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List of Abbreviations

Abbreviation	***Mean***
DES	Dry Eye Syndrome
GRAS	Generally Recognized As Safe
LAB	Lactic Acid Bacteria
MRS	Man-Rogoza Sharp
MIC	Minimum Inhibitory Concentration
NCCL	National Committee for Clinical Laboratory Standard
TSI	Triple Sugar Iron
TBUT	Tear Break Up Time
NAM	N-acetyl muramic acid

قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ

صدق الله العظيم

سورة البقرة "32"

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Linda

Appendix (1): Results of the API Staph kit system used for diagnoses *Staphylococcus aureus* and *Staphylococcus epidermidis*.

Tests	*Staphylococcus epidermidis*	*Staphylococcus aureus*
0	–	–
GLU	+	+
FRU	+	+
MNE	+	+
MAL	+	+
LAC	+	+
TRE	–	+
MAN	–	–
XLT	–	–
MEL	–	–
NIT	+	+
PAL	+	+
VP	+	–
RAF	–	–
XYL	–	–
SAC	+	+
MDG	–	–
NAG	–	+
ADH	+	+
URE	+	+

(+): positive results. (–): negative results.

0: No substrate, Glu: D-Glucose, FRU: D-Fructose, MNE: D- Mannose, MAL: Maltose, LAC: Lactose TRE: D- Trehalose, MAN: D- Mannitol, XLT: Xylitol, MEI: D- Melibiose, NIT: Potassium nitrate, PAL: B-naphthyl-acid phosphate, VP: Sodium pyruvate, RAF: Raffinose, Xyl: Xylose, SAC: Sucrose, MDG: α- methyl-d-glucoside, NAG: N- acetyle-glucosamine, ADH: Arginine, URE: Urea.

Appendix (2): Results of the API Strep kit system used for diagnosis *Streptococcus pneumoniae* and *Streptococcus pyogenes*.

Tests	*Streptococcus pneumoniae*	*Streptococcus pyogenes*
VP	**–**	**–**
HIP	**–**	**–**
ESC	**–**	**–**
PYRA	**V**	**+**
α GAL	**V**	**–**
βGUR	**–**	**–**
βGAL	**+**	**–**
PAL	**–**	**+**
LAP	**+**	**+**
ADH	**V**	**+**
RIB	**–**	**–**
ARA	**–**	**–**
MAN	**–**	**–**
SOR	**–**	**–**
LAG	**+**	**+**
TRE	**+**	**+**
INU	**V**	**–**
RAF	**+**	**–**
AMD	**+**	**V**
GLYG	**–**	**–**
βHEM	**–**	**+**

(+): positive results. (–): negative results. V: variable.

VP: Pyruvate, HIP: Hippurate, ESC: Esculin, PYRA: Pyrrolidonyl- 2-nphthylamide, α GAL: 6-Bromo- 2-naphthyle α- D- galactopyranosides, βGUR: Naphthol AS-BI**β-** D- glucuroonate, βGAL: 2- naphthyl- B- D-galactopyranoside, PAL: 2- naphthyl phosphate, LAP: L- Leucine- 2-naphthylamide, ADH: Arginine, RIB: Ribose, ARA: L- Arabinose, MAN: Mannitol, SOR: Sorbitol, LAC: Lactose, TRE: Trehalose, INU: Inuline, RAF: Raffinose, AMD: Starch(2), GlyG: Glycogen.

Appendix (3): Results of the API 20E kit system used for diagnoses *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa*.

Tests	*Escherichia coli*	*Proteus mirabilis*	*Pseudomonas aeruginosa*
ONPG	+	–	–
ADH	–	–	+
LDC	+	–	–
ODC	+	+	–
CIT	–	–	+
H_2S	–	+	–
URE	–	+	–
TDA	–	+	–
IND	+	–	–
VP	–	–	+
GEL	–	+	+
GLU	+	+	+
MAN	+	–	–
INO	–	–	–
SOR	+	–	–
RHA	+	–	–
SAC		–	–
MEL	+	–	–
AMY	–	–	–
ARA	+	–	–
OX	–	–	+

(+): positive results. (–): negative results.

ONPG: Ortho- nitro- phenyl- β- D- galactopyranoside isopropylthiogalactopyranoside (IPIG), ADH: Arginine, LDC: Lycine, ODC: Ornithine, ClT: Sodium citrate, H_2S: Sodium thiosulfate, URE: Urea, TDA: Tryptophan, IND: Indole, VP: Sodium pyruvate, GEL: Kohns gelatin, GLU: Glucose, MAN: Mannitol, INO: Inositol, SOR: Sorbitol, RHA: Rhamnose, SAC: Sucrose, MEL: Melibiose, AMY: Amygdalin, ARA: Arabinose, OX: Oxidase.

Appendix (4): Results of the API Coryne kit system used for diagnoses *Corynebacterium diphtheriae*.

Tests	*Corynebacterium diphtheriae*
NIT	**+**
PYZ	–
PYrA	–
PAL	–
β GUR	–
BGAL	–
α GLU	**+**
β NAG	–
ESC	–
URE	–
GEL	–
O	–
GLU	**+**
RIB	**+**
XYL	–
MAN	–
MAL	**+**
LAC	–
SAC	–
GLYG	–
CAT	**+**

(+): positive results. (–): negative results. V: variable.

NIT: Nitrate reduction, PYZ: Pyrazinamidase, PYrA: Pyrrolidonyl Arylamidase, PAL: Alkaline phosphotase, BGUR: Beta- Glucuronidase, βGAL: Beta- Galactosidase, α GLU: alpha- Glucosidase, β NAG: N-Acetyl- B- Glucosaminidase, ESC: Essculin, URE: Urease, GEL: Gelatin (hydrolysis), O: Control, GLU: Glucose, RIB: Ribose, XYL: Xylose, MAN: Mannitol, MAL: Maltose, LAC: Lactose, SAC: Sucrose, GLYG: Glycogen, CAT: Catalase.

الخلاصة

شملت الدراسة جمع (٦٠) عينة اخذت كمسحات من مرضى يعانون من اعراض العين الجافة (Dry Eye Syndrome) في مستشفى ابن الهيثم للعيون بمدينة بغداد ولدى زرع العينات على الاوساط الزرعية المنتخبة اعطت (٥٣) عينة منها نتيجة موجبة لتواجد العزلات البكتيرية.

تم تشخيص العينات الموجبة من خلال الفحوصات الزرعية ، والمجهرية، والكيموحياتية اللازمة للتشخيص وبعد ان تم التاكد من التشخيص النهائي باستخدام عدات Api الخاصة بكل نوع من البكتريا، أمكن الحصول على ألأنواع البكتيرية ونسب تواجدها ألأتيه:

Staphylococcus genus (45.9%), *Streptococcus* genus (17%), *Corynebacterium diphtheriae* (9.3%), *Hemophilus influenzae* (7.4%), (5.6%) to each of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, while (3.7%) to each of *Proteus mirabilis* and *Neisseria gonorrhoeae* and (1.8%) to *Escherichia coli*.

بذلك تكون بكتريا *Staphylococcus epidermidis* هي السائدة في العينات الماخوذة من اصابات العين الجافة، تليها في ذلك عزلات *Staph aureus* ثم الانواع الاخرى.

فيما يتعلق بالمرضى، سجلت أعلى نسبة مئوية (45%) لحدوث حالات التهاب العين الجافة فى الفئة العمرية فوق الستين عاما مقارنة ببقية الفئات، وتبعا لجنس المريض فقد كانت النسبة المئوية في الاناث أعلى (56.7%) مما في الذكور (43.3%).

أظهرت اختبارات حساسية عزلات بكتريا إصابات العين الجافة اتجاه (14) مضاد حيوي ان مضادي السبروفلوكساسين والجنتامايسين كانت الاكثر تاثيرا، فيم أقتصرتأثيرمضاد الكلورامفينيكول على عزلات البكتريا الموجبة لغرام. وفي الوقت الذي اختلفت بقية المضادات الحيوية ألأخرى في تاثيرها، واضهرت اغلب العزلات مقاومتها لمضادى الامبسلين وبنسلين ج .

بعد تنمية عزلتي *Lactobacillus acidophilus* و *L. plantarum* في اوساط زرعية سائلة وصلبة ولفترات حضن مختلفة (24, 48, 24 ساعة) ضد عزلات البكتريا المرضية المعزولة من مرضى العين الجافة، لوحظ امتلاك هذة العزلات لأفضل فعالية تثبيطية عند نموها على الوسط الصلب ولفترة الحضن لمدة (72) ساعة ولا سيما ضد البكتريا الموجبة لغرام. وبالرغم من ان

رواشح بكتريا اللأكتيك غير المركزة لم تعط أي تأثير تثبيطي ملحوظ ضد العزلات البكتيرية، لكن الفعالية التثبيطية ازدادت لدرجة كبيرة ضد كل العزلات البكتيرية عند تركيز هذه الرواشح.

لدى تقديرالتراكيز المثبطة الدنيا لرواشح بكتريا *L. acidophilus* و *L. plantarum* المركزة، دلت النتائج بأن قيم التزاكيز المثبطة الدنيا كانت أوطأ لبكتريا *Lactobacillus acidophilus* بالمقارنة مع تلك لبكتريا *L. plantarum* وبذلك أثبتت النتائج هذه أن الفعالة التثبيطية لبكتريا اللأكتيك ألأولى هي ألأفضل ضد المسببات البكتيرية لإصابات العين الجافة.

1.2: Review of Literature

1.3: Anatomy of the eye and Lacrimal Apparatus:

The eye is the organ of sight, a nearly spherical hollow globe filled with fluids (humors). The outer layer or tunic (sclera, or white, and cornea) is fibrous and protective. The middle tunic layer (choroid, ciliary body and the iris) is vascular. The innermost layer (the retina) is nervous or sensory. The fluids in the eye are divided by the lens into the vitreous humor (behind the lens) and the aqueous humor (in front of the lens). The lens itself is flexible and suspended by ligaments which allow it to change shape to focus light on the retina, which is composed of sensory neurons. The adult human eye is a sphere approximately one inch in diameter. Only about one-sixth of the eye's anterior surface is observable; a cushion of adipose tissue and the walls of the bony orbital protect the remainder (Kanski, 2003).

From the anterior each eye is protected by the eyelids. The medial and lateral junctions of the upper and lower eyelids are referred to as the medial and lateral canthus. A mucous membrane; the conjunctiva lines the internal surface of the eyelids and continues over the anterior surface of the eyeball to the outer edge of the cornea where it fuses with the corneal epithelium. The conjunctiva secretes mucus, which functions as a lubricant for the eyeball (Liesegang *et al.,* 2002). The eyelashes project from the edge of each eyelid. Modified sweat glands called ciliary glands lie between the eyelashes and also help in lubrication of the eyeball. The larger meibomian glands, locates posterior to the eyelashes secrete an oily substance.

