterial suspension from that grown on TSA , and mixed with 0.1M NaCL to determine RBCs agglutination test and was castigated on colonies factor antigen type one.

IV. On clean slide mixed one drop of bacterial suspension with one drop of RBCs suspension and one drop of 0.15M NaCl (without D-mannose).

V. Examine the agglutination at room temperature and from 1/2 – 2 min.

B. CFA/II :

To determine this factor the same steps were followed except, chicken blood was used instead of group A blood.

C. CFA/III :

Also the same steps were used except that after growing on TSA mix with 0.1 M of NaCl. And using the tannic acid to visualize the agglutination of human RBCs type A blood group.

### **Results and Discussion :**

Acne is a common inflammatory skin disease due to inflammation of blocked hair follicles. Each hair follicle is associated with a sebaceous gland which secretes a conditioning oil (sebum) into the follicle. The condition usually starts in adolescence in both male and female.

In this study the distribution of acne vulgaris was higher among female than male (62.7% and 37.2% ) respectively.

This result was correlated with those results obtained by Kligman (1974) who had pointed that acne prevalence was more among female than in male, also Burton *et.al.*, 1971 said that acne was developed earlier in females than in males. Furthermore, the distribution of this disease among patient ages was also studied. Most cases were in patients ages (16-20) years old in both sexes. Burton results were identical to our results obtained by this study about the severity of acne in the ages between 15-17 years in females, and 16-20 years in male.

Acne problems appear in older patients in mid-twenties (20.9%), Rea *et.al.*, 1997 said 18% had late-onset (more 20 years).

Also in this study (4.5%) of patients had their acne between (20-35) . This result was close to Stern, 1992, result's which have 5% and 1% of patients who had acne in the same age groups. All these results and others were listed in table ( 1 ).

Table (1 ): Distribution of acne infection among age and sex.

Age			Number	%
	Male	Femal		
10-15	1	17	18	16.3
16-20	33	31	64	58.9
21-25	7	16	23	20.9
26-30	-	5	5	4.5
30>	-	-	-	-
Total	41	69	110	
%	37.2	62.7		

Acne problem in adolescence may be due to excretion of high quantity of sebum than normal people. Ebling (1974), mentioned sebaceous activity is predominantly dependent on androgenic sex hormones.

Table (2 ) showed the number and percentage of bacterial species isolated from acne lesions. All bacterial types in this study correlated with the three major organisms isolated from the surface of skin and duct of acne patients in previous studies of Nester *et.al.*, 1998 which are *Staphylococcus aureus*, *Staphylococcus epidermides* and *Propionibacterium acnes*.

The large group of microorganism universally present on the normal skin is the gram-positive cocci, staphylococcus.

*Staph. aureus* appeared in low percentage in this study (8.6%), this *S. aureus* is the most pathogenic of the staphylococci.

The principal species in skin flora is *Staphylococcus epidermidis* which have high isolated percentage (81.5%) in this study. It is also the percentage isolation in Marples and Nester studies.

*S. epidermidis* was found on surface of skin as well as inside duct of skin that explain the high isolation rate also Nester showed that the chief importance of skin's staphylococcus is probably in preventing colonization by other pathogens and in maintaining the balance among the skin's various flora. These gram-positive cocci have been found to produce antimicrobial substances highly active against *P. acnes* and other gram-positive bacteria, and that *P. acnes* ferments the lactic acid produced by *S. epidermidis* to form propionic and acetic acid.

All these factors and living of *P. acnes* inside hair follicle lead to hard difficulty of isolation of *P. acnes* from skin that lead to only three isolates (3.21%) in this study.

Two isolates of *Acinetobacter* were gram-negative had been isolated. These bacteria were widely distributed in population. This bacteria widely distributed in soil and water can occasionally be cultured from skin (Jawets, 2001). Tiny yeast almost universally inhabit the normal human skin.

Holland *et al.*, 1978 said that *P. acnes* as they live in association with the *S. epidermidis* and fungi species, thus the latter probably have some control over the growth of *P. acnes*.

Table (2 ): The number and percentage of all genera and species of isolates.

