The Effect of Lactic Acid Bacteria on Bacterial Causes of Severe Acne Vulgaris

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SUMMARY

Sixteen isolates of lactic acid bacteria were isolated from dairy products collected from Baghdad markets. The isolates were identified into 4 genera; *Lactobacillus* (6 isolates), *Lactococcus* (2 isolates), *Pediococcus* (4 isolates) and *Leuconstec* (4 isolates). Later, further identification of lactobacillus showed the isolates belonged to *Lb. plantarum* (Lb.1 and Lb.3), *Lb. bulgaricus* (Lb.4), *Lb. acidophilus* (Lb.2), *Lb. brevis* (Lb.5) and *Lb. fermentum* (Lb.6).

Fifty bacterial isolates were obtained from comedones and pustules of twenty patients that suffering from sever acne vulgaris. These isolates were identified as, three species of bacteria isolated from comedone (*Propionibacterium acne*, *Staphylococcus epidermidis* and *Staphylococcus aureus*), and five species isolated from pustules (*Staphylococcus epidermidis*, *Propionibacterium acne*, *Proteus mirabilis*, *Staphylococcus aureus* and *Escherichia coli*).

Antibiotic sensitivity test was performed for *Lb. plantarum* (Lb.1 and Lb.3) isolates. Results showed that both isolates were resistant to streptomycin, penicillin G and trimethprim, but sensitive to amoxicillin, chloromphenicol, cloxacillin, rifampin and ampiclox. Regarding the bacterial isolates causing acne vulgaris, they were sensitive to doxycycline, ciprofloxacin, neomycin, clindamycin and rifampin, but resistant to ampicillin, amoxicillin, ampiclox, methoprim, tetracycline and cefalexine.

The inhibition activity of *Lb. plantarum* (Lb.3) against acne vulgaris isolates was tested on solid and in liquid media. It was found that Lb.3 isolates when grown on solid media had better inhibitory activity against all pathogenic isolates, but the incubation period 36 hr. was chosen as the preferred period in this approach while the result of Lb.3 isolate when grown in liquid media showed that Lb.3 isolate had inhibition activity against gram positive bacteria

tested at 36, 48 and 72 hr. but gram negative bacteria was not effected. When the filtrate was concentrated to 3 times fold, it became had showed markedly inhibition activity against all bacterial isolates of sever acne vulgaris.

When bacteriocin of Lb.3 isolate was extracted and purified to test it's inhibition activity against the sever acne vulgaris isolates, result showed that it had inhibitory effect on Gram's positive and gram negative bacteria. However, the activity greater on gram positive than on gram negative bacteria.

Protein quantity of concentrated filtrate and the bacteriocin was determined. Results showed that it was equal to 4 mg / ml in filtrate and 5 mg / ml in the partially purified bacteriocin.

Minimum inhibitory concentration of concentrated filtrate was estimated and showed that 80 % was the preferred value that inhibited all type of bacterial causes of sever acne vulgaris (*Staphylococcus epidermidis*, *Propionibacterium acne*, *Proteus mirabilis*, *Staphylococcus aureus* and *Escherichia coli*) and used in treating the patients.

The result of treatment showed that after 2 weeks of treatment with filtrate, acne completely or almost completely healing in most patient (up to 95 %), with the presence of simple skin dryness as the only reported side effect during the period of treatment.

List of Abbreviations

ATCC	American Type Culture Collection
D. W.	Distilled Water
FFAs	Free Fatty Acids
LAB	Lactic Acid Bacteria
MIC	Minimum Inhibition Concentration
MRS	Modified Regosa Agar
NCCLS	National Committee of Clinical Laboratory Standard

List of Contents

Chapter one

Introduction

1-1 Introduction

Acne vulgaris is chronic inflammatory disease of the pilosebaceous follicles. It s characterized by comedones, papules, pustules, cysts and nodules, chiefly in certain sites seborrhoeic area of the skin such as the face, chest, upper arms and back (Arnold and Odom, 1982; Ebling and Cunliffe, 1986).

Acne vulgaris, the most common of all skin disease, is a disease of adelescent, which usually resolve by the mid twenties and is of multifactorial etiology (Cunliffe and Cotterill, 1975; Stern *et al*., 1977).

The literature on the acne problem is full of disputes, contradictions and confusions, even the term "Acne" and its origin is unsolved (Kligman, 1974).

Ionthoi (ionthoi mean the first growth of the beard) was the Greek term for acne and was used by Hippocrates. Roman Physician called the disease "Varus". Actius (542 A. D.) first used the word "Acnae". In the third century A. D. Cassius called it "ACME" which means the height of any thing including growth. He mentioned that these spots appear on the face at the time of "ACME"; so it has been suggested that acne has been arisen by an error in transcription from "ACME". The Egyptian word "Aku-t" meaning boils, pimples and pustules may have been accepted by the Greek as a loan word. Wilson in 1842 was the first physician to use the term "Acne Vulgaris" which include the simplex, punctuate and indurate varieties which was previously used to describe acne (Cunliffe and Cotterill, 1975; Goolumall and Andison, 1977).

The discovery of acne bacillus, in acne lesions at 1893 by Unna, paralyzed all researches in the field for a bout thirty years. The organism named an aerobic diphtheroids" *Corynebacterium acnes*" and nowadays called *Propionibacterium acne*.

Later on, in 1903 it was declared that the organism had been proved to be the causative agent of acne (Cunliffe and Cotterill, 1975; Voss, 1976).

In regard to treatment, Hebra in 1868 claimed that he had been unable to find a remedy to prevent acne developing or to banish it once established, but he prescribed that "have to reduce the cutaneous glands to their normal function. So as to prevent their taking on that inflammatory action which lies at the root of the disease". Various attempts were made to treat acne; including diet restriction, marriage, sun light, metals and spaced doses of UV.

Flemins in 1909 used vaccines containing acne *Bacillus* and *Staphylococcus* (Cunliffe and Cotterill, 1975; Voss, 1976; Leyden, 1976; Kerrigan, 1985).

A great attention was made to use microorganism or their metabolites in treatment of some disease. So bacteria and yeast considered as the first two types of microorganisms used in this approach. Among bacterial group is the *Lactobacillus* spp. Which have a great role in probiotic, due to it's presence in mucus membrane of intestine and digestive tract of human as normal flora. Safe used in food industry and it's ability to produce inhibited materials such as organic acid, H_2O_2, CO_2, amino acid, di-acetyl, acetaldehyde and bacteriocins (Runar, 1998).

Despite that several studies concentrated on the use of *Lactobacillus* in treatment of many diseases such as gastrointestinal distribution, hepatic, encephalomegaly, antitumor activity, lactose intolerance and pelvic radiotherapy (Gilliland, 1990; Well *et al*., 1996).

No local study was dealt with using it as topical treatment for sever acne vulgaris for such reason, this study was performed.

1-2 Aims of the Study

1. Isolation and identification of the bacterial cause of sever acne vulgaris.
2. Isolation and identification the *Lactobacillus plantarum* as probiotic bacteria.
3. Studying the inhibition effect of the probiotic bacterial filtrate and its bacteriocin against the bacterial cause of sever acne vulgaris in vitro.
4. Preparing of solution from the probiotic bacteria filtrate and using in treatment the patient that suffering from sever acne vulgaris.

Chapter Two

Literature review

2.1 Anatomy and Physiology of Sebaceous Glands:-

Skin is divided anatomically into three layers, the epidermis, the dermis and the subcutaneous tissue. Accessory epidermal structures include hair follicles, sweat glands, sebaceous gland and nails (Kirby, 1986).

The target organ in acne" sebaceous glands" are present on most areas of the skin (Kligman, 1974).

The sebaceous gland develops about the 12^{th} week of intrauterine life as a rudiment of hair follicle.

The gland is situated in the upper half of the dermis, wrapped round the middle-third of a hair follicle and connected to it by a duct. Through which their secretion gains access to the surface of the skin. See figure (2-1).

The secretion of the sebaceous gland" the sebum" is said to be the first demonstrable glandular secretion of human body. This section is holocrine and is not under nervous control and takes place continuously. The production of sebum being minimal in infancy, suddenly increasing at puberty and remaining fairly constant throough out adult life and decreases again toward old age. Oestrogens cause a decrease in sebaceous activity but only when administered in enormous and entirely unphysiological amounts (Kligman, 1974; Rosenfield, 1986; Kirby, 1986).

2.2 Microbiology of Skin:-

The skin is the largest organ of human body in adults, weighting (5 Kg) and having an area of about (1.75sq. meters). No area of this organ appears to be without a resident microbial flora and all areas have a transient or contaminant flora of bacteria and fungi. Price in 1938 distinguished the "transient flora" consisting of multivarious organism dependent on personal hygine and environmental conditions, from the "resident flora" consisting of organism that more or less constantly present (Noble and Somerville, 1974).

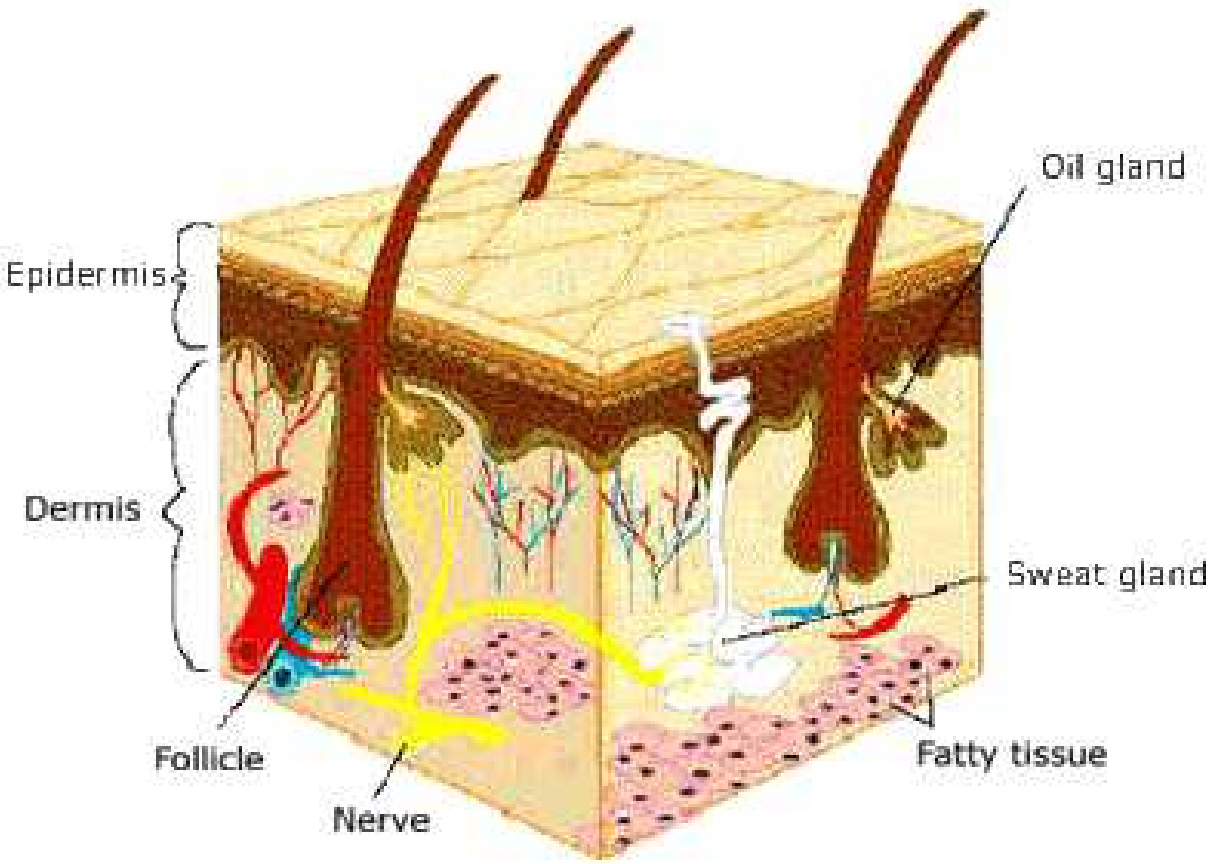


Figure (2-1) Normal Skin

(Orentreich and Durr, 1974)

At birth, the skin flora consists of *staphylococcus epidermidis*, diphtheroid bacilli with few coliform and *Proteus*. Occasional streptococci may be seen. They all may be derived from birth canal (Sarkany and Gaylard, 1968; Alyazachi, 1977).

The healthy skin appears to have some natural self-disinfecting mechanisms that are responsible for disappearance of living organisms implanted on it (Wilson (A), 1983).

The skin flora consisting mainly of: Aerobic and an aerobic bacteria; diphtheroids are classified now a days in the genus propionibacterium. Healthy skin carries large number of this diphtheroids. Evans in 1968 concluded that *Propionibacterium acne*s out numbers the aerobic flora by (10-100) folds; mostly in areas were there are large sebaceous follicles (Marples, 1969; Somerville and Murphy, 1973).

It seems probably that number of *P. acnes* decrease with age as sebaceous activity is reduced in older age group.

Matta (1974) has found that *P. acnes* are rare on the forehead of prepubertal children but increase in number at time or slightly before out ward of puberty are evident. The distribution of *P. acnes* on the skin was studied and found to be in this sequence: forehead axilla, scalp, forearm, shoulder and ear lobe (Marples, 1969; AL-Yazachi, 1977).

Diphtheroid may be isolated from the skin in virtually all parts of the human body, although age plays an important role in determining the incidence of these organisms (Noble and Somerville, 1974; Matta, 1974).

Member of the micrococcus from the second major flora of human skin, they include the genera staphylococcus and Micrococcus.

S. aureus, the pathogenic member, is found in a relatively small number. It is found in (8-20 %) of samples taken from various parts of the skin and in about 40 % of samples from the hand. Skin carriage is usually associated with nasal carriage, it is present in the nostrils of about 25%; such individuals being referred to as carrier (Wilson (B), 1983; Kirby, 1986).

Among the coagulase negative *Staphylococus* species, *S. epidermidis* and *S. homonis* are the most important species found in moist areas, but *S. homolyticus*, *S. saprophyticus* and *S. capitis* have also been isolated from another area. Organism of the genus *Micrococcus* are less numerous than *Staphylococcus* on healthy skin, but are relatively more important on sparsely populated dry sites and in children. *Micrococcus variant* appears to be the dominant species (Nobel and Somerville, 1974; Roberts and Highet, 1986).

Among other microorganisms found as part of skin flora are gram negative rods. They are comparatively rare on normal healthy skin. Customary, in clinical microbiology, these are divided into the following groups: *Proteus, Pseudomonas, Enterobacter aerogenus, Escherichia* and Coli forms. These organisms were isolated from axilla, groin and toewebs.

It was found that children and adults carry Gram negative bacilli more often than do infants or the aged (Somerville, 1969; Somerville and Nobel, 1970; Nobel and Somerville, 1974).

It's generally accepted that *Streptococci* do not form part of the normal skin flora, but although this is true in most circumstances of beta-hemolytic *Streptococci*. Other groups may be founded as resident flora in certain site or in certain group of individuals; alpha and gamma hemolytic *Streptococci* were isolated from healthy skin except on nail and axilla, and were more common in infants than in older age groups (Somerville, 1969; Noble and Somerville, 1974).