The lacrimal apparatus composed of two parts, the secretory (lacrimal gland and ducts) and excretory include (puncti, canaliculi, lacrimal sac and nasolacrimal duct) the lacrimal glands are exocrine glands which derived from cells of epithelium membranes (Fox, 2002), the secretion of these glands are expressed to the outside of the epithelial membranes through ducts; lacrimal glands lie superior and lateral to each eye, they continually release a dilute salt solution (tear) on the anterior surface of the eyeball through several small ducts, The tear flush across the eyeball into the lacrimal canals medially, then into the lacrimal sac and finally into the nasolacrimal ducts which empties into the nasal cavity (Pflugfelder *et al.*, 2005). Figure (1-1) shows anatomy of the eye.

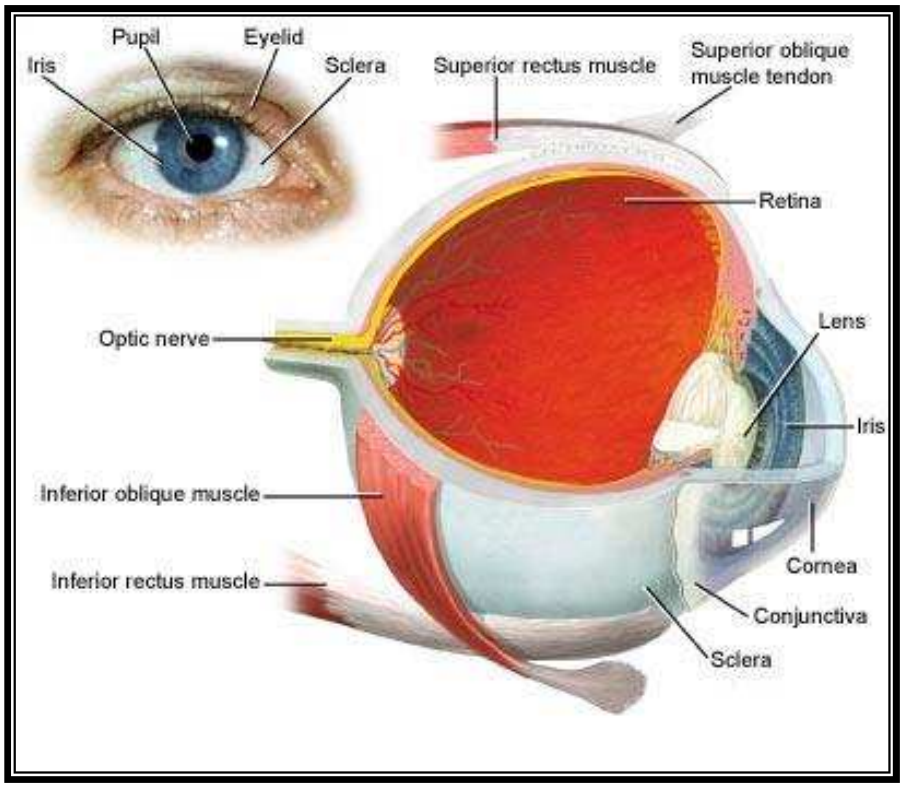

Figure (1-1): Anatomy of the eye (Fox, 2002).

1.4: Tear Physiology

1.4.1: Tear Film Composition

Tear film contains several proteins, electrolytes, aminoacids and metabolic products which have antimicrobial effects, these components maintain and protect ocular surface (Pflugfelder *et al*., 2005).

One of these proteins is lysozyme which forms about 30% of total tear proteins and produce in large quantities in morning; it attacks the protective cell wall of bacteria by breaking bonds that links the alternating N-acetyl-glucosamine and N-acetyl muramic acid molecules and this destroy the structural integrity of the glycan chain, the back-bone of the peptidoglycan molecule. It is active against Gram positive bacteria and accelerates lysis of Gram negative bacteria in presence of antibody and complement (Hancock and Diamond, 2000).

Another important antimicrobial protein is lactoferrin, an 80-kDa, iron-binding glycoprotein found in milk, bile, saliva, tear, and polymorphonuclear granules which exert broad spectrum antimicrobial activity against many pathogens including bacteria, fungi and viruses (Valenti *et al*., 1998; Levay and Viljoen, 1995).

The first antimicrobial functions of lactoferrin is bacteriostatic, due to strong iron-binding properties of the protein and the fact that lactoferrin is found in relatively iron free state in secretions, this permit the protein to bind any available iron at site of infection, there by, sequestering this essential growth nutrient away from iron requiring microbes (Iyer and Lonnerdal, 1993).

The second function is bactericidal by bind to outer membrane of Gram negative bacteria and can cause rapid release of lipopolysaccharides

with an associated increase in membrane permeability, so disrupt the structural integrity of microbial membrane (Hwang *et al*., 1998). In addition to bacteriostatic and bactericidal properties lactoferrin can protect epithelial cells against microbial infections by inhibiting intracellular invasion by pathogenic bacteria (Van□ □t Hof *et al*., 2001).

Tear lipocalins are the major lipids binding to protein in tears leading to increase the surface pressure of aqueous layer by scavenging lipids from hydrophobic surfaces and delivering them to the aqueous phase of the tear film (Glasgow *et al*., 1995).

Electrolyte such as Na^{+} and Ca^{++} ions are very important for epithelial and nerve function and to ensure correct osmolarity.

Complement is one of the humoral non specific defense mechanisms, upon activation; it can lead to increase vascular permeability, recruitment of phagocytes cells, lysis and opsonization of bacteria (Walport, 2001).

Others, such as immunoglobulin IgA and leukocyte, provide antibacterial activity.

Tear composition in chronic dry eye disease is characterized by increased concentrations of proinflamentory cytokines reduce level of growth factors and soluble mucin, and the presence of activated proteases that degrade the extra cellular matrix and the tight junctions between cells of the corneal epithelium (Villarreal *et al.*, 2005).

1.4.2: Tear Film Layers

The human tear film is most often described as consisting of three distinct layers (Rolando and Zierhut, 2001) as shown in figure (1-2). A mucus layer lies on top of the epithelial cells of the cornea, an aqueous layer

is present above the mucus layer, and a lipid or fatty layer covers the aqueous layer, these layers are :-

A-The outer lipid layer:

This is secreted by the meibomian gland, contain fatty acid called lipids, these smooth the tear surface and slow evaporation of the middle watery layer and increase surface tension, when the oil layer is abnormal (McCulley and Shine, 1997), the watery layer evaporates too quickly, patients with lipid phase defects primarily notice their symptoms in the morning (Stein, 2004).

B-The middle aqueous layer:

The middle layer which makes about 90 percent of tears in mostly water with little bit of salt this layer produce by tear gland (lacrimal gland) which clear your eyes and wash away foreign particles or irritant from conjunctiva and cornea, supply atmospheric oxygen to the corneal epithelium, and has antibacterial substance such as lactoferrin and lysozyme (Sharma, 1998), patients with aqueous phase deficiencies typically report symptoms upon awakening and in the evening (Stein, 2004).

C-The inner mucin layer:

This is very thin and is secreted by the globet cells in the conjunctiva, the mucus is 10-100 times more viscous than the overlaying aqueous layer, this contribute to cell adhesion, They’re also a defense against ocular surface damage, allow tears to spread evenly over the surface of eyes and convert the corneal and conjunctiva epithelium from hydrophobic to hydrophilic state by allowing the aqueous layer to adhere to them (Sharma *et al*., 1999).

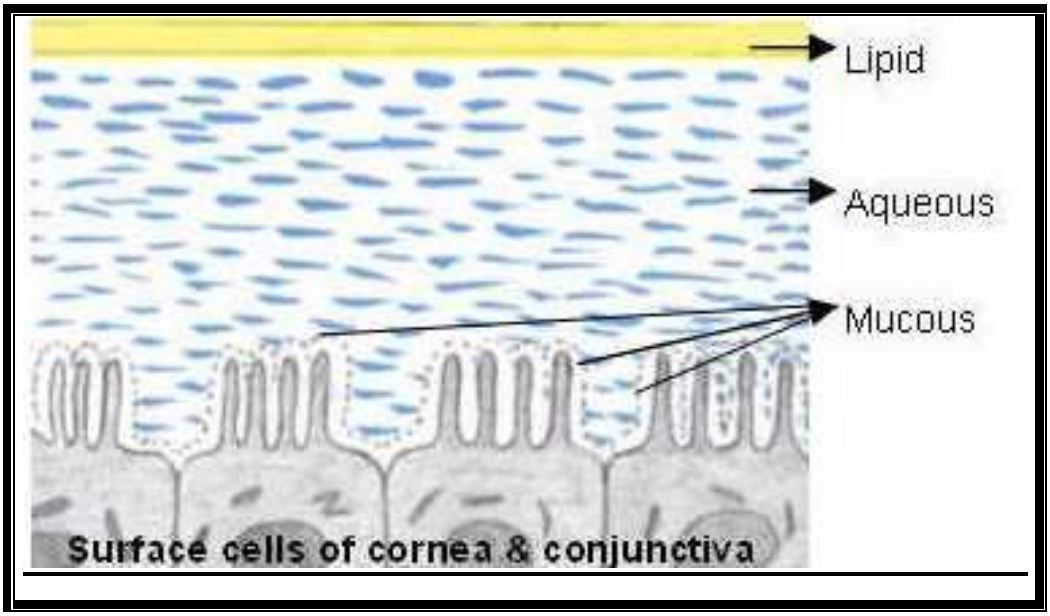

Figure (1-2): Tear Film Layers (Rolando and Zierhut, 2001)

1.4.3: Tear Film Function

Tear film keeps the cornea wet, thus allowing gas exchange between the air and the epithelium. It cleans debris from the transparent surface, providing a clear optical path to the retina, and protects the ocular surface from invasion by bacteria and viruses. The tear film also provides essential metabolites such as retinol, which serves to preserve the transparent nature of the epithelium (Walcott, 1998).

1.4.4: Tear Mechanism

A number of mechanisms have been suggested for maintaining the aqueous and soluble components of tears: the lacrimal gland may have an in-built timed release of fluid, there may be local or systemic biochemical mechanisms or an active neural feedback system may operate (Zhou *et al.*, 2004).

The cornea, conjunctiva, main and accessory lacrimal glands, meimbomian glands and interconnecting innervations act as a functional unit controlling tear secretion, if any part of this functional unit is impaired, lacrimal gland support to the ocular surface is affected, and as a result to that affect tear production (Beuerman, 2005).

1.4.5: Tear Production

Production of tears is under nervous and hormonal control and occurs in two ways. Basic tearing produces tears at slow, steady rate and keeps the eye lubricated. What is called reflex tearing produces large quantities of tears in response to eye irritation or emotions, reflex tears contain much more water than do basic tears, and they are low in mucus and oils (Bron, 2001).

1.5: Dry Eye

1.5.1: Dry Eye Syndrome:

Dry eye is a collection of symptoms that result from insufficient quantity or quality of tears.