<i>Type of bacteria</i>	<i>Number</i>	<i>%</i>
Staphylococcus epidermidis	75	81.5

Staphlococcus aureus	8	8.7
Propionibacterium acnes	3	3.3
Acinetobacter	2	2.2
<i>Fungi</i>	4	4.4
<i>Total</i>	92	100

Only three isolates of *Propionibacterium acnes* were cultured from the lesion of patients with acne vulgaris. All isolates were obtained directly from the pus formed in the bottom part of the inflamed follicle.

In Iraq, this bacteria was isolated from patients with acne vulgaris in Baghdad in Iraqi studies (Sabri ) *et.al.*, 1999 and Al-Anbuky, 2000.

The most important characters of *P. acnes* colonies on anaerobic blood agar are circular enter convex, white or yellowish colonies with weak or no hemolysin.

Direct microscopic examination showed that the bacteria is gram-positive irregular bacilli, highly pleomorphic, showing curved clubbed, or pointed ends.

Perry and Staley, 1997 suggest that this non – sporforming bacilli had typically slow growth on obligate media, however, some strains aerotolerant, but better growth was seen when the bacteria cultured anaerobically, also they observed that these organisms up take the lactic acid fromed by the lactic acid bacteria fermentation and further metabolized it to probionic and acetic acid as well as CO<sub>2</sub>.

All isolates were grown an anaerobically on blood agar and on thioglycolate media. This bacteria can hydrolyze esculin,and it were Catalase and Indol positive, all these tests and others listed in table (3) which are listed in Colle *et.al.*, 1996

Table (3): Some morphological and biochemical test used in identification of Prpionibacterial acnes.

Test	Isolates
Growth on thioglycolate	+
Growth on blood agar	White to yellowish

Gram stain	+
Albert stain	+
Shape of cells	Bacilli, polymorphic
Hemolysis	B(Weak)
Catalase	+
Oxidase	-
Escoline hydrolysis	+
Indol production	+
Nitrate reduction	+
Glucose	+

Only one strain was found to produce hemolysis that come in conformity with Koneman et al.,1997 results that reported only one strain produce a narrow zone of hemolysis.

The isolates of *P. acnes* were tested for their possessing colonization factor antigens and some enzymes , the results showed that all isolates did not have the activity to produce the CFA/1 and CFA/2, as listed in table (4).

Table (4) : Detection of some virulence factors of *P. acnes*

Test	Isolate No.1	Isolate No.2	Isolate No.3
Capsule staining	+	+	+

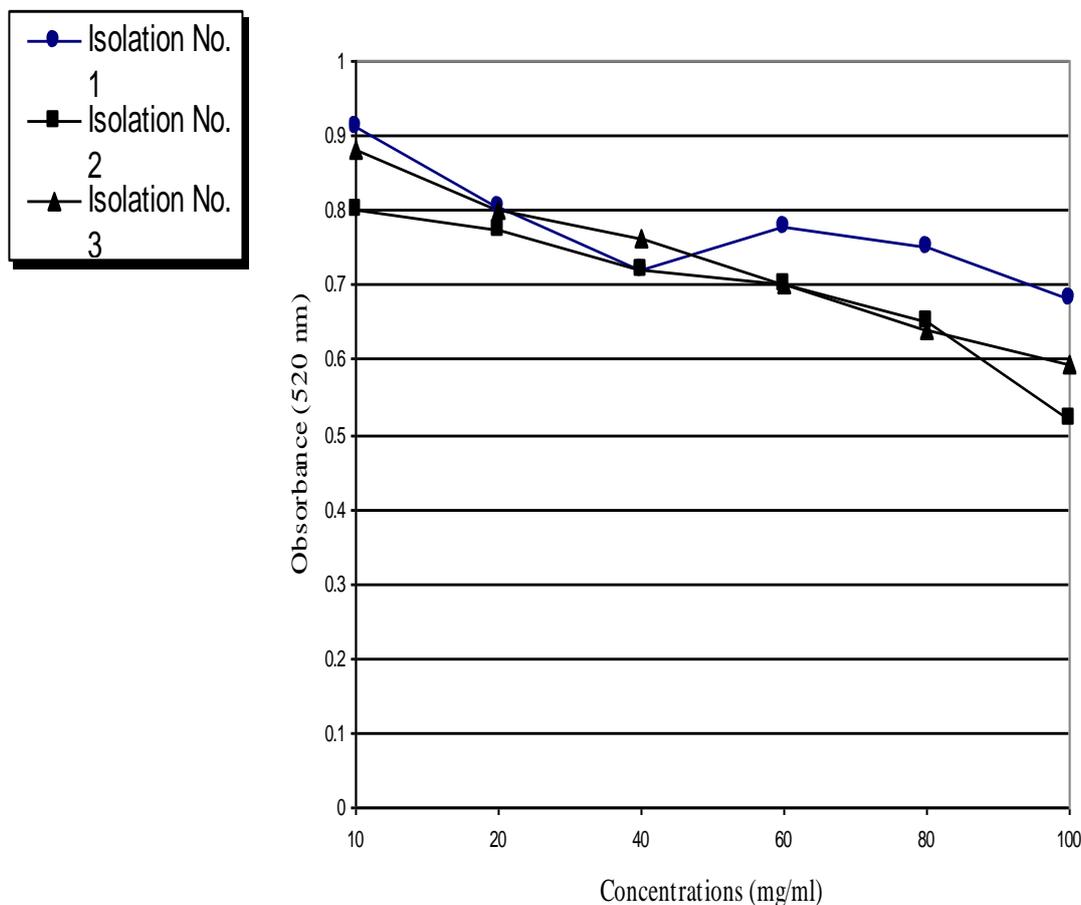
Hemolysin production	+	-	-
Sidophore production	-	-	-
CFA/I	-	-	-
CFA/II	-	-	-
CFA/III	+	+	+
Haemoagglutination test	-	-	-
Lipase production	+	+	+
Protease production	+	+	+