Other cutaneous bacteria that are found either as transient or resident flora of skin were aerobic spore-forming bacilli, a typical *Mycobacterium* and *Neisseria* species. Fungi are frequently isolated from the skin. Among other organisms the lipophilic organism "*Pityrosporum* species", *Pityrosporum ovale* is found on areas of skin which are particularly well supplied with sebaceous glands. It's most commonly isolated from the scalp and face but can also be found on the trunk and arms (Noble and Somerville, 1974; Wilson (B), 1983).

2.3 Bacterial Causes of Acne Vulgaris:-

It's generally considered that the acne microflora of sebaceous follicle, whether normal or acne affected, is limited to four main genera of organisms:

2.3.1 Propionibacterium:-

Propionibacterium was first described by Unna 1896 and cultivated by Sabourand 1897. It was designated as acne bacillus or *Corynebacterium acne*; Douglas in 1946 suggested change the name to propionibacterium due to the ability to ferment glucose producing propionic and acetic acid (Voss, 1976; Holland *et al*., 1981).

The bacteria are G (+), non-motile rods with atypical diphtheroid appearance "club-shaped" and in some cases show evidence of rudimentary branching. It's anaerobic or aerotolerant. Three species are now generally acknowledged as members of the resident flora in adults. *P. acnes* and *P. granulosum* are wide spread but particularly associated with follicles which have large sebaceous glands, over face and upper truck; they have both been associated with acne lesions. The third species *P. avidum* is found in moist sites; particularly the axilla and groin; it is pathogenic potential is unclear (Allen, 1985; Roberts Highet, 1986).

P. acnes forms delicate rods staining unevenly, non-motile, grows best on a slightly acidic medium under anaerobic conditions. After five days incubation at 35˚C, colonies are circular (1-2 mm) in diameter, convex and creamy or yellow. Growth is favored by glucose, glycerol, blood, serum and oleic acid. The culture has a sour smell; forms acid from glucose, dextrin and glycerol but not from lactose or sucrose. *P. acne*s gives α-lysis of human and rabbit blood. Its oxidase and catalaze positive, urease negative, indole production is variable (Wilson (A), 1983; Allen, 1985).

Propionibacterium species isolated from the skin have been reported to produce a variety of exocellular enzymes such as hyaluronate lyase, lipase, neuraminidase, phosphates, protease, gelatinase, lecithinase, hemolysin, DNAs

and RNAse. There are evidences that lipase activity is produced by propionibacterium in vivo, while other enzymes produced by bacteria in vivo are not yet known (Marples *et al*, 1971; Weeks *et al*, 1981; Yee and Cunliffe, 1994).

2.3.2 *Staphylococcus* and *Micrococcus*:-

Staphylococcus is G (+) cocci, occurring singly, in pairs or clusters. They are non-motile, non-spore forming and unencapsulated. Staphylococcus is classified primarily on the basis of coagulase production. The coagulase positive species are more important from pathological point of view; although coagulase negative species form the most common flora of the skin. Among coagulase negative *S. epidermidis* is the most common in normal and acne affected skin, and is isolated from most sites of the body (AL-Yazachi, 1977; Kloos and Jorgensen, 1985; Cunliffe and Simpson, 1998).

Micrococcui are usually coagulase negative, and can be differentiated from coagulase negative *Staphyylococci* by their resistance to lysostaphin (Benzidine test), Bacitracin susceptibility and presence of cytochrome c (as determine by modified oxidase test), furthermore coagulase negative staphylococci are able to produce acid from glucose an aerobically (Faller and Schleifer, 1981; Falk and Guering, 1983; Kloos and Jorgenson, 1985).

2.3.3 Diphtheria:-

In Spite of existence the aerobic cocci and diphtheroids in acne lesions, they form the main flora of normal skin in acne bearing areas. The normal skin flora has been shown by electron microscopy to reside in the hair follicle and on the surface keratin near their orifices, which from a good distribution for their involvement in acne patients with sever acne have higher than average counts of both groups of organisms on the forehead skin (Montes and Wiborn, 1970; Jawetz *et al*, 1989; Brook, 1998).

Each phase of acne lesion needs to be looked separately to assess the role bacterial flora. Several studies have described proportions and distributions of

different organisms in different acne lesions (Marples and Izumi, 1970; Brook, 1998).

2.4 Etiology of Acne:-

The definite etiology of acne is still not well established, however, the cause of acne is obvious to so many, but clinically explained by no one for generations. Texts dealing with acne discussed it's etiology by focusing on poor personal hygiene, bad diet, anxiety and too many hormones Although the current view have been directed toward more scientific areas, the number of possibilities still remains imposing and many etiological factors have been implicated (Cunliffe and Cotterill, 1975; Rasmussen, 1983; Haslett *et al*., 1999).

2.4.1 Hormones (Androgens):-

Acne usually beings when the body starts to produce hormones called androgens. When androgen production goes into high level (about age 11 to 14 years) acne also goes into high level. Androgen causes the sebaceous gland to enlarge, and this is normal. People who develop acne have sebaceous glands that are over – stimulated by androgens- Young women tend to have acne flora –ups that coincide with the hormonal changes associated with their menstrual cycle. These changes affect the sensitivity of their sebaceous glands to androgens (Arnold *et al*., 1990; Freedberg *et al*, 1999).

2.4.2 Increased Sebum Production:-

After the sebaceous gland is stimulated by androgens, it produces more sebum. The oily sebum accumulates in the follicle, and travels up the hair shaft to the surface of the skin when the hair shaft also, mixes with normal skin bacteria and dead skin cell, that have been shed from the lining of the follicle. The greater the sebum production the greater the likehood, that the hair follicle

will become clogged and result in comedones (Voss, 1976; Ebling and Cunliffe, 1986; Thiboutot, 1997).

2.4.3 Changes Inside The Follicle:-

As androgen production increases and sebaceous glands enlarge, the inner lining of skin in the hair follicle also changes. Normally, dead cells inside the follicle shed gradually and get expelled onto the surface. However, during puberty, these cells are shed more rapidly and tend to stick together, when mix with sebum, they can clog the follicle. The cells and sebum form a plug in the follicle (Hunter *et al*., 1990; Haslett *et al*., 1999).

2.4.4 Microorganisms:-

In spite of all emphasis placed on the bacterial flora of the facial follicles, its not likely that acne is purely an infectious disease (Rasmussen, 1978).

The most common and important organism in acne as shown by selective antibiotic studies, is *P. acnes* surface counts of organisms are probably irrelevant in the etiology of acne. In most instances there is no correlation between acne severity and surface counts of *S. epidermidis* and *P. acnes* (Narples and Kligman, 1971; Cove *et al.*, 1980; Leeming *et al*., 1984).

The development of inflamed lesions probably depends upon ductal colonization. To understand the morphogenesis of acne, it's necessary to examine ductal organism. In inflammatory acne lesions (by using a sensitive immunoflorescent technique to locate live or dead *P. acnes*) it was found that there is intense deposit inside follicular canal, but only small amounts in the surrounding dermis were seen. This would indicate non-invasiveness, so the impact of *P. acnes* on the acne process is therefore, not dependent on its invasiveness but on its products (Leeming *et al*., 1984).

The evidences implicating *P. acnes* in the pathogenesis of acne are:

a- Large number of *P. acnes* accumulates in follicles immediately before comedo formation (Kligman, 1974).

b- *P. acnes* produce substances capable of inducing comedo formation in the rabbit ear model (De Young *et al*., 1984).

c- *P. acnes* are responsible for the liberation of FFAs which are comedogenic and capable of inciting pronounced inflammatory reactions (Lygden, 1976).

d- In the absence of *P. acnes*, inflammatory lesions are not seen and antibiotics which suppress *P. acnes* are beneficial in therapy and decrease comedo (Kligman, 1974; Leyden *et al*., 1975).

"Microbial lipase theory ": the most intensively tested was the role of bacterial lipases in the pathogenesis of acne. This theory was based on the idea that lipases produced by *P. acnes* in vivo, hydrolyzed native sebum to produce FFAS, which are both comedogenic and irritant. Support for this theory came from antibiotic studies in which a decrease in percent FFAs in skin surface lipids correlates with an improvement of acne in treated individuals (Cunliffe *et al*., 1973; Holland *et al*., 1981).

In the 1970s the microbial lipase theory came under increased criticism. Isolates of *P. acnes* from acne patients were no more lipolytic in vitro them those from non-acne individuals and it reported that the bacterial lipase inhibitors were unable to control acne vulgaris (Weeks *et al*., 1977).

Important bacterial products produced by *P. acnes*, other than lipase, might include protease, neuraminidase, hyaluronidase and lecithinase. The inflammation produced by intradermal injection of comedones may reflect prior production of toxins or irritants by microbial flora thus it seems quite likely that *P. acnes* and possibly staphylococci and pityrosporum as well may contribute to the pathogenesis of acne lesions other than or in addition to the release of FFAs (Voss, 1976; Holland *et al*., 1981).

Holland *et al.* (1978) proposed that an entirely different approach should be taken to understand the role of bacteria in acne taking into account the following factors:

a- Even in the severest grades of acne only a limited number of the total pilosebaceous until exhibit acne at any one time (Cunliffe and Cotterill, 1975).

b- *P. acnes* are found both in acne and normal pilosebaceous units (Holland *et al*., 1977).

c- There is no dose response, in that more bacteria are isolated from subjects with sever acne (Leyden *et al*., 1975).

d- The growth of bacteria in the hair follicles must be regarded as a continuous culture system, not a batch system because cells are continually removed by natural physical means and being replaced by growth and division of bacteria remaining. (Holland *et al*., 1978).

e- Alteration in the environment within a continuous culture system may not necessarily influence the number of bacteria present if the rate of loss of cells also changes but the growth rate , and the physiology of the bacteria will change.(Holland *et al*.,1978).`

It's suggested that the role of bacteria might be explained by the change of bacterial physiology in response of microenvironmental changes in the pilosebaceaous units. The physiological changes of importance in acne may be growth rate, production rate of exoenzymes and soluble antigens such as lipase, hyaluronidase and protease, and the production rate of directly irritate metabolites. The possible microenvironmental changes from one pilosebaceous unit to the other unit may be pH value, oxygen, carbondioxide tension, limiting nutrient supply, moisture availability and inhibitor substances. The theory has its appeal in that it can account for adjacent follicles being normal or of the acne type, while still possessing similar numbers of the same bacteria (Holland *et al*., 1978; Leeming *et al*., 1984).

2.5 Role of Bacteria in Acne Inflammation:-

There are considerable amount of evidences to implicate *P. acnes* in the pathogenesis of inflammatory stages of acne vulgaris. The potential of *P. acnes*

in evoking an inflammatory response was shown by Kirschbaum and Kligman in 1963 who demonstrated that live *P. acnes*, but not *S. epidermidis*, injected into sterile steatocustoma proliferate vigoursly and converted these quiescent cysts into inflammatory lesions. The mechanisms by which *P. acnes* may cause inflammatory response are unknown. There are, however, several theories to explain how *P. acnes* could initiate and maintain the inflammatory response in acne lesions. Modification of the immune status is one of these theories. For nearly two decades, it has been recognized that patients with acne have some unusual immunologic reactions (Holland *et al*., 1981).

Antibody titers to *P. acnes* increase with age, this rises by the agglutinating antibody levels correlates well with the increase in colonization of individuals by *P. acnes* at puberty. Before puberty, the antibody class is almost exclusively IgM and therefore is confined to blood vessels. During adolescence there is a switch from IgM to IgG which readily penetrates tissues. Higher level of complement fixing antibodies to *P. acnes* occur in patients with sever acne, compared with those patients having mild or no acne, while increased antibody level to *S. epidermidis* do not occur in acne patients (Puhvel *et al*., 1965; Leyden *et al*., 1975; Gowland *et al*., 1978).

A possible mechanism for initiation of inflammation would be *P. acnes* antigens combining with antibody and activating complement in the dermis. In addition soluble proteins produced by *P. acnes* as proteases may also cleave C_3 to activate the alternative pathway. This is demonstrated by detecting immuno-globulins (IgG, IgM and IgA) and C_3 in the follicular epithelium by immunofluorescent technique (Dahl and Mc-Gibbon, 1979; Holland *et al*., 1981).

It's suggested that cellular immune mechanisms are a late event and that complement activation is an early one in the pathogenesis of inflammation; however, there is a correlation between cell-mediated immunity to intradermally injected *P. acnes* antigens and the severity of the patient's acne (Scott *et al*., 1979; Kersey *et al*., 1980).

Puhvel and Sakamoto (1978, 1980) described *P. acnes* derived cytotoxin; a low molecular weight compound which is a potent chemoattractant from polymorphonuclear neutrophils (PMN); the diffusion of it through the follicular epithelium could explain some of the early stages of inflammation in acne, fig (2-2 and 2-3). Extracts of *P. acnes* are also known to be a potent activator of macrophages.

It also produces prostaglandin-like substances which might have role in inflammatory acne (Russel *et al*., 1978; Mills and Kligman, 1983).

The arrival of PMN cell directly and under the influence of complement would allow phagocytosis of bacteria and the release of lysosomal enzymes that would then modify the follicular epithelium. The content of the follicle might thus pass into the dermis; it can create inflammation by foreign body reaction undoubtedly induced by the lipids, hairs and horny cell and being characterized by infiltration with mononuclear cells, macrophages and giant cell (Cullen, 1985).

2.6 Pathogenesis of Acne Vulgaris:-

Acne is a polymorphic disease which exhibits a series of diverse lesions: comedone, papules, pustules, cysts and scars. Despite this diversity, the entire display can be reduced to a two-stage sequence. First, a sebaceous follicle transforms into a comedone without any inflammatory component, then disorganization of the epithelial capsule in whole or in parts and the attempts at healing that follows give rise to all the other lesions of acne (Kligman, 1974).

Acne develops in the sebaceous follicles and begins by an altered keratinization process of the follicular canal results in obstruction of the pilosebaceous unit. When the normal flow of sebum onto the skin surface is obstructed by this follicular hyperkeratosis, comedones are formed which thus starts the process of acne (Hurwitz, 1974).

Two types of comedones are formed, open comedones and close comedones, see figure (2-4 and 2-5).

Figure (2-2) Invasion of follicle by neutrophils (Rubin and Farber, 1998)

Figure (2-3) inflammation and rapture of sebaceous follicle (Rubin and Farber, 1998)

Figure (2-4) Open Comedons (Rubin and Farber, 1998)

Figure (2-5) Close comedones (Rubin and Farber, 1998)

The open comedone is composed of epithelium lined sac that is filled with keratin and lipid. It has a widespread dilated orifice, with a role of melanin in the genesis of the black head tips. The contents of open-comedones easily escape to the skin surface; follicular disruption and inflammation therefore rarely occur (Strauss and Kligman, 1960; Kaidbeg and Kligman, 1974).

The closed comedo has a microscopic opening that keeps its content from escaping, it continuous to form keratin and some sebum, and when the follicular wall ruptures expel sebum into surrounding dermis, thus stating the inflammatory process (Strauss and Kligman, 1960; Klignam, 1974).

The clinical appearance of the resulting inflammatory lesion is dependent not only on size of the comedo in which the rapture occurs, but also on the location of the inflammatory reaction in the dermis. If the inflammatory nidus enclosed to the surface the lesion will be pustule, deeper inflammation result in a larger papule or nodule (Pochi, 1977).