In medical literature, it is called Keratoconjunctivitis sicca, Kerato (corneal) conjunctivitis (conjunctival inflammation) sicca (from the latin sicco, meaning “to dry”). It is disorder of tear film due to tear deficiency or excessive tear evaporation which causes damage to the interpalpebral ocular surface and it is associated with symptoms of ocular discomfort (Brewitt and Sistani, 2001).

1.5.2: Causes of Dry Eye:

A- Aqueous Layer Deficiency:

Aqueous layer deficiency includes several types:

- **Sjögren syndrome:** Sjögren's syndrome (SS) is a systemic autoimmune exocrinopathy (Kassan and Montsopoulos, 2004). It is characterized by progressive dysfunction of the exocrine glands particularly the salivary and lacrimal glands and symptoms of oral and ocular dryness (Petrone *et al*., 2002).
- **Non Sjögren syndrome:** patient with non sjögren syndrome did not produce tears because of disease in tear gland (lacrimal gland) example sarcoidosis, trauma, diabetes, and immune disease which causing systemic and local damaging to the lacrimal gland and decrease tear flow (Rolando and Zierhut, 2001).
- **Aging**: aging lead to dry eye because certain aspect of tear physiology change with age such as reflex by the lacrimal gland, tear volume, tear film stability and osmolarity increase over the years (Graig and Tomlinson, 1995). The tear volume decrease as much as 60% by age 65 from that at age 18, and it is effect 75% of people over age 65 (Moss *et al*., 2000).
- **Increase tears evaporation:** evaporation accounts for small amount of liquid in the normal eye; One –third of resting tear flow evaporates but in dry eye approximately three quarters of the tear film is evaporating (Mathers, 2000). The evaporation of tears is related to either environmental factors such as central heating, dry climate wind, hair dryer, cigarette smoke and contact lens wear or reduce blinking this related to computer user, watching TV, driving, reading

(Meadows, 2005). For example computer users tend to blink much less frequently about 7times/min, a normal rate of around 22times/min, so the tear film is not spread across the surface of the eye and this lead to increase evaporation.

- **Absence of lacrimal gland:** this occur either congenital or after surgical treatment (Kanski, 1994).
- **Scarring of conjunctiva:** severe conjunctiva scarring (as aresult of trauma or infection) lead to destruction of lacrimal glands and ducts (Kanski, 1994).
- **Destruction of lacrimal tissue:** this due to tumours, sarcoidosis and chronic inflammation of the lacrimal gland (Kanski, 1994).

B- Mucin Layer Deficiency:

Mucin layer deficiency caused by deficient in vitamin A supplying which lead to dysfunction of globet cells (cells secreted the mucin layer) and as a result to that occurrence of dry eye syndrome (American Academy of Ophthalmology, 2003).**********

C- Lipid Layer Abnormalities:

Abnormalities in lipid layer result from meibomain gland dysfunction which decreases lipid production in tear, so destabilizes the tear film (Baudouin, 2001).

1.5.2.3: Signs and Symptoms of Dry Eye:

Occasional burning sensation in the eyes in areas of low humidity or high pollution, drifty and scratchy eyes sensation, painful, itching, redness, sensitivity to light, burning sensation that improves with blinking in addition to that decrease tolerance to contact lens (Meadows, 2005).

1.5.2.4: Detection and Diagnosis of Dry Eye:

There are several methods to test for dry eye and measuring production, evaporation and quality of the tear film (Bron, 2001).
The more important tests are (Stein, 2004).

- **Schirmer Test:**

Schirmer Test I: This test I used it for patients to establishing dry eye diagnoses and its one of the oldest test. It performed without anesthesia which measures basic tear secretion and done by placing (35mm*5mm) size filter paper strip folded 5 mm method from one end and inserted at the junction of the middle and outer third of the lower eye lid, and eye was kept open and blink as necessary, after 5 minutes the filter strip was removed and the amount of wetting from fold was measured in mm by ruler, a normal eye will wet between 10mm and 25mm during 5 minute. Measurement between 5 mm and 10 mm were considered borderline and values less than 5 mm indicative of impaired secretion, particularly if obtained on consecutive occasions.

Schirmer Test II: It's quantifies basic secretion and performed as the same procedure for type I but installing a topical anesthesia in the eye and the amount of wetting is measured after 2 minute. Measurement less than 15mm indicates failure of basic secretion. However this test is seldom used because this secretion is usually intact.

- **Rose Bengal Test**:

Rose Bengal (1% conc.) is particularly valuable for identifying combined tear film disorders and also the tiniest epithelial cell lesions by its affinity for devitalized epithelial cells and mucous. This done by applying one drop in the lower fanix by staining the interpalperal conjunctiva in the form of two triangles with their bases at the limbus.

- **Tear Break Up Time Test (TBUT):**

  It's another test help in determining tear film stability (the time from the last blink to the first tear film break up). TBUT reductions are most marked in mucin phase disorders and aqueous phase disorders, while lipid phase disorders generally don't show TBUT reductions.

1.5.2.5: Treatment of Dry Eye:

Unfortunately, there is no way to increase production of tears, either lubricating drop called artificial tears may be used to replace the missing ones or the tear which is produced naturally may be conserved.

The first line of treatment for dry eye should address the cause of dry-eye, rather than simply treating the symptoms with eye lubricants

There are numerous treatment strategies exit to compact the signs and symptoms associated with dry-eye, these are:

- **Conservative**

Conservative include lid hygiene and warm compresses, Lid hygiene must be done for the computer users by taking short breaks about every about 20 minutes to reduce evaporation (Meadows, 2005). Also adjusting the monitor so that it is below eye level will allow the upper lid to be positioned, lower and cover more of eye surface, again to reduce evaporation.

Brewitt and Sistani (2001) noted an increase in the tear film lipid layer thickness by 80% in that eye treated with warm (40°C) moist compressor for five minutes.

Recent research has shown that nutrition supplements containing certain essential unsaturated fatty acid (linoleic and Gamma linolenic acid) reduce ocular surface inflammation and improve dry eye symptoms, also supplement with vitamin A, C and B_6 can decrease dry eye symptoms (Ambrioso *et al*., 2002).

- **Topical agent**

Artificial tears remain the quintessential agent used in the treatment of dry-eye irrespective of cause or type (Murube *et al*., 1998a). They have the ability to lubricate the eye and relief symptoms. Those with out preservative are recommended because they are the most soothing and have fewer additives that could potentially irritate (Murube *et al*., 1998b).

There are two groups, first designed to simply lubricate the ocular surface allowing uninhibited movement of the lids without causing epithelial damage, Second group is designed to minimize the natural tear fluid as closely as possible with properties similar to its natural counterpart such as PH, tonicity and electrolyte balance.

Bio Tear, Murine Tear and Thera Tear are the most used; it contains nutrients show to be helpful in improving tear quality.

The ointments are typically used only before bed time or in sever cases since vision will be somewhat blurred from the ointment, It keep eyes moist longer than drops (Meadows, 2005) and preservative free ointments such as Refresh PM may be of benefit.

- **Punctal occlusion**

When artificial tears and lid hygiene donot work, the next step is to consider punctual plugs. Punctal plugs are silicone implants which block the natural drainage of tears from the eye through the punctum (Nava-Castaneda

et al., 2003). Inserting punctual plugs is a very effective option for treating dry eye syndrome, and it is a reversible procedure. Initially most doctors insert temporary collagen plug which dissolves after several days, so that the treatment may be evaluated before considering permanent punctal occlusion in the electrical cautery (Baudouin, 2001).

1.5.2.6: Complication of Dry Eye

Most people with dry eye don't experience any long term complications. However, if left untreated, severe dry eye may lead ocular surface complications which initiate by instability of tear film to associate with reduced ocular surface defense and increases susceptibility to irritation, allergy, and infection due to tear stagnation.

A major consequence of reduced aqueous volume is reducing antibacterial function due to decreasing levels of lactoferrin and lysozyme (Vant Hof *et al*., 2001).

The complication includes eye infection and inflammation, filamentary keratitis, irritates the surface of cornea and at the end epithelial damage and ulceration (Pflugfelder *et al*., 2004). Conjunctivitis may be bacterial, virus, allergy and chemical.

Bacterial conjunctivitis is the most common and serious type, it can affect one or both eye and usually accompanied by a heavy, yellow discharge cause by variety of bacteria including staphylococci and streptococci (Bohigian, 1987).

Filamentary keratitis occurs when fine filament of epithelium and mucous are attach to cornea. Eye is irritated by prolong and frequent use of eye medication containing preservatives (Murbe *et al*., 1998b).

1.5.2.7: Inflammation Mechanism in Dry- Eye:

Once dry eye has developed; inflammation becomes the key mechanism of ocular surface injury as the cause and consequence of cell damage. Inflammation may be initiated by dryness, hypertonic of tears, microtrauma from eyelids during blinking (Villarreal *et al*., 2005). Inflammation is enhanced by cytokines secreted by damaged surface epithelial cells, and lymphocytes and leucocytes that leak out from dilated conjunctiva blood vessels (Moss *et al*., 2000). Thus patients with severe dry eyes can become trapped in an increasing cycle of inflammation and ocular surface injury. Ocular surface inflammation reduces surface wettability and tears film stability (Stern *et al*., 2002).

1.5.2.8: Pathogenesis of Dry Eye:

The ocular surface and tear-secreting glands function as an integrated unit to refresh the tear supply and to clear used tears. Disease or dysfunction of this unit results in an unstable and unrefreshed tear film that cause the set of symptoms called dry eye syndrome (American Academy of Ophthmology, 2003).

Decrease tear secretion and clearance initiate an inflammatory response on the ocular surface, and researchers suggest that this inflammation plays a role in the pathogenesis of dry eye (Pflugfelder *et al*., 2000).

Dysfunction may develop from aging, a decrease in supportive factors (such as androgen hormones), systemic inflammatory diseases (such as rheumatoid arthritis), ocular surface diseases (e.g., herpes zoster ophthalmicus), surgery that disrupts the trigeminal afferent sensory nerves (including laser in situ keratomileusis [LASIK], extracapsular cataract

extraction, or penetrating keratoplasty), and systemic diseases or medication that disrupting the efferent cholinergic nerves that stimulate tear secretion (Baudouin, 2001).

1.6: Defense Mechanisms of Eye:

Complex mechanisms are involved in mucosal defense at the air/epithelium interface. There are apparently two lines of protection (Brandtzaeg, 1995). The first, which of interest here prevents colonization by micro-organisms on and through the epithelium. It is a non-inflammatory process, mainly mediated by (1) well known defence factors such as lysozyme and lactoferrin (Micallef and Cuschieri, 2001). (2) Specific secretary immunoglobulin A (IgA) appropriates to surface protection, elicited by both local antigen administration and stimulation at related distal sites (Giese and Mondino, 2001). (3) Factors with cysteine proteinase inhibitor activity such as cystatin, these different factors keep potentially harmful microorganisms and antigens out of the body.