All isolates gave positive result in agglutination test with (group A) human RBCs in the presence of tannic acid which may refer to the fact that all isolates had the colonization factor antigen type III , which is a clear evidence that all strains of *P. acnes* have high activity in adhesion that increase the infectivity of human body.

The presence of lipase and protease contribute the virulence of the bacteria , lipase is a very important virulence factor of bacteria associated with acne vulgaris .Gribbon et al.,1993 reported that the adhesion to the internal surface of a continuous culture vessel in the presence of the free fatty acid, and proposed that triglycerides within nascent sebum, which contains no free fatty acid, were partly converted to free fatty acid by *P. acnes* lipase, assisting bacterial adherence and colonization of the sebaceous follicle and induce the development of inflammatory lesions.

*Propionibacterium acnes* also produce follicular proteases, several enzymes that be important in the inflammatory processes. These protease are essential in protein degradation and produces at optimal PH between 6-7 and at optimal temperature between 35-45 C (Alkhafaf,2005) .

**Figure (1)**  
**Effect of Retinoic acid on growth of *P. acnes* Isolates**



It was observed that the addition of Retinoic acid to the growth culture isolates causes decreasing in the growth of these isolates particularly at the concentrations above 20 mg/ml, while significant reduction of bacterial growth was markedly observed in the highest concentrations especially at 100 mg/ml. Retinoic decreases markedly the formation of sebum and reduces the population of *P. acnes*. Which in turn lead to the alteration of the follicular microclimate affecting the growth of *P. acnes*( Bershad,2001).

The effect of some antibiotics on *P. acnes* isolates were illustrated in table (5) which give a primary picture on the source of these isolates.

Prior to 1976, there were few reports of resistance of *P. acnes* to antibiotics, and recent studies, showed that resistance to erythromycin was the most common and less to tetracyclin and also doxycyclin .

Tetracyclin drugs exert favorable influence on acne ,they can inhibit the synthesis of bacterial enzymes including lipase .Furthermore ,neomycin, fusidic acid and gentamycin can be used in treatment of acne vulgaris ( Alkhfaf,2005).

Table (5): Sensitivity of *P.acnes* isolate to deferent antibiotics.

Antibiotics	Isolate No.1	Isolate No.2	Isolate No.3
Erythromycin	+	+	+
Tetracycline	-	-	-
Fusidic acid	+	+	+
Vencomycin	+	+	+
Neomycin	-	-	-
Chloromphenecol	-	-	-
Gentamycin	+	+	+
Lincomycin	+	+	+
Kephlexin	-	-	-
Deoxycylin	-	-	-

(+ Resistant) (- Sensitive)

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