2.7 Clinical Features of Acne Vulgaris:-

Acne vulgaris is an almost universal accompaniment of adolescence. The incidence in adolescence varies between 30-85 % (Emerson and Strauss, 1972; Cunliffe and Cotterill, 1975).

Acne develops earlier in females than in males. This may reflect the earlier onset of puberty in females. However, some subjects may show small non-inflamed lesions by the age of (8-9) years (Burton *et al*., 1971).

The age of greatest incidence and severity is (16-18) years for women and (18-19) years for men. There after, the acne resolves slowly, however, some patients still have acne worthy of treating up to age of (25-35) years (Burton *et al*., 1971; Ebling and Cunliffe, 1986).

Jones (1988) reported that in 5 % of females, acne is persistent and require therapy until the age of forty. While, other studies indicate that sever acne involvement is more common in males (Strauss, 1979).

In young males acne predominantly affects the face and in older males the back. The clinical varieties of acne vulgaris are either non-inflamed lesions which are more frequent in young patients and consist of blackhead, whiteheads or intermediate non-inflamed lesions which show features of black heads and white heads (Burke and Cunliffe, 1984), or inflammatory lesions which include superficial and deep lesions, figure (2-6) (Orentreich and Durr, 1974; Burk and Cunliffe, 1984). The severity grading of inflammatory lesions explain in table (2-1) (Habif, 1996).

Acne usually appears as a variety of lesions in which comedone is pathognomonic. In its mildest form, it's limited to open and closed comedones. As the disorder increases in severity papule, pustule, nodule or cysts may develop in the patient. Nodules and cysts are disfiguring (Hurwitz, 1974).

Scaring of acne is of two sorts and follows only those lesions which have affected deeper dermis: firstly, hypertrophic scar, which in some patients may become frankly keloidal mostly on trunk and secondly consists of superficial atrophic depression (ice-pick scars) mostly on the face (Cunliffe and Cotterill, 1975; Ebling and Cunliffe, 1986).

Acne associated clinical features are: Seborrhoea which may be related to the severity of acne, a greasy scalp, a frequent complains as is dandruff, itching of acne spots, hirutism and melasma which are seen in few female patients and hidradeitis suppurative (Leyden, 1976; Reingold and Rosenfied, 1987).

Table (2-1) Severity grading of inflammatory lesions

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

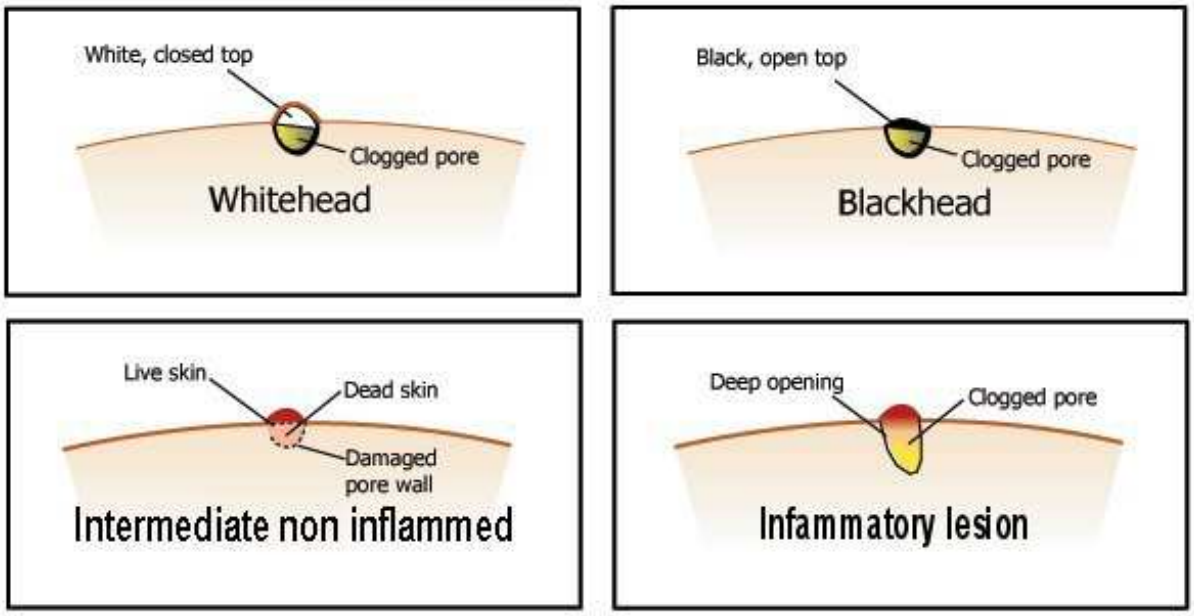


Figure (2-6) The clinical varieties of acne vulgaris

(Orentreich and Durr, 1974)

2.8 Differential Diagnosis:-

Despite that acne is rarely misdiagnosed, some lesions which like acne should be differentiated, and they are: a) Rosacea which occur in an older age group. There are no comedones, without nodules, cysts and scarring. Occasionally, patients may have both rosacea and acne. b) Acne form drug eruptions. c) Milia d) Multiple facial warts e) Adenoma sebaceum f) pseudofolliculitis barbac g) Acne agminata (Cunliffe and Cotterill, 1975; Rasmussen, 1978; Ebling and Cunliffe, 1986).

2.9 Resolution of Acne:-

Acne finally resolves essentially in all cases, in spite of the continued production of sebum and presence of large numbers of bacteria in follicles (Marsden *et al*., 1987).

Remission of acne is unlikely to be due to reduction of changes in composition of sebum. The immune response to *P. acnes* may serve to inhibit the function of bacterial products within the follicle, and thus to limit the development of pathological changes. Alternatively, long-continued exposure to irritants may result in accommodation or hardening of the follicular epithelium, so that no longer respnding to the damaging effect of bacterial products in spite of their continuous presence (Mcosker and Beck, 1976; Voss, 1976).

2.10 Treatment of Acne:-

Acne is the most treatment disease. Mild acne vulgaris requires only topical therapy, while patients with moderate or sever acne need both oral and topical therapy. The most widely used topical therapy are benzoyl peroxide, vitammine A derivatives, and antibacterial agent. The last which include (retinoic acid, antiandrogens, zinc sulphate and certain non-steroid anti-inflammatory agents), constitute the main oral therapeutic regimen (Weimar *et al*., 1978; Swinyer *et al*., 1980; Clanachan *et al*., 1985; Ebling and Cunliffe, 1986).

The main drugs used in the treatment of acne are acting through: a) Reducing sebum production (ostrogens, antiadrogens and retinoic acid) b) Reducing the pilosebaceous duet obstruction (ultraviolet radiation, salycillic acid, sulphur, bezoyl peroxide and vitamin A derivatives) and c) Modification of the follicle microbes and surface lipid composition as antibiotics. (Cunliffe and Cotterill, 1975; Rasmussen, 1983).

2.11 Acne Treatment by Antibiotics:-

2.11.1 Topical Antibiotics:-

Clindamycin, erythromycin, tetracycline and chloramphenicol in concentration are the most commonly used. They are effective to treat moderately sever inflammatory facial acne.

Recovery takes (8-12) weeks of treatment. Topical antibiotics are also equal, but not superior to tetracycline orally for example a 500 mg daily of oral tetracycline is similar in effect to 1% topical clindamycin and (1.5-4%) topical erythromycin (McKenzie *et al*., 1981; Jones and Crumley, 1981; Katsambs *et al*., 1987; Hirschmann, 1988).

The development of antibiotic resistance by *P. acnes* might diminish the efficacy of topical antibiotics in treating acne vulgaris. When topical erythromycin and clindamycin were used for (4-8) weeks, antibiotic- resistant staphylococcus strains increased after therapy initiation, then deceasing when medication discontinued. But *P. acne* remained susceptible (Bernstein and Ahalits, 1980; Borglund *et al*., 1984).

Antibiotic resistance of cutaneous bacteria has not yet emerged as a clinically important problem; However, the potential seriousness of development of resistance and the availability of equally effective, alternative topical agents provide enough reason eschew the use of topical antibiotics for treating acne or restrict them to patients who have failed to respond to other medication (Swinyer *et al*., 1980; Eady *et al*., 1981; Hirschmann, 1988).

2.11.2 Systemic Antibiotics:-

Oral antibiotic are the main therapy used for server moderate acne, including: tetracycline, erythromycin, cotrimoxazole, minocycline and clindamycin. Tetracycline is the drug of choice, erythromycin is satisfactory while clindamycin is the most useful due to its lipid solubility, but it should not be used routinely because of the possible risk of pseudomemberane colitis (Rasmussen, 1978; Ebling and Cunliffe, 1986; Jones, 1988).

Tetracycline and erythromycin are bacteriostatic, especially in large doses. It may also inhibit various enzyme activities and affect chemotaxis and lymphocyte function (Marples and Kligman, 1971; Webster *et al*., 1981; Esterly *et al*., 1984).

Voss (1974) stated that tetracycline action in reducing FFAs level in surface lipids is due to inhibition of *P. acnes* lipase. Any improvement of acne through lowering of FFAs release by tetracycline would be attributable more to inhibit bacterial growth or enzyme synthesis (Marples and Kligman, 1974; Webster *et al*., 1981).

Acne patient treated by tetracycline or erythromycin should be given in dose up to one gram daily for a minimum of six months. However, not all patients respond equally, young males with marked seborrhea and trunkal acne respond less (Leyden *et al*., 1982; Ebling and Cunliffe, 1986; Jones, 1988).

Systemic tetracycline may produce antibiotic resistant bowel flora and vaginal candidiasis in long term therapy (Adams *et al*., 1985).

Gram negative folliculitis due to *Enterobacter*, *Klebsiella* or *Proteus* bacilli colonization may complicate acne treated with long- term antibiotics mainly tetracycline (Leyden *et al*., 1979; Domonkos and Odom, 1982).

2.12 Potential Use of Probiotic:-

2.12.1 Probiotics Definition and History:-

Probiotics (Greek: for life) are defined as mono or mixed culture of live microbes that when applied to animal or human posses beneficial effect on health of the host. These beneficial effects include disease treatment and prevention as well as improvement of nutrients digestion and absorption (Fuller, 1991).

Probiotic microorganisms are generally (but-not only) lactic acid bacteria (LAB) including *Lactobacillus acidophilus, L. casei. L. plantarum, L. bulgaricus and L. rhamnsus.*

Other such as bacillus and bifidobacterium spp as probiotic strains are also used. Several microbes have been used unintentionally in food production such as dairy products and fermented vegetable. Such fermented foods are popular due to their distinct characteristic in terms of taste and aroma. Probiotic microorganisms are used to improve food flavor, for their beneficial aspects in health restoration and for disease treatment. They proposed and used in a wide range of clinical trials, ranging from diarrhea disease to cancer prevention (Fuller, 1994; Kaur *et al*., 2001).

The term "Probiotic" was originally used by Lilley and still in 1965 for substance (s) that stimulates other microorganism growth. The meaning of this term has now been redefined and restricted to a viable microbial agent (s) which, when used in animal or man, beneficially affect the host possibly by improving the balance of the indigenous microflora (Fuller, 1991; Salminem *et al*., 1999).

Based on this meaning, several term such as "friendly", "beneficial", "healthy" bacteria are also commonly know for probiotics. The first study regarding beneficial effect of probiotics was carried out by Metchikoff in the early 1900 when he reported the favorable effects of soured milk in human and suggested that consumption of live microbes (possibly LAB) in such fermented milk may help improve the balance of the gut micro flora, since then, microbial

probiotics have gained an increasing interest and their use in now widely accepted (Fuller, 1994).

2.12.2 Probiotic Microorganism:-

Ideally, microbial probiotics should have a beneficial effect and not cause any harm to the host. Therefore, all strains must have been studied comprehensively prior to use in humans or animals and thus are given GRAS (Generally Regarding As Safe) status (table 1-2). Typically safety evaluation of a proposed or accepted probiotic strain (s) includes: Ability to produce metabolites, Colonization (or adhesion), Effect on the strain survival, Interactions with the host.

Within the LAB group, the genus *Lactobacillus* is the most widely encountered for probiotics (Harenarr *et al*., 1992; Greene and Klaenhammer, 1994; Reid, 1999).

The previously mention species of *Lactobacillus* posses several important properties are used as effective probiotic organism they are: Efficient adherence to intestinal epithelial cells to reduce or prevent colonization of pathogens (Reid *et al*., 1993; Berent *et al*., 1994; Sarem-Damerdji *et al*., 1995; Kirjavainen *et al*., 1998; Ouwehand *et al*., 1999), Production of metabolites to inhibit or kill pathogen (Reid and Burton, 2002) Nonpathogenic (Conzalez *et al*., 1995; Reid, 1999).

Table (2-2) Probiotics microorganisms and their safety status (Donohue and Salminen, 1996)

Organism	Infection potential
Lactobacillus	Mainly non pathogenic
Lactococcus	Mainly non pathogenic
Streptococcus	Opportunistic; only *S. thermophilus* is used in dairy product.
Enterococcus	Opportunistic; some strains exhibit antibiotic resistance.
Bacillus	Only *B. subtilis*, GRAS status, is report in probiotic use.
Bifidobacterium	Mainly non pathogen; some strains are isolated from human infections.
Propioibacterium	Dairy propionibacterial group is a potential candidate for probiotic.
Saccharomyces	Mainly non pathogens, some strain are isolated from human infection.

2.12.3 Lactic Acid Bacteria (LAB):-

The term lactic acid bacteria (LAB) firstly are associated with the milk souring organism. It was classify by Orla-Jensen in 1919 into four main spp. (*Streptococcus, Pediococcus, Leuconstoc* and *Lactobacillus*) they have common properties of G (+), non spore forms, catalase negative, anaerobic or aerotolarate, fastidious bacteria and produce lactic acid as main product of fermentation process (Hozapfel *et al*., 1998).

LAB classified by Bergy's manual in 1994 into: *Aerococcus, Lactobacillus, Leuconstoc, Pediococcus* and *Streptococcus.* Later it found that there are new kinds of LAB which have the ability to moving, so it is put into new species called *Vogococcus* spp. other newest *Tetragenococcus* to have the ability to tolerate high salt concentration (18 % NaCl). After that new kind of LAB not tolerates high acid concentration was named *Ornobacterium*. But in recent year, the modern classification of LAB was according to the sequence of rRNA and hybridization, for this reason lactic streptococcus divided into two genera; *Streptococcus* and *Lactococcus* (Axels son, 1995).

LAB was classified by Wood and Holzapfed (1995) into cocci (*Aerococcus, Enterococcus, Lactococcus, Pediococcus, Leuconestoc, Vagococcus, Tetragenococcus* and *Streptococcus*) and bacilli (*Cornbacterium, Lactobacillus and bifidbacterium*). LAB use in maintenance of food according to its ability to produce: acid (lactic acid and acetic acid), ethanol, H_2O_2, diacetyl, acetaldhyte and bacteriocin (Runar, 1998).

Also, it used in medicine for treatment of many diseases such as gastrointestinal distribution, hepatic encephalomegaly, antitumor activity, lactose intolerance and pelvic radiotherapy (Suskovic *et al*., 1997).