The second line of protection triggers an inflammatory response. This takes place when foreign material has penetrated the epithelium. It consists of a non-specific action of various systems and of a specific amplification of humeral and cellular immune response (Beutler and Hoffmann, 2004). The composition of tear secretion will be tuned appropriately to play numerous roles: wetting of the surface, exchanging gases, intake of nutrition and excretion of waste products, chemical and microbial defense, mechanical entrapment, and removal of potentially harmful microorganisms and compounds (Walcott, 1998).

The anterior part of the eye is protected by the mechanical action of blinking and the washing action of tears (Forbes *et al*., 2002). The tear film maintains comfort and serves as the optical surface (Rolando and Zierhut, 2001). The first line of protection depends on the nature of the protein components present in the secretion: besides secretary IgA four proteins (lysozyme, lactoferrin, cystatin, and tear lipocalin) make up nearly 50% of the protein content. This site specific secretion composition enables tears to perform their protective function.

1.7: Microorganisms of Eye Infection:

The eye is infected by a number of organisms that enter through many parts and cause disease such as conjunctivitis, keratitis, endophthalmitis, dacryocystitis and others diseases.

These microorganisms are *Staphylococcus epidermidis, S. aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Corynebacterium diphtheriae, Haemophilus influenzae, Pseudomonas aerugenosa, Klebsiella pneumoniae, Proteus mirabilis, Escherichia coli* and *Neisseria gonorrhoeae.* Major related microorganisms to eye infection will be discussed in some detail below.

Genus *Staphylococcus*:

The organism is Gram positive cocci which posses no flagella and don't form spores, it is facultative anaerobic and catalase positive which distinguish it from *Streptococcus, Enterococcus* and *Lactococcus* species despite they are also Gram positive cocci but obligate fermenters that lack the enzyme catalase (Carrity, 2001). On blood agar pathogenic *Staphylococcus* causes hemolysis of the erythrocytes. Rabbit and sheep

erythrocytes are the most sensitive to the Stapyylococcal haemotoxin (Jensen and Wright, 1989).

Several species of *Staphylococcus* are notable for their medical significance and even that are typically part of the normal flora they can cause infection in people who have underlying medical problems (Nester *et al*., 2001).

Species *Staphylococcus aureus*:

It was discovered by R. koch (1878) and it is major cause of dacrycyctitis and dacryoadenitis, conjunctivitis and keratitis (Baron *et al*., 1994). One of the most identifying characteristics of *Staphylococcus aureus* is producing coagulase (coagulates plasma); fermenting mannitol and ability to grow under quite high salt condition (Nester *et al*., 2001).

Virulence factors of *Staphylococcus aureus* are; capsule (inhibit phagocytes), production of Alpha, Beta, Gamma and delta hemolysis (characterized by lethal, hemolytic and necrotic activity), leucocidin (destroy leukocyte) (Kingsbury and Wanger, 1991).

Species *Staphylococcus epidermidis*:

It is one of the most important bacteria normal flora which lives normally on the skin and mucous membrane and may cause conjunctivitis, plepharitis and dacryocystitis (Baron *et al*., 1994). Its virulence factors is produce a kind of slime that cements the growing colony to the plastic in the biofilm, protecting it from attack by phagocyte and other host defense mechanisms as well as antibacterial medication (Forbes *et al*., 2002).

Genus *streptococcus*:

The *Streptococci* are large group of bacteria belong to the family streptococcaceae. Some members of the genus are notable human pathogens, whereas others are indigenous flora of the gastrointestinal tract. The most important human pathogens are; *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae* and the viridans *Streptococci* (Kingsbury and Wagner, 1991).

Species *Streptococcus pneumoniae*:

It is considered as one of the important normal flora found in conjunctiva of the eye (Presscot *et al.*, 1990). It causes conjunctivitis and keratitis (Baron *et al*., 1994) and bacterial pneumonia in human, also frequent cause of otitis media and meningitis.

It is Gram positive diplococcus, alpha hemolytic and faculitative aerobic or anaerobic in presence of CO_2 (Talaro and Talaro, 1996).

The most striking characteristics of *Streptococcus pneumoniae* is its thick polysaccharide capsule (which responsible for organism virulence factor), Thus encapsulated strains are virulent and able to invade the lung, while non capsulated strains are readily removed by phagocytes and non virulent.

Species *Streptococcus pyogenes:*

It was discovered by T. Billroth (1874) in tissue erysipelas and wound infections. More specifically it is called beta hemolytic group A Streptococci because member of this species give beta hemolytic on blood agar (Carrity, 2001), it causes eye infection and found in conjunctiva (Prescott *et al*., 1990) but it rarely found as normal flora, It cause an array of supportive disease

and toxins in addition to some autoimmune or allergic disease and it is also the main streptococcal pathogen for man.

Current evidence indicates that *Streptococcus pyogenes* causing invasive disease is more virulence than other strains by at least two extracellular products; the first is pyrogenic exotoxin A which is super antigen and cause streptococcal toxic shock, second is exotoxin B, which is a protease breaking down protein to destroy the tissues (Nester *et al*., 2001).

Species *Corynebacterium diphtheria*e:

It is one of bacteria normal flora found in mucous membrane of conjunctiva (Prescott *et al*., 1990), it is straight or slightly curved Gram positive rod producing no spores, capsules or flagella (Nester *et al.,* 2001). It is fastidious, grows readily on media contain protein such as coagulase serum, blood agar and serum agar.

Toussen *et al*., (2000) found that diphtheriod had been regarded as causative agents of serious ocular diseases. In broth culture *Corynebacterium diphtheria* produce potent exotoxins (histotoxin, dermonecrotoxin, hemolysin). Histotoxin is the most important and play the principle role in pathogenesis of diphtheria which blocks protein synthesis in the cells of mammalian and inactivate transferase, the enzyme responsible for the formation of peptidoglycan chain (Leoomes *et al*., 1990).

Species *Hemophilus influenzae*:

It causes conjunctivitis, endophthalmitis, cellulites (Baron *et al*., 1994) and dacryocystitis (Hatikainen *et al*., 1997). It is short Gram negative bacillus, non motile, non sporeformer and facultative unaerobic (Rollins,

2000). It is a very fastidious growth organism which can not grow on common nutrient media unless fortified by two factors: the so called X-factor (hemin) and V factor (NAD).

The virulence smooth strains are capsulated which facilitate attachment to host cells (Forbes *et al*., 2002). This bacterium produce no exotoxin, instead, their pathogenisity is associated with an endotoxin.

Hemophilus influenzae give arise to acute catarrhs of the upper respiratory tract in combined action with other bacteria such as *Staphylococcus* and *Streptococcus.*

Species *Pseudomonas aeruginosa*:

It is an opportunistic pathogen that widespreads in the environment (Forbes *et al*., 2002). It is a major cause of nosocomial infections and an occasional cause of community-aquired infections include eye infections from contaminated contact lens solusions, keratitis endophthalmitis (Baron *et al*., 1994) and dacryocystitis (Hartikainen *et al*., 1997).

It is gram–negative pigments producing rod, motile by a single polar flagella. In addition, it cultures of have distinct fruity odor, and oxidase positive serve (Brook *et al*., 1998).

It has many virulence factors such as exotoxin which have toxic effect on corneal tissue (Ijiri *et al*., 1993). Also this bacteria has the ability to produce proteolytic enzymes and hemolysin that destroy cells and tissue.

Species *Escherichia coli:*

The organism was isolated from faeces in 1885 by T. Escherich, and exerted in great numbers with the faeces and always present in the external

environment (Mange *et al*., 2001). It is straight Gram negative rod, single or pairs, marked by polymorphism, and motile or nonmotile (Kingsbury *et al*., 1991). Definite *E. coli* serogroups are capable of causing various acute intestinal diseases in human and observed in conjunctiva of the eye (Prescott *et al*., 1990). It is facultative anaerobic and grows readily on ordinary media at room temperature (Carrity, 2001). It possesses two important virulence factors; enterotoxin production and adhering ability to small intestine are coded by plasmid (Forbes *et al*., 2002).

Species *Proteus mirabilis:*

Proteus is one the important pathogenic genera. Which returns to the family Enterobacteriaceae (Swiezkoet *et al*., 2000). *Proteus mirabilis* is one of the important pathogenic species of this genus which infects the eye and found in conjunctiva (Prescott *et al*., 1990), causing damage to eyelid (Baron *et al*., 1994). It is considered as opportunistic pathogens that causing many infections when move from their normal site (Davis *et al*., 1990). It is Gram negative rod, motile by peritrichous flagella, not forming either spores or capsules, facultative anaerobic, grows on common media, and gives very strong odor. The most important feature that differentiates members of *Proteus* genus from other genera of Enterobacteraceae is swarming phenomena (Mobley and Belas, 1995), *Proteus* plays an important role in putrefactive processes owing to it's ability to produce proteolytic enzyme (Leoomes *et al*., 1990), Most of its strains are sensitive to aminoglycoside antibiotics (Nester *et al*., 2001).

Species *Neisseria gonorrhoeae:*

It belongs to the family Neisseriaceae. Which have cocus- paired shaped (0.6 – 1 micron in diameter). It is gram negative occurs inside and outside of cells, aerobic or facultative anaerobic, grows on ordinary medium and no proteolytic activity(Garrity, 2001).

Neisseria gonorrhoeae has strong affinity of columnar and transitional epithelium and having attachment to mucosal cells begins to produce substance like proteases, elastases that play an important role in pathogenicity (Stainer *et al.*, 1986). It does not produce soluble toxins, while an endotoxin is released as a result of disintegration of bacteria cell (Forbes *et al*., 2002).

Species *Klebsiella pneumoniae:*

This genus, which returns to the family Enterobacteriaceae, causes conjunctivitis and responsible for pneumonia bronchopneumonia involves one or several long lobes some times producing fused foci and long abscesses which quit high death rate (Kingsbury and Wanger, 1991).

It is capable of producing capsules when present in the host's body or on nutrient media. It is Gram negative thick short bacilli with rounded ends, non-motile and devoid of spores (Carrity, 2001). Its cells occur mainly in pairs, but may be seen as singles, facultative anaerobic, capable of synthesizing all amino acids essential for their growth (Nester *et al.*, 2001). Virulence its factor was associated by producing thermostable exotoxin (Stainer *et al*., 1986).

1.8: Antibiotics Treatment of Eye:

Many criteria considered for choice of drug for treatment includes, the drug is active against the infecting organisms, non toxic, the antibiotic concentration obtained and the effect little or no on normal flora.

For this important it's divided to many divisions on the bases of mechanisms of inhibition:

1. Antibiotic inhibit cell wall synthesis

These include β-lactam drugs which are bactericidal against a variety of bacteria; inhibit pencillin-binding proteins. Its resistance is due to synthesis of β-lactamases.

Pencillins a family of antimicrobial medications; there are different group of it vary in their spectrum of activity and their susceptibility to B-lactamases, some must be injected, but others can be taken orally. Pencillin G is natural penicillin active against Gram- positive organisms and Gram negative cocci, pencillin G is destroy by stomach acid, and so it usually must be administered by injection (Jensen and Wright, 1989). Pencillinase resistant such as methicillin and dicloxicillin similar to natural penicillin, but resistant to inactivation by the pencillinase of *Staphylococci*.

Amoxcillin is one of the broad- spectrum penicillin it is similar to natural penicillin, but more active against gram negative bacteria (Jensen and Wright, 1989).

Ampicillin is semi synthetic penicillin which has bacteriocidal action against both gram positive and gram negative it affects cell wall synthesis by inhibition transpeptidases enzymes involve in cross linkage of polysaccharides chain which active cell wall lytic enzymes (Prescott *et al.*, 1990).

Vancomycin is bacterioacidal against gram positive bacteria but not gram negative bacteria, consequently, these organisms are innately resistance (Nester *et al.* 2001). Vancomycin binds to the terminal amino acids of the peptide side chain of N-acetyl muramic acid molecules (NAM) that are assembled to form glycan chains. By doing so, it blocks synthesis of peptidoglycan resulting in weakling of the cell wall and ultimately, cell synthesis (Nester *et al.,* 2001). It is, however, Avery important medication for treating infections caused by gram positive bacteria that are resist to B-lactam drugs.

Acquired resistance to vancomycin is most often due to an alteration in the peptide side chain of the NAM molecules that prevent vancomycin from binding (Yao and Moellering, 1999).

Bacitracin it is bactericidal against Gram positive bacteria, inhibit cell wall biosynthesis by interfering with the transport of peptidoglycan precursors across cytoplasmic membrane. Its toxicity limits its use to topical applications, However it is a common ingredient in non-prescription first aid ointments (Nester *et al.,* 2001).

2. Antibiotic inhibit protein synthesis

Aminoglycosides are Bacteriocidal against aerobic and facultative bacteria, it irreversibly bind to the 30_s ribosomal subunit, causing translation and causing misreading of the mRNA by ribosome's that have already passed the initiation step (Nester *et al.,* 2001).

Aminoglycosides are actively transported into bacterial cells by a process that requires respiratory metabolism (consequently; that are generally not effective against anaerobic, enterococci, and streptococci) (Edwards *et al.,* 1992).

Example of aminoglycosides includes gentamicin, amikacin, streptomycin, and tobramycin. Unfortunately, these all can causes severe side effects including hearing loss and kindly damage; consequently, they are generally used only when other alternatives are not available (Nester *et al.,* 2001).

Amikacin has less intrinsic antibacterial activity than gentamicin (Edward *et al.,* 1995). It has broad spectrum activity against organism resist to gentamicin and tobramycin (Joklik *et al.,* 1992) because it is chemically modified semi synthetic antibiotic with resist to inactivating enzymes engaged in the destruction of the activity of gentamicin and tobromycin (Nester *et al.,* 2001).

Neomycin is too toxic for systemic use; however it is a common ingredient in non prescription topical ointments.

Tetracycline is effectively against certain gram-positive and gram-negative bacteria. It is reversibly bind to the 30_s ribosomal subunit, blocking the attachment of tRNA to the ribosome and preventing protein synthesis so it is bacteriostatic (Nester *et al*., 2001).

(Jawetz *et al.,* 1998) mentioned that, all tetracycline are readily absorbed from intestinal tract and distributed widely in tissue and the newer tetracyclines such as doxycyclin have a longer half life, allowing less frequent doses.

Chloramphenicol is bacteriostatic and broad spectrum against a wide range of bacteria, it binds to the 50_s ribosomal subunit, preventing peptide bonds from being formed and, consequently, blocking protein synthesis (Atlas *et al.,* 1995). Chloramphenicol is effective against Gram-positive cocci including Staphylococci such as *Staphylococcus epidermidis* and some

strains of *Staphylococcus aureus,* and *Streptococci* such as *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and the viridans *Streptococci*. Gram–negative cocci such as *Haemophilus influenzae* are usually highly sensitive. *Moraxella catarrhalis,* a Gram negative aerobic diplococcal.

(Nester *et al.,* 2001) found that in rare case chloramphenicol causes potentially lethal condition a plastic anemia, in which the body is unable to make white and red blood cells. For this reason, chloramphenicol is usually used only when no other alternatives are available. Resistance to chloramphenicol is often due to a plasmid encoded inactivating enzyme called chloramphenicol acetyltransferase which is chemically alter this antibiotic to render it ineffective (Atlas *et al.,* 1995).

Lincomycin and clindamycin have a spectrum of activity similar to pencillin but different chemical structure they are very effective antistaphylococcl compounds with relatively low toxicity and are useful in treating patients who are allergic to penicillin, their mechanism of action is similar to that of chloramphenicol and erythromycin (Jensen and Wright, 1989).

3. Antibiotic inhibit nuclic acid synthesis

Ciprofloxacin is most important of new fluroquinolones. It has relatively bactericidal against a wide variety of bacteria, including both Gram-positive and Gram negative organisms. Acquired resistance is most commonly due to an alteration in the DNA gyrase target (Edwards *et al.,* 1995).

1.9: Probiotics

Microbial cultures have been used for thousands of years in food and alcoholic fermentations, and in the past century have undergone scientific scrutiny for their ability to prevent and cure a variety of diseases (Drisko *et al*., 2003).

Probiotic microorganisms are generally (but not only) lactic acid bacteria which including *Lactobacillus acidophilus, L. casei*, *L. plantarum, L. buligaricus and L. rhamnosis* .Others such as *bifidobacterium* spp are also used.

Fermentation of food with lactic acid bacteria has been shown to increase folic acid content of yogurt, Bifidus milk and kefir are used to increase niacin and riboflavin levels in yogurt, vitamin B_{12} in cottage cheese and vitamin B_6 in chedder cheese (Alm, 1882). In addition to nutrient synthesis, Probiotic may improve the digestibility of some dietary nutrients such as proteins and fats (Friend and Shahani, 1984; Anthony, 1997). Also short chain fatty acids such as lactic, propionic and butyric acid produce by lactic acid bacteria may help in maintaining an appropriate pH and protect against pathological changes in colonic mucosa (Drisko *et al*., 2003).

Evidence from in vitro systems show that probiotics can enhance both the specific and non specific immune response, possibly by activating macrophage, increasing level of cytokines, increasing natural killer cell activity and /or increasing levels of immunoglobulins (Perdigon *et al.*, 1995)

Also probiotics may exert abeneficial effect on allergic reaction by improving mucosal barrier function (Majamaa *et al*., 1997). In vitro studies proposed and used probiotic in a wide rang of clinical traits, ranging from diarrhea disease to cancer preventions (Rafter, 2003). Probiotic posse’s

antibiotic properties, maintaining a low and acidic pH in the intestine and vigina that inhibit the growth of harmful bacteria (Reid *et al.,* 2003), Bacteriosin and microcins kill microbes and bacteria (Sabler *et al.,* 2000, ocana *et al.,* 2004) but antibiotic indiscriminately destroy bacteria both good and bad leaving intestine without its normal probiotic flora

1.9.1: Important Probiotic Microorganisms:

Microbial probiotics are extremely safe and not associated with any significant for determined side effects, thus they are consider as GRAS (Generally Considered As Safe) (Mcfarland and Elmer, 1995).

Within the lactic acid bacteria group, the genus *Lactobacillus* is the most widely encountered for probiotics (Reid, 2002). Some members of this genus may enhance specific and non specific immune response, inhibit pathogen growth and reduce chance of infection (Reid *et al*., 2003). Production of metabolites and efficient adherence to intestinal epithelial cells to reduce or inhibit colonization of pathogens (Drisko *et al*., 2003) are characterized by pure viable culture of well identified species of these organisms.

1.9.2: *Lactobacillus* as Probiotics:

The *Lactobacilli* include over 25 unique species. The first level of differentiation is based on end-product composition; some are homofermentatives, whereas others are hetrofermentative. The former are classified as organisms that produce over 85% lactic acid as their end product from glucose. The latter include organisms that produce approximately 50% lactic acid as an end product with considerable amount of carbon dioxide, acetate and ethanol (Chakraborty, 1996).

The next major criterion for distinguishing the *Lactobacilli* is the production of gas from carbon source including glucose and gluconate in addition there is a great degree of diversity in the ability of various *Lactobacilli* spp to ferment pentose sugar including ribose and xylose (Batt, 1999).

Lactobacillus is Gram negative, non spore-forming, catalase negative, aerobic or aerotolerant, fastidious bacteria and producing lactic acid as the main product of fermentation process (Holzapfel *et al.,* 1998). It may be found in a number of fermented food products and the occurrence of it contributes to the preservation, nutrition availability and flavor. Number of dairy products are produced using *Lactobacillus* either alone or in combination with other lactic acid bacteria (Alm, 1982), also vegetable are fermented with *Lactobacilli* to produce products including pickles, olives, and sauerkraut, as well as play an essential role bread making. *Lactobacillus* spp. inhibits the activities and proliferation of pathogenic bacteria by several ways such as production of lactic acid, bacteriocin, hydrogen peroxide and other metabolites.

For example, *Lactobacillus acidophilus* is naturally occurring bacteria that reinforce protective mucosal surface and prevent enhancement and attachment of harmful microorganism and allergens (Sander *et al*., 2001). In addition, production of lactase enzyme that breakdown lactose (Mcdonough *et al.,* 1987) and acidophilin that inhibits pathogenic bacteria. While *Lactobacillus plantarum* produces lactocidin that has action and inhibited several bacterial like *E. coli* and *proteus* (Hirayma and Rafter, 1999).

3.1: Dry Eye Syndrome (DES) Patients:

3.1.1: Distribution of (DES) Patients According to Gender:

From a total of 60 specimens belong to patients suffering of dry eye syndrome (DES) and after making schirmer test type I for them, the result shown that 41 (68.3%) patients were suffering from severe DES (measurement of wetting less than 5mm/5min) and 19 (31.6%) patients with moderate dry eye (measurement of wetting between 5-10mm/5min). Figure (3-1) show that 34 (56.7%) were females and 26 (43.3%) were males. Bacterial growth was detected in 31 (91.1%) of female specimens, while only 2 (5.8%) showed no growth. Regarding male specimens, bacterial growth appeared in 22 (84.6%) specimens, no growth in 3 specimens (11.5%) and 2 speciemens were return to fungal culture

The relatively high percentage of female DES patients as compared with that of male patients came almost in agreement with some other studies. For instance, Lin *et al*., (2003) found that 33.7% of a sample of Taiwanese patients had DES often or at all times, and women were more likely than men to be affected, While Schein (1999) reported that dry eye affect 3.2 million American woman compared to 1.6 million American men.

Schaumberg *et al*., (2003) found that the prevalence of the dry eye syndrome increased with age; from 5.7% for women who are less than 50 years old to 9.8% in women above 75 years old. Such ratio may be due to various reasons, first possibly due to the hormone flactuation that occur during menopause, at menopause, a decrease in circulating sex hormones occurs which may affect the functional and secretory aspects of the lacrimal gland, according to Mathers (2000) studies about 28% of the menopausal women have dry eye symptoms.