2.12.4 LAB Bacteriocins:-

The study of bacteriocin begins with the discovery of Gratia in 1925 to specific antibiotic was produced from *E. coli* when found that it inhibited another strain of the same species of bacteria. Then Gratia and Fredrieq in 1946 named this anitibiotic colicine. Later Jocob *et al* in 1953 used the term bacteriocine. After that, Holland in 1967 changes the terms (colicine and bacteriocine) into bacteriocin and colicin (without "e" at the end) (Mayer-Haring *et al*., 1972).

Tagg *et al*., in 1976 introduced bacteriocin as a protein material act against bacteria, which affects the same group that produced it (Piared and Sesmazeazeaud, 1992).

Piard *et al* (1992) referred to bacteriocin as multipeptide it produce from some types of lactic acid bacteria and which inhibits different types of microorganism found in fermented foods. Bacteriocins have cidal effect and ability to bind with receptor of differentiation cell, so the cytoplasm membrane is the main side of bacteriocin effect, when a cell is affected by a bacteriocin specific secretion of amino acid and positive ions will increase causing blasting cellular membrane, and then finally, the death of sensitive cell occurs.

Lactic acid bacteria produce many types of bacteriocin such as diplococcin, nisin, lactostrepsin and lactocin (Contreras *et al*., 1997).

There are many kinds of bacteriocin such as A, B, C, D, E and this different from each other by the types of amino acid and its biological activity (Piard *et al*., 1992).

Nicin is the first type of bacteriocin is known and it found in ferment food and it's known by its ability to inhibit G (+) spore formers as *Clostridium botulinum*, and thermophilic bacteria which polluted food (Morris, 1991).

Bactriocin classify by Piard *et al*., (1992) according to biochemical properties into two classes:

Class 1: have usual amino acid such as **lacticin F** which produce from *Lb. acidophilus* and have 54 amino acid, and **lactococcin** which produce from *Lc. lactic* and have 37 amino acid, and **leucococcin** which produce from *Leu. gelidum* and have 57 amino acid.

Class 2: it refers as lantibiotic have unusual amino acid example lanthanide as Nisin.

Chapter three

Materials and Methods

3.1 Materials:

3.1.1 Apparatus:

Apparatus	Company / Country
Autoclave	Gallenkamp (U.K.)
An aerobic jar	Rodwell (England)
Balance	Ohaus (France)
Centrifuge (1000-10000 rpm / min).	Hermlxe Labortech Nik (Germany)
Compound light microscope	Olympus (Japan)
Cooling centrifuge	Harrier (U.K.)
Distillater	GFL (Germany)
Electrical incubator	Kallenkamp
Electrical oven	Gallenkamp
Freeze-Dryer	Virtis (U. S. A.)
Glass Pasteur pipettes	John poutten Ltd. (England)
Millipore filter unit (0.22μm)	Millipore and whatman (England)
Micropipette	Oxford (U.S.A)
PH-meter	Metter GmbH-Teledo (U.K)
Sensitive balance	Delta Range (Switzer land)
Spectrophotometer	Aurora instruments Ltd (England)
Vortex (mixer)	Stuart scientific co. Ltd (U.K.)
Water bath	Gallenkamp.

3.1.2 Chemicals:

The chemicals used in this study, are the following; classified according to the manufacturing company:

1. BDH (England):

$CaCO_3$, NaCl, Xyloze, Glycerol, phosphoric acid, Ethyl alcohol, Sodium hydroxide, HCl, peptone, Glucose, Iodine, Arganine, Mono hydrochloride, Bovine Serum albumin.

2. Biolife (Italy):

Meat extract, Yeast extract, Agar, skim milk (powder) and Gelatin.

3. Difco (USA):

Sorbitole, Sallicin, Mannitole.

4. Fluka (Switzer land):

Lactose, Sucrose, phenol red, Methylene blue, $NaH_2PO_4.2H_2O$, Na_2HPO_4, $MnSO_4.4H_2O$, Hydrogen peroxide, Crystal violet, Counter stain, Ammonium Sulphate.

5. LKB (Sweden):

Commassie blue G.

6. Merck (Germany):

$MgSO_4.7H_2O$

7. Riedel-DeHaeny (Germany)

Sodium acetate trihydrate, triammonium citrate.

8. Sigma (England);

Tween 80

9. Antibiotics:

The following antibiotic discs were provided from AL-Razi Diagnostic Center in Iraq:

Antibiotic name	Symbol	Concentration (µg)
Doxycycline	DO	30
Neomycin	N	30
Rifampicin	RA	30
Clindamycin	DA	10
Lincomycin	MY	2
Chloramphenicol	CM	30
Erythromycin	E	15
Tetracycline	TE	30
Ampiclox	AX	30
Ampicillin	AM	10
Amoxycillin	AMX	10
Methoprim	SXT	25
Fluxacillin	FU	5
Ceflexin	KF	30
Cefotaxime	CTX	30
ciprofloxacin	CIP	5

3.1.3 ApI System Kits (API- Bio Merieux, Lyon, France):

Three types of API- system kits were used in this study which includes:

3.1.3.1 API 20E:

It consists of:

- Gallery contains 20 microtubes having dehydrated substrates
- Reagents: TDA, IND, VP, OX.

3.1.3.2. API Staph:

It consists of:

- Gallery contains 20 microtubes having dehydrated substrates
- Reagents: VP1, VP2, N1T1, N1T2, ZYMA, ZYMB.

3.1.3.3. API 20A:

It consists of:

- Gallery contains 20 microtubes having dehydrated substrates
- Reagents: BcP, HER, XYL.

3.1.4 Culture Media:

3.1.4.1 Ready to Use Media:

- MacConkey agar (Oxoid)
- Nutrient broth (Oxoid)
- Thiogly collate broth (Oxide)
- Nutrient agar (Biolife)
- Muller-Hinton agar (Biolife)
- Motility medium (Difco)
- MRS- agar (HI media laboratory limited)

3.1.4.2 Laboratory Prepared Media:

I- Blood agar:

It was prepared according to Atlas *et al*., (1996), by dissolving 37 g of blood base agar in 1 liter of D.W. and autoclaved. After cooling to 45ºC, 5% of the blood was added to it, mixed well and distributed in Petri-dishes. It was used for enrichment and isolation of aerobic cocci.

II- MRS Broth:

This media was used for enrichment and growth o f lactic acid bacteria.

It was prepared according to Harrigan and McCance (1976) by dissolving:

Materials	Quantity
Peptone	19 g
Meat extract	10 g
Yeast extract	5 g
Sodium acetate trihydrate	5 g
D-Glucose	20 g
Tween 80	1 ml
Triammonium citrate	2 g
$MgSO_4.7H_2O$	0.2 g
$MnSO_4.4H_2O$	0.05 g

In 1 L of D. W. after that pH was adjusted to 6.0-6.5. The medium was autoclaved.

III- Fermentation Media:

MRS broth without glucose and meat extract was used after the addition of 0.004% of phenol red regent and 1% of each carbohydrate source (xyloze, sucrose, sorbose, lactose, sallicin and mannitol).

After PH of the medium was adjusted to 6.5, it was sterilized by Millipore filter unit (0.22 μm) (De Man *et al*., 1960).

IV- MRS Arganine Broth:

It was prepared by adding 0.3% W/V of L-arganine monohydrochloride to the MRS broth, and autoclaved.

It was used for identification of LAB species (Harrigan and MacCance, 1976).

V-Litmus Milk Medium:

It was prepared by dissolving 100g of powdered skim milk and 5g of litmus media in 1 liter of D.W. then sterilized by tyndalization. It was used for identification of LAB (Baily *et al*., 1990).

VI-Gelatin Medium (Stolp and Gadkari, 1984):

Gelatin (12% w / v) was added to MRS broth and autoclaving.

3.1.5 Solutions:

3.1.5.1 Phosphate Buffer Solution 10 mM and pH 7.2:

It was prepared as follow:

Solution A: 1.56 g of $NaH_2PO_4.2H_2O$ was dissolved in 1 liter of D. W.

Solution B: 1.4 g of Na_2HPO_4 was dissolved in 1 liter of D. W.

Then a portion of 28 ml from solution A was mixed well with 72 ml of solution B, then the volume was completed by D. W. to 200 ml (Griuchsank *et al*., 1975).

3.1.5.2 Normal Saline Solution:

It was prepared by dissolving 0.85 g of NaCl in 100 ml of D. W. then autoclaved after the pH adjusted to 7.0.

3.1.6 Sterilization:

3.1.6.1 Moist Heat Sterilization:

A- Autoclave was used to sterilize media, buffer and solution, at 121ºC for 15 min.

B- Tyndalization was used to sterilize litmus milk agar, by steaming at 100ºC on three successive days for 60, 45 and 30 min. (Baily *et al*., 1990).

3.1.6.2 Dry Heat Sterilization:

Electrical oven was used to sterilize glassware and other by heating at 160-180ºC for 3-2 hrs.

3.1.6.3 Filtration (Membrane Sterilization):

It was used Millipore filter unit (0.22 μm) to sterilize the extract of *Lactobacillus plantarum* and the fermentation media.

3.1.7 Reagents:

3.1.7.1 Catalase Reagent (Atlas *et al*., 1996):

A concentration of 3 % H_2O_2 was prepared for this purpose.

3.1.7.2 Dye Reagent (Bradford, 1976):

It was prepared by dissolving 100 mg of commassie blue G in a mixture consisted of 100 ml of phosphoric acid (85 %) and 50 ml of ethyl alcohol (95 %). Then volume was completed to 1 liter by D. W.

3.1.7.3 Nessler Reagent:

It was provided from the chemical department of AL-Nahreen University.

3.2 Methods:

3.2.1 Samples Collection:

Twenty samples of dairy product (cheese, crude milk, yoghourts) were collected from local market in Baghdad in sterile containers and carried to the laboratory under aseptic conditions.

3.2.2 Isolation of Lactic Acid Bacteria (LAB):

Two steps were used to isolate the bacteria from samples (Harrigan and McCance, 1976).

First step: by addition of 1 % of samples to 10 ml of MRS broth in a test tube, then incubated at 37ºC for 24 hrs.

Second step: After incubation serial dilutions were made from growth in the MRS broth. A portion of 0.1 ml from the last dilution was streaked on the surface of MRS agar (containing 1% $CaCO_3$) in a petridish, and then incubated for 24 hrs at 37ºC.

After incubation a colony that surrounded by clear zone was transferred and streaked on surface of plate containing MRS agar for purification. Plates were then incubated at 37ºC from 24 hrs. After that, part of the growth was transferred to MRS broth in test tubes and incubated under the same condition. After 1% of $CaCO_3$ was added to tubes, they were stored at 4 ºC until use.

3.2.3 Identification of Lactic Acid Bacteria (LAB):

The isolated bacteria had been identified by the following tests:

3.2.3.1 Gram's Stain (Harely and Prescott, 1996):

It was used to detect the gram reaction, cell shape and spore forming of the isolates.

3.2.3.2 Catalase Test:

This test was performed by adding drops of H_2O_2 (3%) on a single bacterial colony grown on MRS agar. The production of gaseous bubbles indicates the ability of bacteria to produce catalase enzyme (Baily *et al*., 1990).

3.2.3.3. Production of Acidity and Formation of Clot in Litmus Milk Test:

Tubes containing litmus milk media (10 ml) were inoculated by 1 % of the suspected bacteria, and then incubated at 37ºC for 24 hr. (Kandler and Wiess, 1986).

3.2.3.4 Growth on Nutrient Agar:

Nutrient agar was inoculated by the suspected bacteria, and then incubated at 37ºC for 24 hr.

3.2.3.5 Motility Test:

It was performed by using semi-solid agar media, motile bacteria "swarm" and gave a diffuse spreading growth that was easily recognized by the naked eye (Cruickshank *et al*., 1975).

3.2.6.3 Gelatin Hydrolysis Test (Harely and Prescott, 1996):

Inoculated the tubes of gelatin medium with isolated bacteria by stabbing and then incubated at 30ºC for 5 days. This test was performed to demonstrate the ability of isolated bacteria to hydrolyze gelatin.

3.2.4 Further Identification of LAB:

3.2.4.1 Tubes containing MRS broth (with 0.004 % phenol red reagent) and Durham Tubes were inoculated with two loop full of the isolated LAB; then incubated at 37ºC for 24 hr. after incubation the gas production (CO_2) and the color changes of the medium to yellow, indicated positive results.

3.2.4.2 LAB was inoculated in MRS broth at pH 4 and 9. After incubation at 37ºC for 24 hr, the density of growth was observed and compared with the control (pH 7.0).

3.2.4.3 LAB was inoculated in MRS broth containing NaCl at concentration 6.5% and 18 %. After incubation at 37 ºC for 24 hr, the density of growth was observed and compared with the control (with out NaCl).

3.2.4.4 LAB was inoculated in MRS broth and incubated at 10ºC and 40ºC. After incubation the density of growth was observed and compared with the control (37ºC)**.**

3.2.5 Identification of LAB Species:

3.2.5.1 Fermentation of Carbohydrate Source:

Tubes containing fermentation media was inoculated by the LAB isolates, and incubated with the positive control tube (contained fermentation media) and the negative control tube (contained MRS broth) at 37 ºC for 24 hr (DeMan *et al*., 1960).

After incubation, the indication was done by changing the color from red to yellow.

3.2.5.2 Formation of Ammonia from Arganine:

Tubes of MRS-arganine medium was inoculated by LAB isolates and incubated at 37 ºC for 24 hr. After incubation, 1 ml of the nesseler reagent was added to 1 ml of the culture. Inability of the isolate to change medium color to orange indicates that the bacteria are not producing ammonia (Briggs, 1953).

3.2.5.3 Growth at 15 ºC and 45 ºC:

LAB isolates was inoculated in tubes containing MRS broth, and incubated at 15 ºC or 45 ºC for 24 hr. After incubation, turbidity was observed in the tubes and compared with control (grow at 37 ºC).

3.2.6 Maintaining of The Selected LAB Isolate:

2.2.6.1 Working culture: MRS broth was inoculated by *Lactobacillus plantarum* and incubated at 37 ºC for 24 hr. After incubation, $CaCO_3$ (1 %) was added to the tubes and stored in 4ºC.

3.2.6.2 Stock culture: MRS broth that contains 20 % glycerol was inoculated by *Lactobacillus plantarum* and incubated at 37 ºC for 24 hr, then stored at -20 ºC.

3.2.7 Isolation, Identification and Maintaining of Acne Vulgaris Causing Bacteria:

3.2.7.1 Samples Withdraw:

Fifteen samples of pustule from different sites were taken from 10 patients after the skin was cleaned with 70 % ethyl alcohol, sterile blood lancets were used to puncture the acne pustules. The pus was taken by cotton swab and inoculated on blood agar, thioglycollate broth and MacConkey agar. Moreover, 15 comedones were extracted from 10 patients (5 with open comedones and 5 with closed comedones) by using sterile comedone extractors, after cleaning the skin with 70 % ethyl alcohol. The comedo materials were held in thioglycollate and nutrient broth media.

3.2.7.2 Samples Culturing :

All samples that taken from acne patients inoculated on:

- Blood agar: This incubated aerobically at 37 ºC for 18-24 hr for isolation of aerobic cocci.
- Thioglycollate broth: that incubated at 37 ºC for 5-7 days, then subcultured on blood agar and incubated at 37 ºC for 5-6 days, an aerobically, in gas pack jars for isolation of *Propionibacterium acne.*
- MacConkey agar: that was used for isolation of gram negative bacteria.