Second reason is the contact lenses which wearied by female more than by male for cosmetics aspects. There is a possibility that contact lens use could lead to lacrimal gland damage and as a result dry eye (Mathers, 2000). In this regard, McMonnies and Ho (1986) were among the first to report that contact lens wearers report significantly drier eye symptoms than do non-contact lens wearers. Begley *et al.*, (2001) and Galion *et al.*, (2002) also showed that dry eye disease was more frequent or intense in contact lens wearers than in non wearers.

Third reason is due to the successive using of hair dryer by woman which leads to increase evaporation of tear from eye.

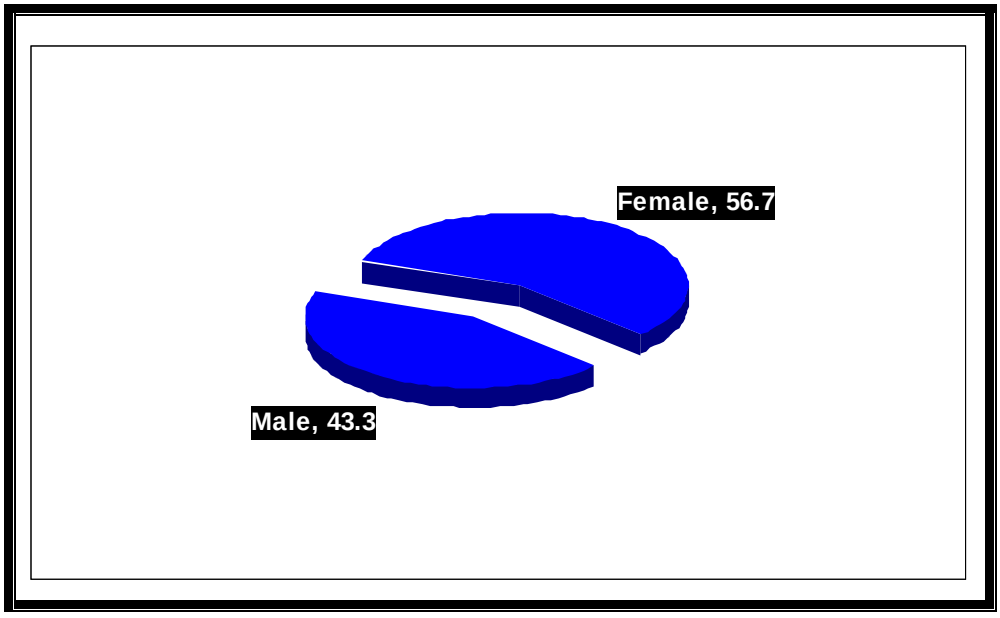

Figure (3-1): Percentage of Dry Eye Syndrome Patients According to Gender.

3.1.2: Distribution of (DES) Patients According to age:

Results in table (3-1) show that:

- 27 patients were 61 years and above.
- 23 patients aged between (41-60) years.
- 6 patients aged between (21-40) years.
- Only 4 patients were below 20 years.

Same figure also shows the percentage of infection for each age group. High rate of infection patients in old age may be due to certain causes such as physiology and biochemical change in tear with age which includes decrease in reflex secretion by the lacrimal gland, decrease in tear volume, and tear film instability.

Such results agree with these of Mathers (2000) who found that osmolarity increases over the years, and tear volume decreases. As tear flow decreases, the percentage of evaporation goes up and the Schirmer score goes down. An average tear flow of 24 mm in 20 years old might drop to 8 mm in 80 years old.

Schepens Eye Institute researcher Debra Schaumberg, ScD, presented data estimating that the prevalence of dry eye in people aged 50 or older in the United States is 7.8% for women and 4.8 % for men.

Lee *et al*., (1993), Pietsch and Pearlman (1973) concluded that lacrimal gland proteins lysozyme and lactoferrin decline linearly and progressively with age. Goblet cells of the conjunctival epithelium produce the mucous components of the tears, which enable tears to adsorb to the corneal surface that modified with age (Kessing, 1968). While the tear

evaporation rate has not been found to be correlated with age, this returns to that the evaporation is primarily controlled by the lipid layer of the tear film and lipid layer thickness appears to be constant for different age groups (Tomlinson and Giesbrecht, 1994).

Moss (2000) noticed that after age 59, prevalence of dry eye is more than doubled. The thickness and area of the lacrimal gland decreased with age in women, but not in men (Obata *et al*., 1995).

A separate Australian study of 926 subjects at age 40 and older showed higher prevalence of dry eye in women, generally (Mc Carty, 2000).

One of the reasons reason that most patient are of old ages may be returned to using several antibiotic to control other diseases. In this regard, Crandall and Leopold (1979) reported that a variety of medications for various conditions, mostly outside the field of ophthalmology, are known to be inhibitory to tear production, also Van'Haeringen and Glasius (1980) declared that even aspirin exerts an inhibitory effect on the tear production and a change in the composition of tears.

Table (3-1): Distribution of Dry Eye Syndrome Patients According to Gender and Age.

Age group (year)	Gender		Total No. of patients	Percentage (%)	Infected patients		
	Male	Female			Number	Percentage* (%)	Percentage** (%)
<20	1	3	4	6.6	2	3.7	50
21- 40	4	2	6	10	5	9.4	83.3
41- 60	9	14	23	38.3	20	37.3	86.9
> 60	12	15	27	45	26	49	46.2
Total	26+34		60	100	53	-	-

* Percentage of age-group patients to the total number of infected patients.

** Percentage of age-group patients to the total number of infected patients at same age group.

3.2: Isolation and Identification of Bacterial Isolates:

Swabs from eye of patients suffering from dry eye syndrome (DES) were cultivated on common and selective media specialized for each suspected bacterial genus and species. Then, the isolates were primary identified according to their cultural and microscopical characteristics, while the final identification was performed throughout the biochemical tests as shown in table (3-2). Api system was also used to ensure identification of the isolates. Below are the most common bacterial isolates detected:-

Table (3-2): Biochemical Tests for Bacterial Species Isolated From Patients Suffering From Dry Eye Syndrome.

Species	Catalase	Oxidase	urease	Gelatin liquification	TSI	Co2	H2S	Manit-ol	Coag-ulase	X	V	Opta-chin
Staphylococcus epidermidis	+	–	–	+	N	N	N	–	–	N	N	N
Staphylococcus aureus	+	–	–	+	N	N	N	+	+	N	N	N
Streptococcus pneumoniae	–	–	–	–	N	N	N	N	N	N	N	+
Streptococcus pyogens	–	–	–	–	N	N	N	N	N	N	N	–
Corynebacterium diphtheriae	+	–	–	–	N	N	N	N	N	N	N	N
Hemophilus influenzae	+	–	N	N	N	N	N	N	N	+	+	N
Klebsiella pneumoniae	+	–	+	–	A/A	+	–	N	N	N	N	N
Pseudomonas aeruginosa	+	+	+	+	Alk /Alk	–	–	N	N	N	N	N
Proteus mirabilis	+	–	+	+	Alk /A	+	+	N	N	N	N	N
Neisseria gonorrhoeae	+	+	N	N	A/A	+	–	N	N	N	N	N
Escherichia coli	+	–	–	–	A/A	+	–	N	N	N	N	N

(+): positive result. (−): negative result. N= not tested.

(Alk): alkaline reaction. (A): acid reaction.

***Staphylococcus* spp**

When the swab specimens was cultured on blood agar incubated at 37°C for 24 hrs, colonies grown were small to medium in size diameter (0.5-1.0) mm and white to gray in color, such characteristics are consider with those of *Staphylococcus epidermidis*, other colonies were (2-3) mm in diameter having white to yellow color which were similar to those of *Staphylococcus aureus* (Forbes *et al*., 2002).

Regarding microscopical characteristics, gram staining showed gram positive cocci, all grouped mainly in clusters which are the properties of *Staphylococcus*.

Ressults of biochemical tests showed that *Staphylococcus aureus* is positive to coagulase and catalase, but negative to oxidase. Furthermore, it was able to ferment mannitol aerobically and able to produce β-hemolysis on blood agar. On the other hand, *Staphylococcus epidermidis* isolates were unable to ferment the mannitol and change his color from red to yellow.
Morphological and biochemical characterization agreed with these stated by Holt *et al.,* (1994) and Atlas *et al.*, (1995).

Results of Api staph kit were are shown in the appendix (1) confirmed the previous conventional identification.

Streptococcus* spp*:

Microscopical examination of the gram stained smears taken from suspected colonies of streptococcal isolates grown on blood agar showed the cells were gram positive, non motile cocci, grouped mainly as short or long chains, Some colonies gave beta hemolysis on the blood agar, which were suspected to be belong to *Streptococcus pyogenes*, while other gave alpha hemolsis which may belong to *Streptococcus pneumoniae.*

Biochemical characteristics of all suspected streptococcal isolates were; catalase and oxidase negative, and ferment carbohydrates without production of gas. *Streptococcus pyogenes* was differentiated from *Streptococcus pneumoniae* by the optachin disc (optachin sensitivity) which inhibit the later without affecting *Streptococcus pyogenes*.

Upon application of api 20 strep kit system. The identification was ensured as shown in appendix (2).

Corynebacterium diphtheriae

At the time that the suspected isolates were unable to grow on MacConkey agar, they grew on blood agar giving red to gray colonies with (0.5-1) mm diameter. Their cells appeared under the oil-immersion lens as small, club shape end, gram positive and arranged as Chinese letter containing distinctive granules. After subjecting the isolates of *Corynebacterium diphtheriae* to the related biochemical tests, they gave negative results for oxidase, gelatin liquification and urease but positive for catalase.

When api system was applied through the api coryne kit, same identification characteristics were obtained. Appendix (4) illustrates that.

Hemophilus influenzae:

Cultural examination showed that colonies of the species, after incubation on chocolate agar at 37°C for 24 hrs, were small, smooth and moist. On blood agar they exhibit no any hemolytic type, and did not grow on MacConkey agar.

Moreover, under the oil immersion objective of the compound light microscope, cells of the suspected *Hemophilus influenzae* were gram negative rods or coccobacilli, occur singly or in pairs.

Results of biochemical tests showed that they were positive to each of catalase and special requirement X, V factors (Nester *et al.*, 2001).

Pseudomonas aeruginosa

After incubating suspected isolates on blood agar, colonies appeared mucoid with flat ends, possessing distinct odor and produce beta-hemolysis, while on nutrient agar they were blue to green with colony diameter of (3-5) mm. Microscopical examination of suspected colony showed that their cells were small gram negative rods found separately but close to each other. Such cultural and morphological characteristics are similar to those of *Pseudomonas aeruginosa* stated in (Brooks *et al*., 1998).

Biochemical characterizations of the suspected isolates shown that they were positive to both of oxidase, catalase and non fermentative for (TSI). They were negative to CO_2 and H_2S production. Such biochemical results are the characterizations of *Pseudomonas aeruginosa* (Collee *et al*., 1996). The identification was confirmed by api kit system as shown in appendix (3).