Then gram stain and catalase test was performed for all isolates that grow in above media.

Presumptive identification of isolates made on basis of gram reaction, cell shape and catalase test. But further identification of any growth was detected by using ApI-system.

3.2.7.3 ApI System Identification:

Further conformation of the acne causing organisms was performed by using three type of ApI system which includes:

3.2.7.3.1 ApI 20E: This was used for identification of enterobactereaceae and other gram negative bacteria. It consists from 20 microtubes containing dehydrated substrates, with the using of a sterile Pasteur pipette. These microtubes were inoculated with a bacterial suspension which reconstitutes the media. According to the manufactures instruction both the tube and the tube section of CIT, VP and GEL microtubes were filled. After that couple section of the ADH, LDC, ODC, H_2S and URE microtone were completely filled with sterile mineral oil. Then the gallery was incubated at 37 ºC for 18 hr. during incubation, metabolism products produces color changes that were either spontaneous or revealed by the addition of reagents.

3.2.7.3.2 ApI Staph: which was used fro identification of *Staphylococcus* and *Micrococcus*. It consist from 20 microtubes each one containing dehydrated substrates. With the using of a sterile Pasteur pipettes. This microtubes were reconstituted by adding an aliquot of ApI Staph medium that had been inoculated with the isolates to be identify, only filled the tube, not the tube section. After inoculation, the tube section of ADH and URE tests were completely filled with sterile mineral oil. Then the gallery was incubated at 37 ºC for 18 hr. During incubation, metabolism products produced color changes that were either spontaneous or revealed by the addition of reagent.

3.2.7.3.3 ApI 20 A: which was used for identification of anaerobic bacteria. It consists from 21 microtubes. Containing dehydrated substrate, with the using of a sterile Pasteur pipette, these microtubes were reconstituted by adding an aliquot of ApI 20 A medium that had been inoculated with the isolates, only filled the tubes. After inoculation, the IND tubes were overlaid with mineral oil. Then the gallery was incubated anaerobically by the using of jar and incubated at 37 ºC for 18 hr then the color changes was read spontaneously or revealed by the addition of reagent.

3.2.8 Maintenance of Pathogenic Bacteria:

3.2.8.1 Working Culture:

The following bacterial isolates: *Staphylococcus epidermidis, Staphylococcus aureus, Proteus mirabilis* and *Escherichia coli* were inoculated separately in nutrient broth, and incubated aerobically at 37ºC for 24 hr, while thioglycollate broth was incubated by *Propionibacterium acne*, then incubated at 37 ºC for 24-72 hr under anaerobic conditions. Then all isolates were stored at 4ºC until use.

3.2.8.2 Stock Culture:

Nutrient broth that containing 20 % glycerol was inoculated by culture of *Staphylococcus epidermidis, Staphylococcus aureus, Proteus mirabilis* and *Escherichia coli* and incubated at 37 ºC for 24 hr under aerobic condition. While thioglycollate broth containing 20 % glycerol inoculated by *Propionibacterium acne*, then incubated at 37ºC for 48-72 hr under anaerobic conditions. After that all isolates were stored at -20 ºC.

3.2.9 Antibiotic Sensitivity Test:

3.2.9.1 Antibiotic Sensitivity Test of *Lb. plantarum*.

Disc method was used according to Bauer *et al*., (1966) as follows:

A loop full of the overnights inoculums of Lb.1 and Lb.3 isolated grown in MRS broth were put separately on plate containing MRS agar and spread evenly over the whole plate by a sterile cotton swab. Then antibiotic discs fixed, gently, on the surface of medium. Then incubated at 37^{o}C for 24hr. After incubated, the diameter of the inhibition zone around the disc was measured and estimated in (mm).

3.2.9.2 Antibiotics Sensitivity Test of the Pathogenic Isolates:

Method that previous stated and mention by Bauer *et al*., (1966) was used to test the sensitivity of *Staphylococcus epidermidis Staphylococcus aureus, Proteus mirabilis* and *Escherichia coli* to the antibiotics used, but by using Muller-Hinton agar instead of MRS agar and using nutrient broth instead of MRS broth.

But to study the antibiotics sensitivity of *Propionicbacterium acne*, broth disc method was used according to Kurzynski *et al*., (1976) as follows:

Appropriate number of antibiotic discs was added into screw capped tubes containing 5 ml of thioglycollate broth. Then, the inoculated tubes were kept at room temperature for 2 hr to allow diffusion of the antibiotic into the medium. After that the tubes were inoculated with 0.1 ml of broth containing *Propionicbacterium acne* and screw capped tightly, they were incubated at 37ºC for 48-72 hr. Controls of non-inoculated tubes and tubes without antibiotic were prepared.

Interpretation done by observing the turbidity. Susceptibility was indicated when there was turbidity.

3.2.10 Determination of the Inhibitory Effect of *Lactobacillus plantarum*:

3.2.10.1 On Solid Media:

A culture of lb. 3 previously grown in MRS broth was streaked on MRS agar, and then incubated under anaerobic conditions at 37 ºC for 48 hr (Silva *et al*., 1987).

After incubation, With the aid of cork porer (5 mm), disc of the grown culture were put on the surface of nutrient agar that was inoculated (before) with 0.1 ml of pathogenic bacteria, then incubated at 37 ºC for 24 hr. After that, the inhibition zone around the disc was measured and estimated in (mm) the same procedure was repeated by using different incubation times of LB3 (18, 24, 36 and 72 hr) to determine the optimum incubation time that gives greater inhibition effect.

3.2.10.2 In Liquid Media:

MRS broth was inoculated by 1 % (10 cell / ml)of Lb.3 culture, then incubated anaerobically at 37ºC for various periods of times (18, 24, 36 and 72 hr) (Schillinger and Luck, 1989; Lewus *et al*., 1991).

After incubation the culture was centrifuged at 4500 rpm to get supernatant which contained the filtrate of grown cells. After adjusting the pH to 6.5 by using NaOH (1 M), it was filtered through millipore filter unit (0.22 μm).

Then the well diffusion method that mention by Vignolo *et al* (1993) was used on nutrient agar. The plate was inoculated with 0.1 ml of pathogenic bacteria by using spreader. Then by the cork porer (5 mm) wells were made in agar and filled by the filtrate of Lb. 3 before incubated at 37 ºC for 24 hr. The inhibition area around the well was measured by (mm) and compared with that of the control which contains MRS broth without bacteria (Vignolo *et al*., 1993). The filtrate was concentrated by the freeze-dryer and the well diffusion method was repeated to detect the effect of concentrated filtrate against the pathogenic

bacteria. The control in concentrated filtrate was contains MRS broth that concentrated also.

3.2.11 Purification of Bacteriocin by Ammonium Sulphate:

A culture of Lb. 3 was inoculated in MRS broth (100 ml) (pH 5.8) at 30 ºC for 18 hr (Yang *et al*., 1994). After incubation placed the culture vessel in a water bath at 70ºC for 15 min. Ammonium sulphate (10.6 g) was added to the supernatants in cold water bath with stirrer. Then recentrifuged for 6000 rpm at 4 ºC for 30 min then centerfuged at 4500 rpm. Ammonium sulphate (31.2 g) was added to the supernatant under same conditions mention above. Then the supernatant was left at 4ºC for overnight (Segel, 1975). After that it was centrifuged at 6000 rpm for 30 min. later, the precipitate was dissolved in little volume of the phosphate buffer solution (0.01 M, pH 7.2), then concentrated by the lyophylization to get powder form, then its weight was measured. Its activity was applied against pathogenic bacteria by using well diffusion method as in (3.2.10.2).

3.2.12 Quantitative Estimation of Protein (Bacteriocin) in Concentrated Filtrate and in Purified Bacteriocin:

Bradford method (1976) was used to estimate protein in the concentrated filtrate and purified bacteriocin (ammonium sulphate). A standard curve of bovine serum albumin was drawn by preparing different concentrations (from 0.20 mg / ml). a portion of 20 µl was transferred from each concentration in test tubes, and then 50 µl of NaOH (1 M) and 1 ml of commassie blue reagent were added to the tubes. After well mixing, it was left at room temperature for 5 min. then the absorption was read for each concentration at a wave length of 590 nm. Absorption was drawn against protein concentration.

Then the same steps were used to determine the protein in concentrated filtrate and purified bacteriocin by the using of the same standard curve.

3.2.13 Determination of the Minimum Inhibitory Concentration (MIC) for Concentrated Filtrate:

Different concentrations of each concentrated filtrate and were made in tubes containing sterile nutrient broth, they were ranged from 10 % to 100 %, then each concentration was inoculated by pathogenic bacteria (1%) and incubated at 37ºC for 24 hr. After incubation the turbidity of tubes was noticed, and MIC was calculated as the lower concentration of concentrated filtrate that prevented the growth of pathogenic bacteria (no turbidity).

3.2.14 Application of LAB Filtrate in Treatment of Acne Vulgaris:

Concentrated filtrate at concentration 80 % was used in treatment the patients with sever acne vulgaris; this by taken to patients as topical solution and by cotton was spread on the infected area. This treatment was used once daily. During the treatment period the patient was seemed and the positive and negative effect was notice in patients.

Chapter four

Results and Discussions

4.1 Isolation and Identification of Lactic Acid Bacteria (LAB):

Sixteen isolates of Lactic acid bacteria were isolated from dairy products that collected from Baghdad markets. The isolates were identified depending on their cultural, morphological and biochemical characteristic. Results showed that all isolates formed clear zone around the colonies when cultured on MRS agar containing $CaCO_3$ (1%). Such clear zone indicates that the isolates have the ability to produce acid which dissolved the $CaCO_3$. The isolates were unable to grow on nutrient agar and they were non-motile. Microscopical examination showed that cell shape mainly long rods, but in some cases curved or short rods, they were either singles or in pairs but mostly grouped as chains containing (5-8) cells. The isolates were gram positive and non-spore forming. Regarding the biochemical characteristics, they were able to produce acid and form clot when grew in litmus milk.

4.2 Identification and Differentiation between LAB Genera:

Table (4-1) shows results of the tests used to differentiate between the four genera of LAB (*Lactococcus*, *Lactobacillus*, *Pediococcus* and *Leuconstoc*) according to the properties that mentioned by Salminion and Wrigh (1992) which included: ability of isolates to produce CO_2 form glucose and to grow at different temperature degree, pH value and NaCl concentration.

Depending on such tests, six isolates were belonged to the genus *Lactobacillus* which given symbols of (Lb. 1, Lb. 2, Lb. 3, Lb. 4, Lb. 5 and Lb. 6), while four isolates were belonged to each of the genera *Pediococcus* (pd. 1, pd. 2, pd.3 and pd. 4) and *Leuconstec* (Lu. 1, Lu. 2, Lu. 3 and Lu. 4). The remaining two isolates were belonged to the genus *Lactococcus* (Lc. 1 and Lc.2).

Such results indicated that isolates belonging to the genus *Lactobacillus* were the most frequently isolated among other LAB genera. In this approach Hammes

and Vogel (1995) mentioned that *Lactobacillus* was the most genera found among Lactic acid bacteria obtained form dairy products.

Table (4-1) Differentiation properties of Lactic acid bacteria (LAB) isolates

Isolates	CO_2 production form glucose	Growth at		Growth in NaCl		Growth at pH	
		10 °C	45 °C	6.5 %	18 %	4	9
Lb. 1	+	+	–	+	–	+	–
pd. 1	+	+	–	+	–	+	–
Lb. 2	–	+	+	–	–	+	–
Lu. 1	+	+	+	+	–	+	–
pd. 2	–	–	–	+	–	+	–
Lu. 2	–	+	+	–	–	+	–
Lb. 3	–	–	–	+	–	–	–
Lc. 1	+	–	+	–	–	–	–
Lb. 4	+	+	–	+	–	+	–
pd. 3	–	+	–	–	–	+	–
Lb. 5	+	–	+	–	–	+	–
Lu. 3	–	+	+	+	–	+	–
Lu. 4	–	+	+	+		–	–
Lc. 2	+	–	+	–	–	–	–
Lb. 6	–	+	+	–	–	+	–
pd. 4	–	+	–	–	–	+	–

(+) positive result,

(–) negative result

4.3 Identification of LAB Species:

The identification of *Lactobacillus* spp. was achieved by applying the following tests: fermentation of carbohydrate source, growth at (15 and 45) °C and formation of ammonia from arginine.

Table (4-2) shows that two of the six isolates of *Lactobacillus* (Lb. 1 and Lb. 3) were belonged to the species *Lactobacillus plantarum*, while each of *Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus brevis* and *Lactobacillus fermentum* was represented by only one isolate namely (Lb. 2, Lb. 4, Lb. 5 and Lb. 6) respectively.

Vescovo *et al*., (1996) found that *Lactobacillus plantarum* was the most common bacilli present in the dairy product due to its ability to tolerate high acidity when compared with other species.

Table (4-2) Identification of LAB species

Isolate	Acid production						NH_3 form Arginine	Growth at		Lb. species
	Lac.	Suc.	Sal.	Mn.	So.	Xy.		15 °C	45 °C	
Lb. 1	+	+	+	+	–	–	–	+	+	*Lactobacillus pluntarum*
Lb. 2	+	+	+	+	–	–	–	+	+	*Lactobacillus acidophilus*
Lb. 3	+	+	+	+	–	–	–	+	+	*Lactobacillus pluntarum*
Lb. 4	+	–	–	–	–	–	–	–	+	*Lactobacillus bulgaricum*
Lb. 5	+	+	+	–	–	+	+	+	–	*Lactobacillus brevis*
Lb. 6	+	–	–	–	–	+	+	–	+	*Lactobacillus fermenter*

4.4 Isolation and Identification of Severe Acne Vulgaris Pathogens:

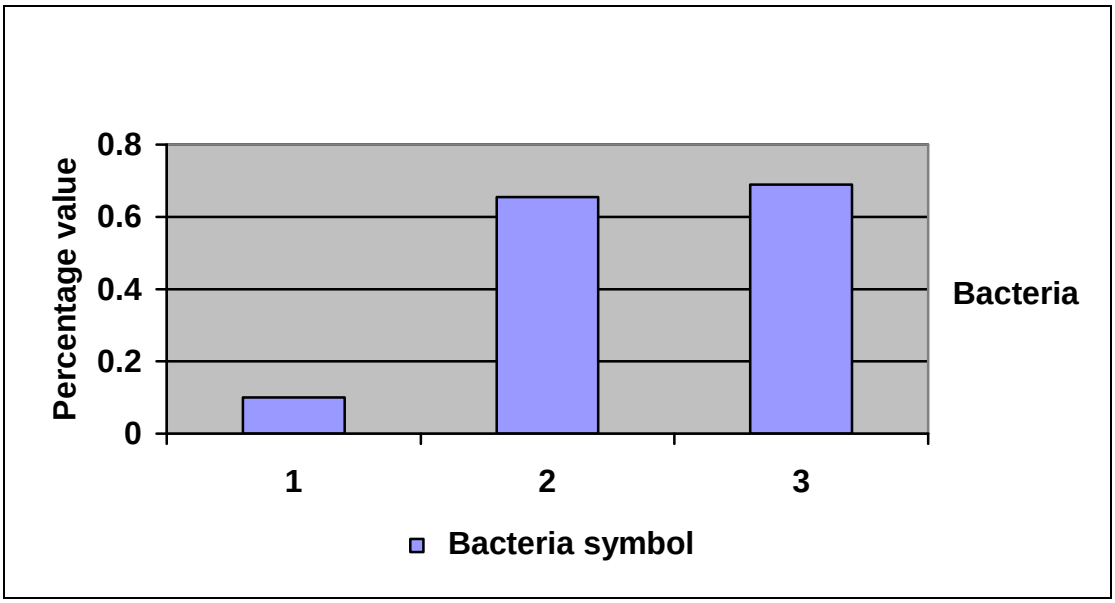
Fifty bacterial isolates had been obtained from comedone and pustule of the twenty patients that included in this study. Primarily identification of these isolates was performed according to the cultural characteristic (after growth on blood agar, thioglycollate media and MacConkey agar media), morphological characteristic (through gram reaction and cell shape), and by the catalase test. Moreover, identification was confirmed by applying ApI Kit-system.