Escherichia coli

On MacConkey agar, suspected *Escherichia coli* colonies appeared pink, mucoid, and lactose fermenter, while on blood agar they were large (2-4) mm diameter gray and smooth producing hemolysis type β.

Under the oil immersion objective of the microscope, cells of *Escherichia coli* were gram negative rod, mainly found as singles, but some of them grouped in pairs. According to Forbes *et al*., (2002) such cultural and morphological characteristics are suspected to be belong to the species *Escherichia coli.*

Final identification of *Escherichia coli* was achieved by biochemical tests. The suspected isolates were negative to oxidase, urease but positive for catalase, ability to grow on (TSI) medium changing the color of its surface and bottom to yellow and producing CO_2 but not producing H_2S. Previous biochemical characterizations were concerted with those identified by Prescott *et al*., (1990).

The identification was confirmed by using api 20E. As shown in appendix (3).

Proteus mirabilis

On blood agar, colonies produced were characterized by swarming phenomena, while on MacConky agar; they were small in size (1 mm in diameter) and pale in color. Gram staining examination showed that cells of suspected isolates were purple, non spore formers rods found separate near each other. Forbes *et al*., (2002) declared that such morphological and cultural properties were similar to those described for *Proteus spp.*

Biochemical characterization indicated that the suspected isolates were negative to oxidase but positive to each of urease and gelatinase. It was also able to grow on (TSI) medium changing its bottom color to yellow (acid reaction) and produced CO_2 gas and H_2S.

Results (appendix 3) of api 20E system gave same identification of those obtained by the conventional.

Klebsiella pneumoniae

After incubating suspected isolates, they formed on MacConky agar large opaque mucilaginous colonies. While gram staining examination

showed that cells were gram negative, thick rod bacilli, having rounded ends, non motile and devoid of spores.

They occur mainly in pairs but may also see as singles. They were surrounded by large capsules. Such cultural and morphological characteristics are similar to those for *Klebsiella pneumoniae* described by (Nester *et al*., 2001). Final identification of *Klebsiella pneumonia*e was achieved by biochemical tests. It is negative to gelatin liquefaction positive to urease. It was able to grow on (TSI) medium changing its surface and bottom color to yellow (acid reaction) and producing CO_2 gas. Previous biochemical characterizations came in accordance with Prescott *et al*., (1990) for *Klebsiella pneumoniae.*

Neisseria gonorrhoeae

Culturing the suspected isolates on chocolate agar showed that their colonies were transparent, circular colonies, with diameters of (1-3) mm. In ascetic broth, they formed pellicles settles at the bottom of test tubes after few days of incubation.

Microscopical examination of the isolates cells showed that they were gram negative cocci grouped mainly in pairs.

Several biochemical tests were done to characterize *Neisseria gonorrhoeae.*

Results of biochemical tests indicated that the suspected isolates were oxidase positive and ability to grow on (TSI) medium changing its surface and bottom color to yellow (acid reaction) and produced CO_2 gas. Such biochemical results are the characterizations of *Neisseria gonorrhoeae* (Collee *et al*., 1996).

3.3: Occurrence of Bacterial Isolates in DES Patients:

Out of the sixty dry eye syndrome cases included in this study, 53(88.3%) gave positive result for bacteria growth, while the rest 7 (11.6%) showed no growth. Identification of the suspected isolates declared that gram positive bacteria was predominant with a total of 38 isolates (79.2%), compared to only 15 isolates (20.8%) for gram negative (Figure 3-3). Occurrence of this percentage for Gram positive bacterial isolates return to skin contamination by attack the hand of patients to his eyes.

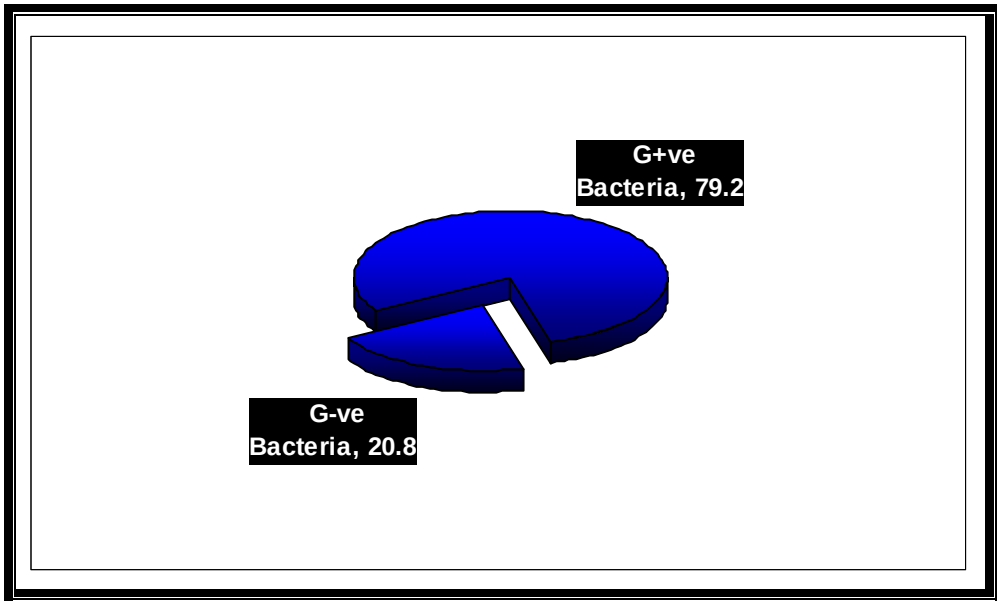

Figure (3-2): Percentage of Bacterial Isolated According to Gram Stain.

Results of the isolated bacterial species in figure (3-4), showed that *Staphylococcus epidermidis* was the most frequent bacteria isolated from patients suffering from DES. Baum and Barza (1998) reported that the majority of postoperative eye infections were due to coagulase negative *Staphylococcus*, While Pflugfelder and Solomon (2006) found, their study,

that the most frequent cultured bacteria of DES was belonged to *Staphylococcus epidermidis.* Same result was also achieved by Tortora *et al*., (1986) when they found that *Staphylococcus epidermidis* was the most common bacteria associated with eye infection. Infection of this bacterium is due to its production of slime layer that facilitates attachment and ability to acquire resistance to most of antimicrobial agents used in hospitals environment (Baron *et al*., 1994).

Same table (3-4) also shows that *Staphylococcus aureus* was the second predominant bacteria. This came in agreement with that achieved by Mannis *et al.*, (1990) who found that *Staphylococcus aureus* was the most common organism associated with bacterial infection of the eye.

From the above results, genus *Staphylococcus* which is represented by *Staphylococcus epidermidis* and *Staphylococcus aureus* resembled the highest percentage (45.9%) among other bacterial isolates of DES patients included in this study. Regarding pathogenicity, *Staphylococcus aureus* was the most common pathogenic bacteria causing eye infection.

On the other hand, *Streptococcus pneumoniae* comes as the third ranked bacteria when its occurrence in the eye patients formed 12%. However, such result was different from that obtained by Ryan and Ray (2004) who found that *Streptococcus pneumoniae* and *Hemophilus influenzae* were the most frequently bacteria associated with the infection of dry eye. Another species of *Streptococcus* was *Streptococcus pyogenes* who isolated in a percentage of 5%.

So, the total isolates belonged to the genus *Streptococcus* reached 17% making them as the second predominant genus just after *Staphylococci.* Seal *et al*., (1982), in this regard, it was found that *Staphylococcus* species

were the most common pathogens of bacterial infection in dry eye, followed by *Streptococcus pneumoniae* and *Haemophilus influenzae.*

Regarding *Corynebacterium diphtheriae*, only 5 isolates were obtained which form 9.3% such percentage is closed (9.5%) to that obtained by Morrow and Abbott (1998).

Other bacterial species (*Hemophilus influenzae, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, Neisseria gonorrhoeae* and *Escherichia coli*) were represented by low percentages (7.4, 5.6, 5.6, 3.7, 3.7 and 1.8) %, respectively. *Neisseria gonorrhoeae* consider one of the dangerous bacteria which infect eye because this organism can invade intact epithelium causing pressure necrosis of the epithelium due to extensive purulent exudates under the closed eyelid (Baum, 1997).

While the previously mentioned bacterial isolates were obtained from patients of the DES included in this study, 5 patients specimens were free of any bacterial growth which may be referred either to use of antibiotics, or to that those patient have diagnosable disease but without infection. Such finding came in agreement with that reached by Brewitt and Sistani (2001). Unfortunatly, the rest 2 specimens were ruturn to fungal culture which were avoided.

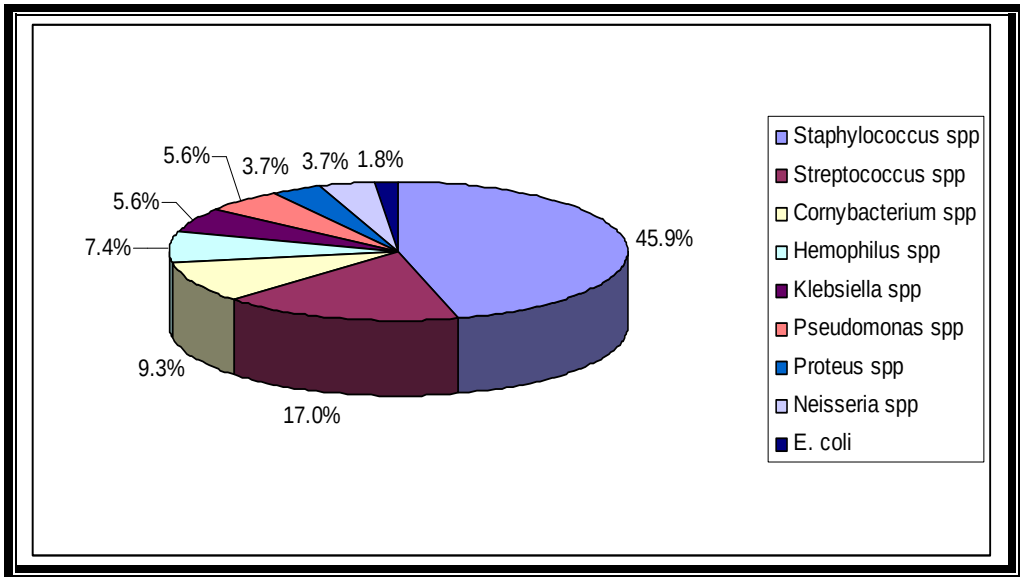

Figure (3-3): Percentage of Bacterial Isolated Obtained From Dry Eye Syndrome Patients Distributed According Their Genera.