Result show that despite *Propionibacterium acne* and *Staphylococcus epidermidis* were the main frequent bacteria isolated from the comedons cases, *Propionibacterium acnes* was the most common and dominant (68.96 %) compared to (65.5 %) of *Staphylococcus epidermidis* as shown in figure (4-1).

Predomination of *Propionibacterium acnes* is due to with standing the lower oxygenic pressure found inside the comedone which helps in the creation of anaerobic conditions that preferred by these bacteria (Izumi, 1970; Marples, 1975; Leeming *et al*., 1985).

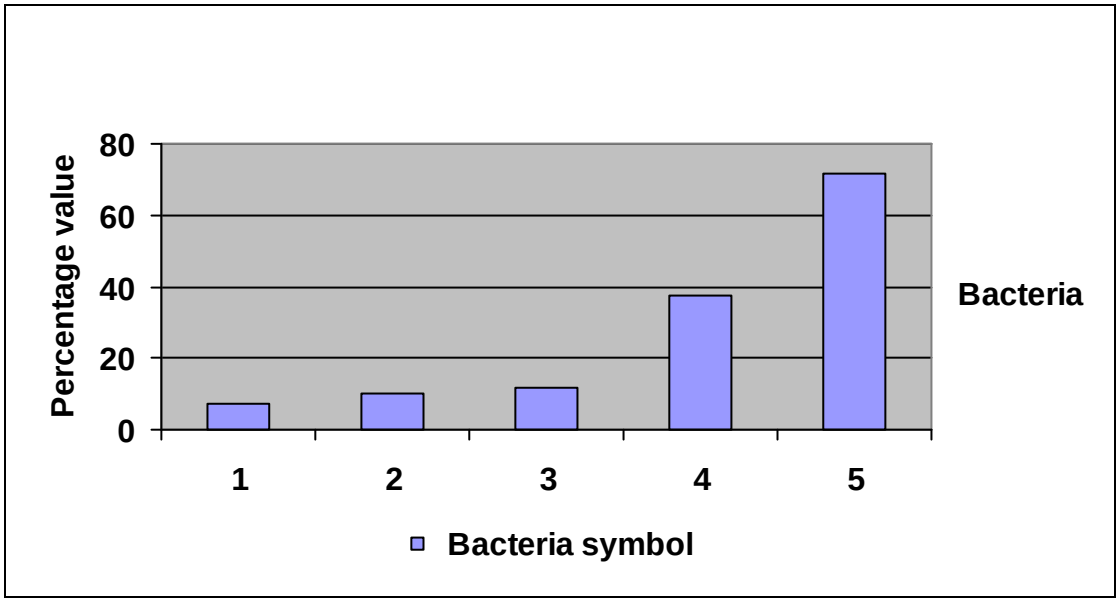
In the pustules adverse situation occurred when *Staphylococcus epidermidis* was the predominant bacteria with a percentage of 71.42 %, in compare only 37.26 % existence for *Propionibacterium acnes*. Other species were also isolated but in lower percentages. They were; *Proteus mirabilis* (11.49 %), *Staphylococcus aureus* (9.93 %) and *E. coli* (7.14 %) as shown in figure (4-2).

However, such predominance to the *Staphylococcus epidermidis* may be related to the high oxygenic pressure in the pustule and to the changes in pH value of media which leads to form favorable conditions for these bacteria. Such condition, on the other hand, is not much favorable by *Propionibacterium acnes*. Moreover, there is another factor governing this existences, it's the permeability of neutrophils in the infected area, which lead to reduce the presence of *Propionibacterium acnes*. This bacteria is able to produce chemically attracted materials of low molecular weights to the neutrophils so, neutrophils prefer *Propionibacterium acnes* more than other types of bacteria (Marples and Izumi, 1970; Holland, 1974; Knecth *et al*., 1995).



1- *S. aureus* 2-*S. epidermidis* 3- *P. acnes*

Figure (4-1) the types of bacteria that isolated from comedone



1- *E. coli* 2- *S. aureus* 3- *Proteus mirabilis*
4- *P. acne* 5- *S. epidermidis*

Figure (4-2) The bacteria types of pustule

Isolation of gram negative bacteria (*Escherichia coli* and *Proteus mirabilis*) in a sum percentage of 17.07 % was agreed with the study of Marples and Izumi (1970) who found that gram negative bacteria in patient of acne vulgaris was detected in small percentages.

Presence of gram negative bacteria in patient suffering from acne vulgaris may be due to the taking of antibiotic for long time. This will lead to growth and duplication of these microorganisms in pustules, a state which is called G-ve folliculitis (Holt *et al*., 1994; Goven, 1996; Champion, 1998).

4.5 Antibiotics Sensitivity:

4.5.1 Antibiotics Sensitivity of *Lactobacillus plantarum*

Effect of 12 different antibiotics on two selected isolates of *Lactobacillus plantarum* (Lb. 1 and Lb. 3) were studied by using the standard disk diffusion method. Diameter of inhibition zone around the colony was estimated (in millimeter) and compared in according with Nccls (1991).

Table (4-3) shows result of antibiotic sensitivity of the two isolates of *Lactobacillus plantarum*. They were resistant to streptomycin, penicillin G and trimethprin. Such results agreed with those found by Suskovic *et al*., (1997) when *Lactobacillus* were resistant to penicillin G and streptomycin. The twelve different antibiotics that used in this test were generally used in treatment of cattle disease. These antibiotics may release with milk or accumulate in muscle of cattle (Sozzi and Simtey, 1980).

Tamine and Deeth (1980) concluded that 64 % of the disease infected cattle were treated by streptomycin and penicillin G. Moreover, resistance of *Lactobacillus* spp. To these two antibiotics made the bacteria able to stay alive and its resistance increases upon releasing the milk.

Results also shows that the two isolates were sensitive to amoxicillin, chloromphenicol, cloxacillin, rifampin and ampiclox, but in various degrees they were highly sensitive to ampiclox when diameter of the inhibition zone reached

30 mm, while its resistance to rifampin was in small extent after reaching 15 mm. Such resistance may be due to the limited use of the latter antibiotics in the treatment of cattle diseases. Sensitivity of the two isolates of *Lactobacillus plantarum* to the tetracycline, erythromycin, ampicillin and gentamycin was also different. At the time that Lb. 1 was sensitive to four mentioned antibiotics, Lb. 3 was resistance to them.

Resistance or sensitivity of any bacteria to antibiotic is usually governed by the method of test used, isolated type, previously used of antibiotic in the treatment and later their presence in milk and the genetic changes of the isolates (Sozzi and Simely, 1980) upon the above results, Lb. 3 isolates was chosen to study its inhibitory activity against the isolated pathogenic causes of acne vulgaris.

Table (4-3) Antibiotic sensitivity of *Lactobacillus plantarum* isolates

Antibiotic	Lb. 1	Lb. 3
Tetracycline	S	R
Erythromycin	S	R
Ampicillin	S	R
Amoxicillin	S	S
Chloromphenicol	S	S
Gentamycin	S	R
Penicillin G	R	R
Streptomycin	R	R
Trimethprin	R	R
Cloxacillin	S	S
Rifampin	S	S
Ampiclox	S	S

S = sensitive, R= resistant

4.5.2 Antibiotic Sensitivity of Pathogenic Bacteria:

The emergence of prevalence of antibiotic resistance strains is consider as a major therapeutic problem that can be explained by several hypothesis such as, the influence of excessive and / or inappropriate antibiotic use (Sotto *et al*., 2001).

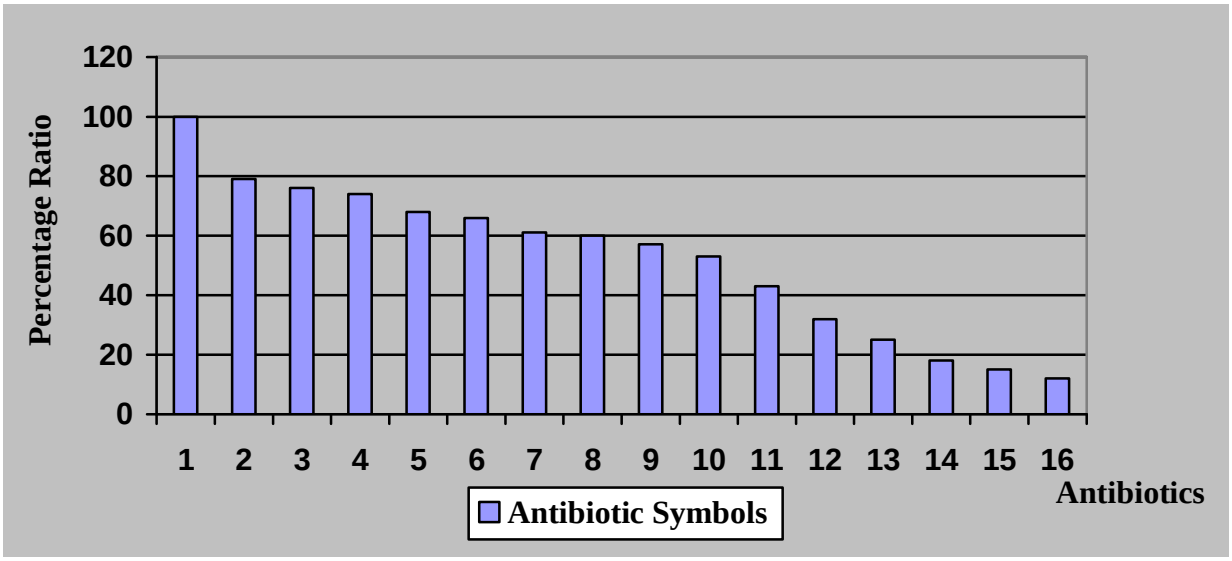
Standard disk diffusion method was used to detect the sensitivity of pathogenic bacteria.

Figure (4-3) shows the antibiotic sensitivity to 5 isolates of *Staphylococcus epidermidis*. All isolates were sensitive totally to doxycyline (100 %). This result may be related to the lower randomly use of this antibiotic by patients. Later the antibiotics ciprofloxacin, Neomycin and rifampin at percentage 79 %, 76 % and 74 % respectively arranged sequently after doxycycline in sensitivity. Other antibiotics were arranged after the previous mentioned antibiotic until reach to less sensitive isolates to ampiclox (18 %), ampicillin (15 %) and amoxicillin (12 %). The resistance of isolates to these antibiotics was belonging to their common using in treatment.

These results were disagreed with local studies (Faraj, 1989; Douri, 1997) and with international studies (Beard-Pegler *et al*., 1989; Etienne *et al*., 1989).

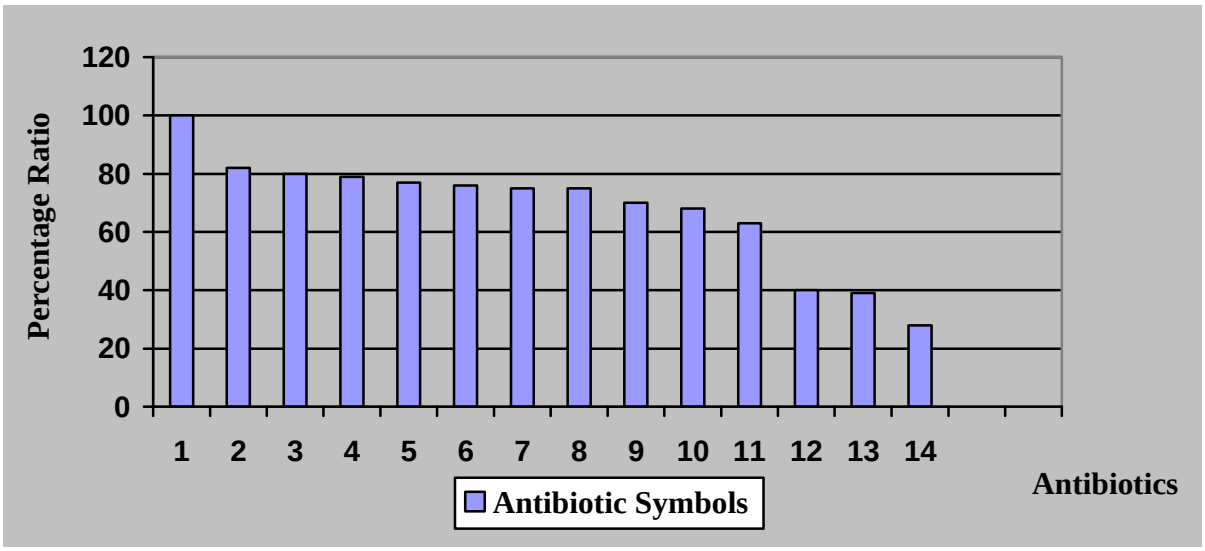
Figure (4-4) shows the result of antibiotic sensitivity to 5 isolates of *Propionibacterium acnes*. From results noticed that all isolates were sensitive to doxycycline (100 %). Later rifampin (82 %), clindamycin (80 %) and neomycin (79 %) were arranged sequently after doxycycline in sensitivity. This result was belong to the lower using of these antibiotics by patients because these antibiotics unknown by the patients. Other antibiotics were arranged sequently until reached to less sensitive isolates to methoprin (40 %), amoxicillin (39 %) and ampicillin (28 %). This resistance belongs to the randomly using of these antibiotics by patients.

Cooper (1998) mention that the take of antibiotic for short time and in small dose lead to increase the development of isolates resistance to these antibiotics.



1- Doxycycline
2- Ciprofloxacin
3- Neomycin
4- Rifampicin
5- Clindamycin
6- Lincomycin
7- Cefotaxime
8- Chloranphenicol
9- Erythromycin
10- Tetracyclin
11- Cefalexin
12- Fluxacillin
13- Methoprim
14- Ampiclox
15- Ampicillin
16- Amoxycillin

Figure (4-3) Antibiotic Sensitivity of *S. epidermidis*



1- Doxycycline
2-Rifampicin
3- Clindamycin
4- Neomycin
5- Chloranphenicol
6- Lincomycin
7- Erythromycin
8- Ciprofloxacin
9- Cefotaxime
10- Cefalexin
11- Tetracyclin
12- Methoprim
13- Amoxycillin
14- Ampicillin

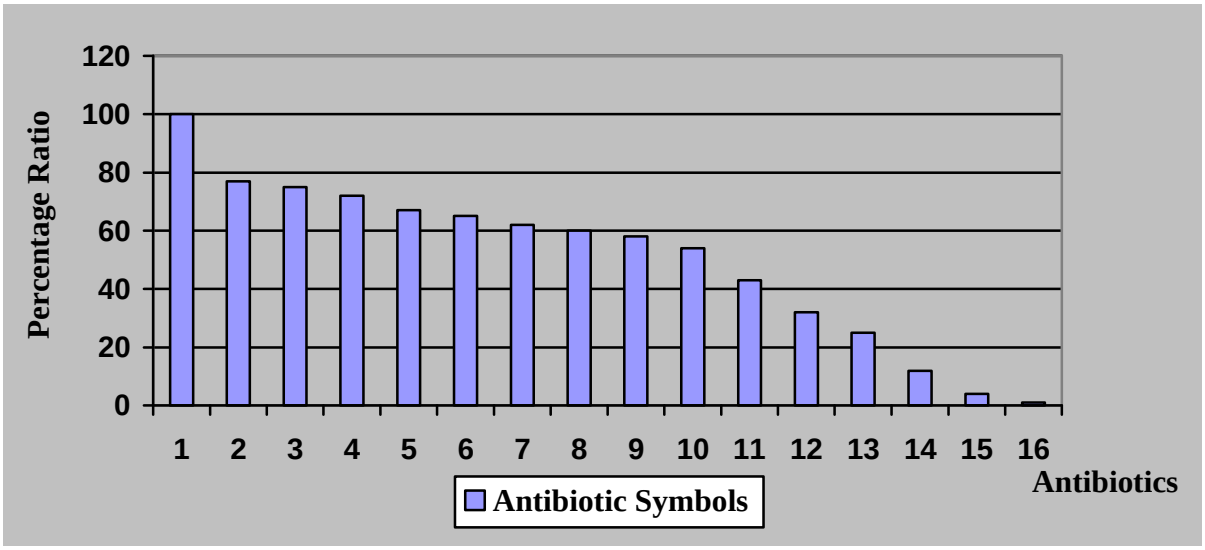
Figure (4-4) Antibiotic Sensitivity of *P. acnes*

The sensitivity results of *Propionibacterium acnes* to the previously mentioned antibiotic was agreed with international studies (Hoffler *et al*., 1976; Sutter *et al*., 1976; Wang *et al*., 1977; Webster *et al*., 1981; Brown and Poston, 1983; Komagata *et al*., 1998; Kurokawa *et al*., 1999; Tan *et al*., 1999; Goldsteine *et al*., 2000).

Figure (4-5) shows the result of antibiotic sensitivity to 5 isolates of *Staphylococcus aureus*. From result noticed that all isolates were sensitive to doxycycline (100%). Later neomycin (77%), rifampicin (75%) and clindamycin (72%) were arranged sequently after doxycycline. Other antibiotics were then arranged until reached to less sensitive isolates to ampiclox (12%) ampicillin (4%) and amoxicillin (1%). These resistances belong to randomly used of these antibiotics by the patients. These results were disagreed with international studies (Schmitz *et al*., 2000).

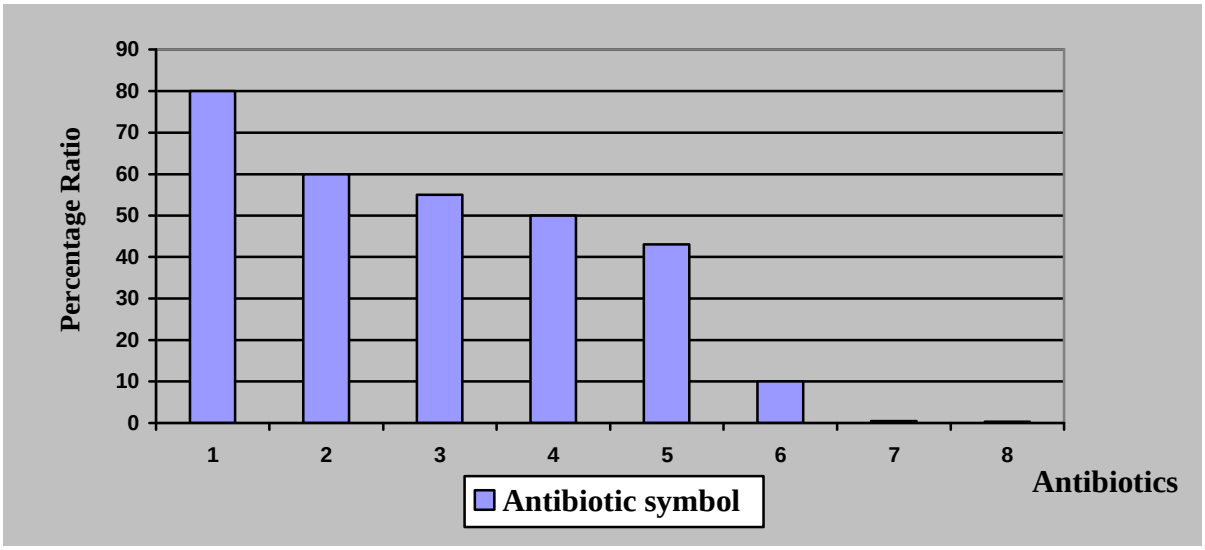
Figure (4-6) shows the result of antibiotic sensitivity to 5 isolates of *E. coli*. Form result noticed that most isolates were sensitive to doxycycline at percentage (80%). Later ciprofloxacin (60%) and neomycin (55%) arranged sequently after doxycycline in sensitivity. Other antibiotics were arranged sequently until reached to less sensitive isolates to cefelexin (0.5%) and methoprim (0.3%) these resistance results belong to randomly using of these antibiotics by the patients.

Figure (4-7) shows the result of antibiotic sensitivity to 5 isolates of *Proteus mirabilis*. From results noticed that most isolates were sensitive to doxycycline at percentage (85 %). Later ciprofloxacin (82 %) and neomycin (77 %) arranged sequently after doxycycline in sensitivity. Other antibiotics would arranged until reached to cefelexin (12 %) and methoprim. These results belong to their common use in treatment. From result noticed that the increasing in resistance of *Propionibacterium acnes* and *Staphylococcus epodermidis* to ampicillin, chloromphenicol and amoxicillin was disagreed with the result of local study (Faraj, 1989; Douri, 1997).



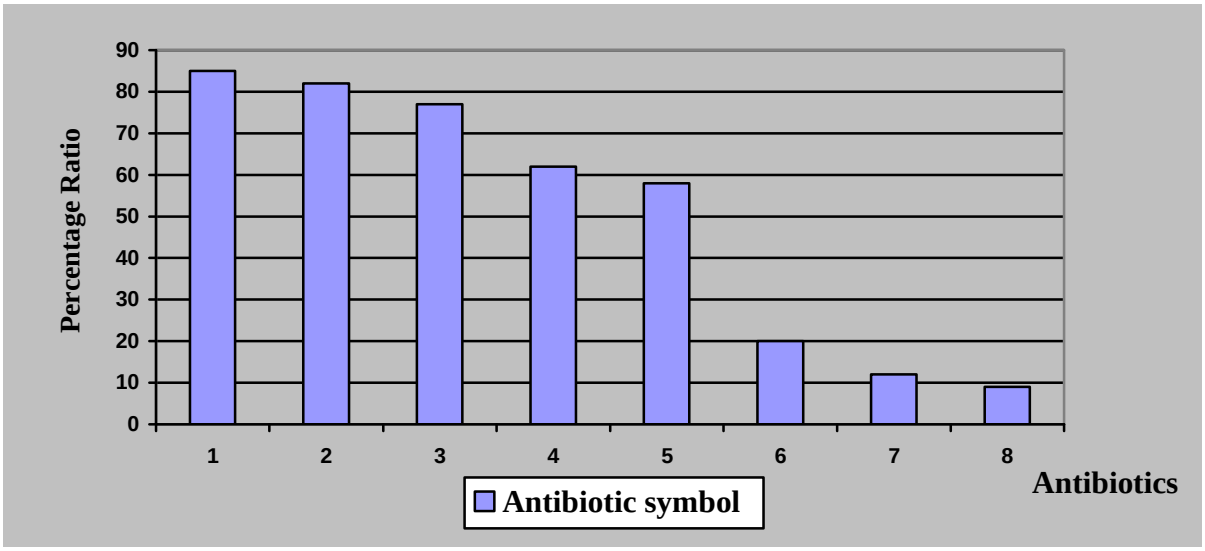
1- Doxycycline
2- Neomycin
3- Rifampicin
4- Clindamycin
5- Lincomycin
6- Ciprofloxacin
7- Chloranphenicol
8- Cefotaxime
9- Tetracyclin
10- Erythromycin
11- Cefalexin
12- Fluxacillin
13- Methoprim
14- Ampiclox
15- Ampicillin
16- Amoxycillin

Figure (4-5) Antibiotic Sensitivity of *S. aureus*



1- Doxycycline
2- Ciprofloxacin
3- Neomycin
4- Cefotaxime
5- Tetracyclin
6- Fluxacillin
7- Cefalexin
8- Methoprim

Figure (4-6) Antibiotic Sensitivity of *E. coli*



1- Doxycycline
2- Ciprofloxacin
3- Neomycin
4- Cefotaxime
5- Tetracyclin
6- Fluxacillin
7- Cefalexin
8- Methoprim

Figure (4-7) Antibiotic Sensitivity of *Proteus mirabilis*

In conclusion noticed that doxycycline was the efficient antibiotic towards all pathogenic bacteria. These antibiotic had active inhibition effect against G +ve and G–ve bacteria, because it enter in bacterial protein synthesis by attached with bacterial ribosome and this prevent it from connecting with amino-acyl transfer (tRNA). Doxycycline belong to the second evolution of tetracycline and given to patient with acne vulgaris who not response to first evolution of tetracycline. Doxycycline is lipophilic, so, it accumulated in sebaceous gland and had cidal effect against bacteria.

Doxycycline was used as anti-inflammatory because it had antioxidant effect on neutrophile (Akamatsu *et al*., 1999; Greenwood, 1996; Corral, 1997; Welsey *et al*., 1999).

But the resistance of isolates to tetracycline was belong to common using in topical and systemic treatment of acne vulgaris, this because it inhibit the bacterial growth and also inhibit the lipid hydrolysis by *Propionibacterium acnes*, in addition to its using as anti-inflammatory (Human-Ackah, 1976; Champion *et al*., 1998).

The resistance of bacterial strain to tetracycline also belongs to the presence of β-lactam ring and at end made all members of this group was an active (Weinrich and Bene, 1976; Nishine and Nakazawa, 1976; Wanger and Murray, 1990; Graummlich and Sidhu, 1997; Epstein *et al*., 1997; Kerndole *et al*., 1998; Hoiby *et al*., 2000).

The resistance of isolates to tetracycline belongs to the presence of resistance plasmid and its ability to transfer from one bacterium to another lead to make the sensitive bacteria to resistance. And at end the treatment became difficult (Watanakunakorn *et al*., 1982; AIM, 1982; Novick, 1987; Lyon and Skurray, 1987; Karawya and Raymod, 1990; Espersen, 1998; Falace *et al*., 1999; Martineau *et al*., 2000).

The similarity in resistance of *E. coli* and *Proteus mirabilis* belong to that the both bacterial isolates had external membrane which had mechanism that

prevent the permeability of antibiotics inside bacteria cell (Pumbwe and Piddock, 2000; Leflon-Guibout *et al*., 2000; Poole, 2000).

4.6 Determination of Inhibitory Effect of *Lactobacillus plantarum* on Pathogenic Bacteria:

4.6.1 On Solid Media:

Ability of the LAB isolate to produce inhibition activity on pathogenic bacteria was tested by growing the isolate on MRS agar medium. In this approach, AL Kassb and AL Khafaji (1992) mentioned that using MRS agar medium in studying the ability of LAB isolates to produce inhibited materials when it grown under anaerobic condition is the choosing procedure that gives reasonable result.

Table (4-4) shows the inhibitory activity of *Lactobacillus plantarum* isolate (Lb. 3) grown on MRS agar against five species of pathogenic bacteria isolated from patients suffering of acne vulgaris at five different incubation periods.

Results shows that Lb.3 isolate exhibited noticeable inhibitory activity against all pathogenic bacteria tested at most of the periods of incubation used. In general, it had more effect on gram positive bacteria than on gram negative (Plates 4.1, 4.2, 4.3, 4.4 and 4.5). Despite that incubation periods of 36, 48 and 72 hr. gave inhibitory activity against all species of pathogenic bacteria, 24 hr. period had also such effect but against gram positive bacteria only. Adversely, incubation period for 18 hr. had no inhibitory effect on both gram positive and gram negative bacteria.

Depending on the just mentioned results, it may be concluded that 36 hr. incubation period was chosen as the preferred period for the Lb. 3 isolate grown on MRS agar against all species of bacteria isolated from acne vulgaris cases.

Garver and Muriana (1994) mentioned that production of inhibited materials by LAB is dependent on the medium used for growth, and they found also that tween 80 induced the production of protein (bacteriocin) by increasing the production activity of bacteria.

Nes *et al*., (1996) found that there is an induction factor (1F), which is a protein of short chain of amino acid found in medium, it is function to increase the production of bacteriocin continuously.

The view of research about the inhibition activity of *Lactobacillus plantarum* against pathogenic bacteria is different from one to another, for instance, Jimenez-Diaz *et al*., (1993) found that *Lactobacillus plantarum* effect was only on gram positive bacteria as *Clostridium*, while Nigatu and Gash (1994) reported that all genus of LAB that isolated from the fermenting foods had effect on gram negative bacteria such as *Pseudomonas*, *E. coli*, *Proteus* and *Salmonella*.

Table (4-4) Inhibitory activity of *Lactobacillus plantarum* (Lb. 3) grown on MRS agar against pathogenic bacteria of acne vulgaris for different incubation periods

Bacteria	Incubation period (hr)				
	18	24	36	48	72
Staphylococcus aureus	–	++	++	++	++
Staphylococcus epidermidis	–	+	+	+	+
Propionibacterium acnes	–	+	+	+	+
Escherichia coli	–	–	+	+	+
Proteus mirabilis	–	–	+	+	+

(–) no effect = inhibition zone (zero mm)

(+) slightly effect = inhibition zone (≤10 mm)

(++) moderate effect = inhibition zone (10-13) mm

Plate (4-1) The inhibition effect of *Lactobacillus plantarum* (lb. 3) incubated for 36 hr. on *Staphylococcus aureus*

Plate (4-2) The inhibition effect of *Lactobacillus plantarum* (lb. 3) incubated for 36 hr. on *Staphylococcus epidermidis*

1- Filtrate
2- Concentrated filtrate
3- Purified bacteriocin
4- When grown on solid media

Plate (4-3) The inhibition effect of *Lactobacillus plantarum* (lb. 3) incubated for 36 hr. on *Propionibacterium acnes*

Plate (4-4) The inhibition effect of *Lactobacillus plantarum* (lb. 3) incubated for 36 hr on *Escherichia coli*

1- Filtrate
2- Concentrated filtrate
3- Purified bacteriocin
4- When grown on solid media

Plate (4-5) The inhibition effect of *Lactobacillus plantarum* (lb. 3) incubated for 36 hr. on *Proteus mirabilis*

1- Filtrate
2- Concentrated filtrate
3- Purified bacteriocin
4- When grown on solid media

4.6.2 In Liquid Media:

Well diffusion method had been used to determine the inhibition activity of *Lactobacillus plantarum* (Lb.3) filtrate grown at different incubation period against the causative bacteria of acne vulgaris.

Table (4-5) shows the results of inhibition activity of Lb.3 filtrate grown at different incubation period (18, 24, 36, 48 and 72) against causative bacteria of acne vulgaris. Filtrates of Lb.3 isolate exhibited noticeable inhibition activity against gram positive bacteria tested at 36, 48 and 72 hr. incubation periods. Gram negative bacteria, on the other hand, may not effect (plates 4.1, 4.2, 4.3, 4.4 and 4.5).

Table (4.5) Inhibition activity of *Lactobacillus plantarum* (Lb.3) filtrate grown at different incubation period against pathogenic bacteria of acne vulgaris

Bacteria	Incubation period (hr)				
	18	24	36	48	72
Staphylococcus aureus	–	–	+	+	+
Staphylococcus epidermidis	–	++	++	++	++
Propionibacterium acnes	–	–	+	+	+
Escherichia coli	–	–	–	–	–
Proteus mirabilis	–	–	–	–	–

(–) no effect = inhibition zone (zero mm)

(+) slightly effect = inhibition zone (≤10 mm)

(++) moderate effect = inhibition zone (10-13 mm)

At 24 hr. incubation period only *Staphylococcus epidermidis* was affected. Adversely, incubation period of 18 hr. was not enough for the isolate (Lb.3) to exhibit inhibitory effect on both gram positive and gram negative bacteria.

These results belong to that concentration of bacteriocin in liquid media was very little when compared with its concentration on solid media (Deklerk and Smit, 1967).

Inhibitory activity of *Lactobacillus plantarum* (Lb.3) was markely increased against all acne vulgaris causative bacteria when the filtrate of Lb.3 was concentrated for three folds after isolate was propagated at 30 °C for 36 hr at anaerobic condition, table (4-6) (plates 4.1, 4.2, 4.3, 4.4 and 4.5).

Table (4.6) Inhibition activity of *Lactobacillus plantarum* (Lb.3) concentrated filtrate against pathogenic bacteria of acne vulgaris

Bacteria	Inhibition effect of Lb.3 concentrated filtrate
Staphylococcus aureus	+
Staphylococcus epidermidis	+++
Propionibacterium acnes	+
Escherichia coli	+
Proteus mirabilis	+

(+) slightly effect = inhibition zone (≤10 mm)

(+++) good effect = inhibition zone (13-20 mm)

4.7 Purification of Bacteriocin (Plantaracin) from Filtrate of Lb.3 Isolate:

Isolate *Lactobacillus plantarum* (Lb.3) had been grown under anaerobic conditions and at conditions, which were mentioned in item (2.2.11), to improve the production of bacteriocin. Then the bacteriocin was purified by precipitating

with ammonium sulphate, which increased the saturation percentage from 30 % to 70 %. After final purification, 8 mg / ml bacteriocin was obtained. Geis *et al*., (1983) mentioned that the first step of purification was done by using ammonium sulphate. White *et al*., (1973) stated that ammonium sulphate precipitated the protein by neutralization the charge that found on protein surface, through salt and disruption by the layer of water that surrounding the protein molecules. Then, this led to decrease protein dissolving and at end protein was precipitated. Vaughen *et al*., (1992) used precipitation of protein at two saturation percentages 30 % and 60 % and later the protein nature of precipitated was detected by using the hydrolyzed protein enzymes.

4.8 Inhibitory Effect of Partially Purified Bacteriocin of Lb.3 Isolate Against Acne Causative Bacteria:

Result in table (4-7) show the inhibitory activity of purified bacteriocin of Lb.3 isolate on bacteria isolated from acne vulgaris. The bacteriocin possess inhibitory activity against all bacterial causative isolated from patients suffering of acne vulgaris. Its activity was different from one bacteria type to another such as there was simple effect at inhibition zone 10 mm but in another 30 mm inhibiton zone was showed. In general its activity on gram positive bacteria isolates was greater than on gram negative isolates (Plates 4.1, 4.2, 4.3, 4.4 and 4.5).

Suskovic *et al*., (1997) reported that the bacteriocin obtained from *Lactobacillus plantarum* had inhibition activity against *Staphylococcus aureus*, *E. coli* and *Bacillus subtilus.*

Wolfson (1999) mentioned that all bacteriocin of the probiotic strain that isolated from milk products were able to inhibit the growth of some, if not all, of the microbial isolates which include *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa,* and *Salmonella typhi.*

Catanazaro J. and Green L. (1997) find that the bacteriocin (plantaracin) that purified from *Lactobacillus plantarum* was had bactericidal effect against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Propionibacterium acnes* and *Proteus mirabilis*.

Table (4-7) Inhibition effect of partially purified bacteriocin obtained from Lb.3 isolate against bacterial causative of acne vulgaris

Bacteria	Inhibition effect of Lb.3 bacteriocin
Staphylococcus aureus	+++
Staphylococcus epidermidis	++++
Propionibacterium acnes	++
Escherichia coli	+
Proteus mirabilis	+

(+) slightly effect = inhibition zone (≤10 mm)

(++) moderate effect = inhibition zone (10-13 mm)

(+++) good effect = inhibition zone (13-20 mm)

(++++) excellent effect = inhibition zone (≥20 mm)

4.9 Protein Quantities in Lb.3 Concentrated Filtrate and Its Bacteriocin:

In this study, both the concentrated filtrate of Lb.3 isolate and it bacteriocin, were analysis by the dye binding method for quantity of protein in each once a quantity of 4 mg / ml was found in the concentrated filtrate (three fold) and 5 mg / ml in its partially purified bacteriocin.

4.10 Using the (Lb. 3) Concentrated Filtrate in Treatment of Sever Acne Vulgaris:

After studying the inhibitory effect of bacteriocin in the concentrated filtrate (three fold) and compared that with the inhibitory effect of purified bacteriocin (by ammonium sulphate) on microbial cause of sever acne vulgaris. It was greater than that of concentrated filtrate. But due to the small differences in concentration of protein in concentrated filtrate and purified bacteriocin, in addition to the cheapness, easy and fast in prepared concentrated filtrate comparing to purified bacteriocin concentrated filtrate (three fold) was used as topical treatment for patient suffering from acne vulgaris, after measured its minimum inhibitory concentration.

Result of measuring the minimum inhibitory concentration of concentrated filtrate (three fold) on bacterial cause acne vulgaris from result noticed that the MIC 80 % was chosen to treatment all bacterial type of acne vulgaris, table (4-8).

Table (4-8) The minimum inhibitory concentration of microbial cause of acne vulgaris

Bacteria	MIC
Staphylococcus aureus	40 %
Staphylococcus epidermidis	40 %
Propionibacterium acnes	50 %
Escherichia coli	80 %
Proteus mirabilis	80 %

After two weeks of treatment with filtrate, acne completely or almost completely healing in most patients (up to 95 %). During the treatment period the redness, swollen (size) and symptoms of inflamed cysts and nodules were reduced, figure (4-8, 4-9, 4-10, 4-11). The side effect of this prepared topical

treatment includes only the drying of skin. Comparing with other topical treatment that prescription by doctor such as Salicylic acid, Azelaic acid, Benzol peroxide, tretinoin and many other which have some adverse effects that can be sever, such as inflammation of the lip, skin itching, redness, stinging, burning, peeling and scaling or discoloration of the skin (Arnold *et al*., 1990).

It's known that mild to moderate acne is treating by using topical treatment alone, but to sever acne the doctor often prescribes oral antibiotic in addition to topical medication. Oral antibiotics are though to help control acne by curbing the growth of *Propionibacterium acnes* and decreasing inflammation. Common antibiotics used to treat acne are tetracycline, minocycline, doxycycline and erythromycin. Some people have side effects when taking these antibiotics such as photosensitivity (higher risk of sun burn), upset stomach, dizziness or light headedness and skin discoloration. Tetracycline is not given to pregnant women because it can discolor developing teeth. Tetracycline and minocycline also decrease the effectiveness of birth control pills, also patient must take oral antibiotics for as long as 4 to 6months to effectively treat acne (Leyden, 1997).

But the prepared topical treatment in this study lasting 2 weeks for complete healing of mild and moderate acne but for sever acne 3 weeks were need to complete healing without any using of oral antibiotics with it.

A- Before probiotic treatment

B- After 2 weeks of probiotic treatment

Figure (4-8) A patients suffering from mild acne vulgaris in face

A- Befor probiotic treatment

B- After 2 weeks of probiotic treatment

Figure (4-9) A patient suffering from moderate acne vulgaris in face

A- Before treatment B-After 2 weeks of probiotic treatment

C- After 3 weeks of probiotic treatment

Figure (4-10) A patient suffering from sever acne vulgaris in back

A- Before treatment B-After 2 weeks of probiotic treatment

C- After 3 weeks of probiotic treatment

Figure (4-11) A patient suffering from sever acne vulgaris in face

Chapter five

Conclusions and Recommendations

5.1 Conclusions

1. Five species of bacteria were isolated from sever acne vulgaris include: *Staphylococcus epidermidis*, *Propionibacterium acne*, *Proteus mirabilis*, *Staphylococcus aureus* and *Escherichia coli*).

2. Sixteen isolates of lactic acid bacterial (LAB) were isolated from dairy products that collected from Baghdad markets and identified other wise two isolates of lactic acid bacteria (LAB) were identified as *Lactobacilus plantarum* (Lb.1 and Lb.3).

3. An isolate Lb.3 was resistance to streptomycin, penicillin G and trimethprim, but sensitive to amoxicillin, chloramphenicol, cloxacillin, rifampin and ampiclox.

4. The bacterial isolates of sever acne vulgaris were resistance to ampicillin, amoxicillin, ampiclox, methoprim, tetracycline and cefatexine, but sensitive to doxycyclin and rifampin.

5. An isolate of Lb. Plantarum (Lb.3) had good inhibition activity against acne vulgaris isolates.

6. The concentrated filtrate of Lb. plantarum isolate (Lb.3) had great role in treatment the patients that suffering from sever acne vulgaris after 2 weeks of treatment with it.

5.2 Recommendations

1. Studying the inhibitory activity of bifidibacterium against sever acne vulgaris microbes.
2. Using the purified bacteriocin in treatment of severs acne vulgaris.
3. The role of bacteriocin in association with antibiotics in treatment of acne vulgaris disease.

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المصادر العربية:

القصاب، عبد الجبار عمر والخفاجي، زهرة محمود (١٩٩٢). تأثير الظروف المختلفة على الفعالية التثبيطية للعصيات اللبنية المعوية تجاه البكتيريا المعوية المسببة للاسهال. مجلة العلوم الزراعية العراقية. المجلد ٣. العدد (١). ص ١٨-٢٦.

الملخص

جمعت (١٦) عزلة تعود لبكتيريا حامض اللأكتيك من منتجات الألبان المأخوذة من الاسواق المحلية في بغداد. شخصت هذهِ العزلات الى اربعة أجناس شملت (٦) عزلات تعود لجنس*Lactobacillus* و (٢) عزلة لجنس *Lactococcus* و (٤) عزلات لجنس *Pediococcus* و (٤) عزلات لجنس *Leuconstec*.

شخصت عزلات جنس الـ *Lactobacillus* حتى النوع الى *Lb. plantarum* (Lb.1, Lb3) و *Lb. Acidophilus* (Lb.2) و *Lb. Bulgaricus* (Lb.4) و (Lb.5) *Lb. brevis* و *Lb. Fermentum* (Lb.6).

كما و جمعت (٥٠) عزلة بكتيرية من الفدام و البثور لعشرين مريضا يعانون من حب الشباب الالتهابي الشديد، و بعد تشخيص هذهِ العزلات وجد ان تلك التي عزلت من الفدام تعود لثلاثة انواع من البكتيريا *Propionibacterium acnes* و *Staphylococcus aureus* و *Staphylococcus epidermidis* لكن تلك التي عزلت من البثور تعود لخمس انواع من البكتيريا و هي:

Propionibacterium acnes و *Staphylococcus aureus* و *Staphylococcus epidermidis* و*Escherichia coli* و *Proteus mirabilis*.

لدى أختيار تأثير المضادات الحيوية على العزلتين اللتين تعودان الى*Lb. plantarum* وجد ان كلتا العزلتين (Lb.1, Lb.3) كانتا حساستين لمضادات Streptomycin وPenicillinG و Trimethprim و لكنهما مقاومتان الى Amoxicillin وChloromphenicol و Cloxacillin و Rifampin و Ampiclox.

اما بالنسبة للبكتيريا المرضية المسببة لحب الشباب الالتهابي الشديد فقد وجد انها كانت حساسة لمضادات Doxycycline و Ciprofloxacin وClindamycin و Neomycin وRifampin و مقاومة لمضادات Ampicillin و Amoxicillin و Ampiclox و Methoprim و Tetracyclineو Cefalexine.

بعد تنمية العزلة Lb.3 في اوساط زرعية سائلة و صلبة لفترات زمنية مختلفة لأختبار قدرتها على اعطاء افضل فعالية تثبيطية ضد عزلات البكتيريا المرضية المسببة لحب الشباب. لوحظ امتلأكها فعالية تثبيطية جيدة عند نموها على الوسط الصلب ضد جميع انواع البكتيريا المرضية و الفترات الزمنية المستخدمة، و كانت فترة الحضانة ٣٦ و ٤٨ و ٧٢ ساعة، فيما لم لكن لها تأثير مثبط للبكتيريا السالبة الكرام. ولدى تركيز راشح العزلة هذهِ لثلاث مرات، أمتلك فعالية تثبيطية عالية ضد البكتيريا المرضية.

تم استخلص و تنقية بكتريوسين هذه ِالعزلةLb.3 ايضاً (plantaracin) لأختبار تأثيره المثبط، فوجد انه يملك فعالية تثبيطية عالية جداً ضد جميع البكتيريا المرضية ولاسيما الموجبة منها.

تم قياس كمية البروتين في الراشح المركز و البكتريوسين المنتقى، ووجد انه الاول كان ٤ ملغم / مل و الثاني ٥ ملغم / مل.

كما وتم تقدير التركيز المثبط الادنى للراشح المركز ووجد ان ٨٠% هو الافضل لتثبيط جميع انواع البكتيريا المرضية، لذا فقد استخدم هذا التركيز في علاج المرضى الذين يعانون من حب الشباب الالتهابي الشديد، و كانت نتيجة العلاج شفاء المرضى نهائياً بعد الاسبوع الثاني في اغلب الحالات (٩٥%) مع وجود جفاف بسيط في البشرة.

تاثير بكتريا حامض اللاكتيك على

المسببات البكتيرية لمرض حب الشباب

الالتهابي الشديد

رسالة مقدمة الى كلية العلوم في جامعة النهرين

جزأ من متطلبات نيل درجة ماجستير علوم في علوم التقانا ت الحياتية

من قبل

رنا قاسم نعيم الدليمي

بكلوريوس تقانة احيائية 2002

جامعة النهرين

شوال ١٤٢٥

تشرين الثاني ٢٠٠٤