3.4: Occurrence of Bacterial Isolates in Normal Eye:

From twenty normal eye people at different gender and age group included in this study, 13(65%) gave positive culturing results, while the rest 7(35%) were negative. 7(54%) of positive culture were return to female and 6(46%) were male. Figure (3-4) show that *Staphylococcus epidermidis* was represented by highest percentage 53.9% of the total bacterial isolates. On the other hand, *Corynebacterium diphtheriae* come as the second bacteria when its occurrence in the normal eyes formed 30.7%. While *Streptococcus pneumoniae* was exit in a percentage of (15.4%). Existence of the last species in normal eyes may return to its occurrence as normal flora which is important to the overall health of the human host (William and Wilkins, 2004). However, results obtained were near those of Nester *et al.*, (2001) who found that normal flora of the eye consists mainly of staphylococci (>60%) which is mostly represented *Staphylococcus epidermidis*, in addition to *Streptococcus pneumoniae* and diphtheroids. Despide that the conjunctiva

and ocular adnexae are rapidly colonized by bacteria at birth (Bohigian, 1987).

Microflora isolated from healthy individuals consist primarily of *Staphylococcus epidermidis* and diphtheroids, Species of greater virulence, such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and even *Neisseria meningitides* have been also reported (Limberg, 1991).

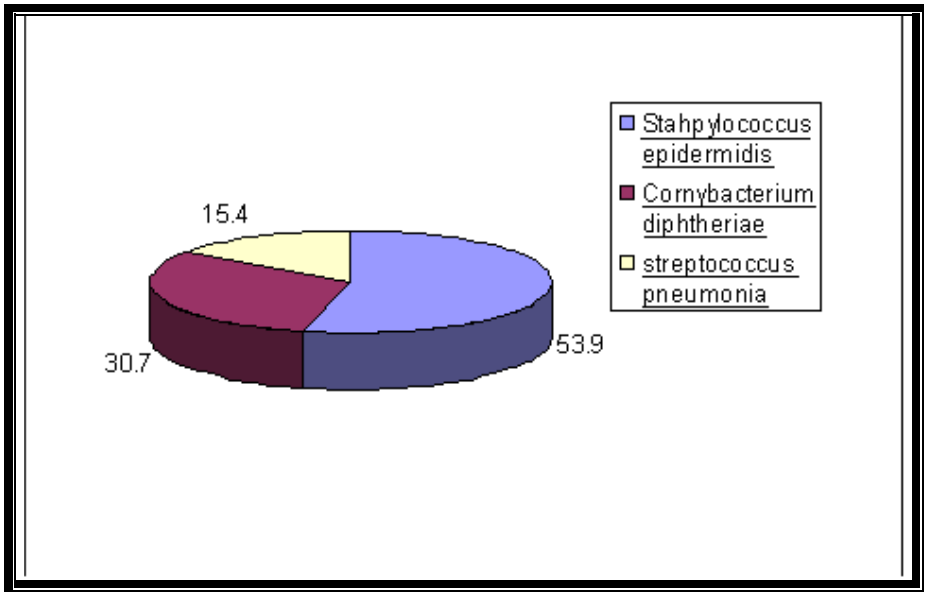

Figure (3-4): Percentage of Bacterial Isolated From Normal Eyes Individuals.

3.5: Antibiotic sensitivity of (DES) Isolates:

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). Two criteria must be considered when choosing a therapeutic agent for dry eye infection; the resistance rate and the penetration of the antibiotic at the level of infection. The disc susceptibility method provides quantitative measurements that are critical for epidemiology and drug resistance surveillance to detect sensitivity of pathogenic bacteria.

Sensitivity of the DES bacterial isolates was tested toward the 14 mentioned ocular antibiotics Ciprofloxacin (cF), Chloroamphenicol, (C) Gentamicin (CN), Cefotaxime (CTX), Neomycin (N), Vancomycin (VA), Tetracycline (TE), Clindamycin (CM), Amikacin (AK), Erythromycin (E), Cloxacillin (OB), Pencillin G (G), Ampicillin (AM), and Bacitracin (Ba) by using the modified disc diffusion method. Since no criteria for the sensitivity of bacterial causatives of DES are established, and in accordance with the recommendations of NCCLS (1994), the cut-off values for the systemic bacteria of septicemia infections were applied.

Table (3-3) shows sensitivity of the isolates of gram positive bacteria for antibiotics. The isolates were different in their sensitivity according to the genus and species. In general, they were highly sensitive to chloramphenicol (94.7%), ciprofloxacin (89.4%), gentamicin (81.5%), cefotaxime (78.9%) and neomycin (73.6%). The isolates were moderate in their sensitivity toward vancomycin (65.7%), clindamycin (63.1%), tetracycline (57.8%), (36.8%) to each of erythromycin and amikacin. Adversely, they were very resistance to the rest of the antibiotics, especially to the ampicillin (2.6%). penicillin (5.2%), (10.5%) for bacitracin and cloxacillin.

Results of sensitivity of gram-negative isolates which are tabulated in table (3-4) show that ciprofloxacin was the most effective antibiotic against the isolates when the sensitivity percentage reached (93.3%). Followed by gentamicin, amikacin and cefotaxime with percentages of 86.6%, 66.6% and 53.3%, respectively. At the time that gram-negative isolates were less affected by other antibiotics, all were totally resistant to ampicillin and penicillin.

Table (3-3): Sensitivity of Gram Positive Bacterial Isolated for Antibiotics.

Antibiotic	**Isolate**											
	S. aureus		***S. epidermidis***		***S. pneumoniae***		***S. pyogenes***		***Coryne-bacterium spp.***		**Total No.**	**% of Total No.**
	No.* of isolate *(8)*		***No.* of isolate *(16)***		***No.* of isolate *(6)***		***No.* of isolate *(3)***		***No.* of isolate *(5)***			
	S	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**		
Ciprofloxacin (cF)	**8**	**0**	**14**	**2**	**4**	**2**	**3**	**0**	**5**	**0**	**34**	**89.40%**
Chloroamphenicol (C)	**7**	**1**	**16**	**0**	**5**	**1**	**3**	**0**	**5**	**0**	**36**	**94.70%**
Gentamicin (CN)	**7**	**1**	**14**	**2**	**5**	**1**	**2**	**1**	**3**	**2**	**31**	**81.50%**
Cefataxime (CTX)	**6**	**2**	**16**	**0**	**4**	**2**	**1**	**2**	**3**	**2**	**30**	**78.90%**
Neomycin (N)	**5**	**3**	**14**	**2**	**4**	**2**	**2**	**1**	**3**	**2**	**28**	**73.60%**
Vancomycin (VA)	**6**	**2**	**16**	**0**	**2**	**4**	**0**	**3**	**1**	**4**	**25**	**65.70%**
Tetracycline (TE)	**4**	**4**	**9**	**7**	**5**	**1**	**1**	**2**	**2**	**3**	**21**	**57.80%**
Clindamycin (CM)	**7**	**1**	**10**	**6**	**4**	**2**	**1**	**2**	**2**	**3**	**24**	**63.10%**
Amikacin (AK)	**4**	**4**	**8**	**8**	**2**	**4**	**0**	**3**	**0**	**5**	**14**	**36.80%**
Erythromycin (E)	**1**	**7**	**5**	**11**	**4**	**2**	**3**	**0**	**1**	**4**	**14**	**36.80%**
Cloxacillin (OB)	**0**	**8**	**3**	**13**	**1**	**5**	**0**	**3**	**0**	**5**	**4**	**10.50%**
Pencillin G (G)	**0**	**8**	**2**	**14**	**0**	**6**	**0**	**3**	**0**	**5**	**2**	**5.20%**
Ampicillin (AM)	**0**	**8**	**1**	**15**	**0**	**6**	**0**	**3**	**0**	**5**	**1**	**2.60%**
Bactracin (Ba)	**1**	**7**	**2**	**14**	**0**	**6**	**0**	**3**	**1**	**4**	**4**	**10.50%**

***** Total number of isolates to the same genus and species number.

Table (3-4): Sensitivity of Gram Negative Bacterial Isolates From DES for Antibiotics

Antibiotic	Isolate													
	Hemophilus influenzae		***Klebsiella pneumoniae***		***Pseudomonas aeruginosa***		***Proteus mirabilis***		***Neisseria gonorrhoeae***		***E. coli***		**Total No.**	**% of Total No.**
	No. (4)*		***No. (3)***		***No. (3)***		***No. (2)***		***No. (2)***		***No. (1)***			
	S	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**		
Ciprofloxacin (cF)	**4**	**0**	**2**	**1**	**3**	**0**	**2**	**0**	**2**	**0**	**1**	**0**	**14**	**93.3%**
chloroamphenicol (C)	**2**	**2**	**1**	**2**	**2**	**1**	**0**	**2**	**1**	**1**	**1**	**0**	**7**	**46.6%**
Gentamicin (CN)	**4**	**0**	**3**	**0**	**2**	**1**	**2**	**0**	**2**	**0**	**0**	**1**	**13**	**86.6%**
Cefataxime (CTX)	**3**	**1**	**2**	**1**	**1**	**2**	**1**	**1**	**1**	**1**	**0**	**1**	**8**	**53.3%**
Neomycin (N)	**2**	**2**	**1**	**2**	**1**	**2**	**0**	**2**	**1**	**1**	**0**	**1**	**5**	**33.3%**
Vancomycin (VA)	**0**	**4**	**2**	**1**	**0**	**3**	**1**	**1**	**0**	**2**	**0**	**1**	**3**	**20.0%**
Tetracycline (TE)	**2**	**2**	**2**	**1**	**0**	**3**	**0**	**2**	**0**	**2**	**0**	**1**	**4**	**26.6%**
Clindamycin (CM)	**1**	**3**	**0**	**3**	**0**	**3**	**0**	**2**	**1**	**1**	**1**	**0**	**3**	**20.0%**
Amikacin (AK)	**3**	**1**	**2**	**1**	**0**	**3**	**2**	**0**	**2**	**0**	**1**	**0**	**10**	**66.6%**
Erythromycin (E)	**0**	**4**	**2**	**1**	**0**	**3**	**0**	**2**	**1**	**1**	**0**	**1**	**3**	**20.0%**
Cloxacillin (OB)	**1**	**3**	**0**	**3**	**1**	**2**	**0**	**2**	**0**	**2**	**0**	**1**	**2**	**13.3%**
Pencillin G (G)	**0**	**4**	**0**	**3**	**0**	**3**	**0**	**2**	**0**	**2**	**0**	**1**	**0**	**0.0%**
Ampicillin (AM)	**0**	**4**	**0**	**3**	**0**	**3**	**0**	**2**	**0**	**2**	**0**	**1**	**0**	**0.0%**
Bactracin (Ba)	**0**	**4**	**0**	**3**	**1**	**2**	**0**	**2**	**0**	**2**	**0**	**1**	**1**	**6.6%**

***** Total number of isolates to the same genus and species number.

When sensitivity to antibiotics was compared between gram positive and negative bacterial isolates, results in figure (3-4) show that ciprofloxacillin and gentamicin were the most effective antibiotics against both types, but their effect on gram negative was more than on gram positive isolates.

Chloramphenicol had also good effect on gram positive bacteria, when (94.7) % of the isolates were sensitive to it, while had quite lower effect against gram negative bacteria with a sensitivity of (46.6%). In addition, antibiotics: clindamycin, vancomycin, cefataxime, neomycin, tetracycline and erythromycin were more effective against gram positive than gram negative isolates.

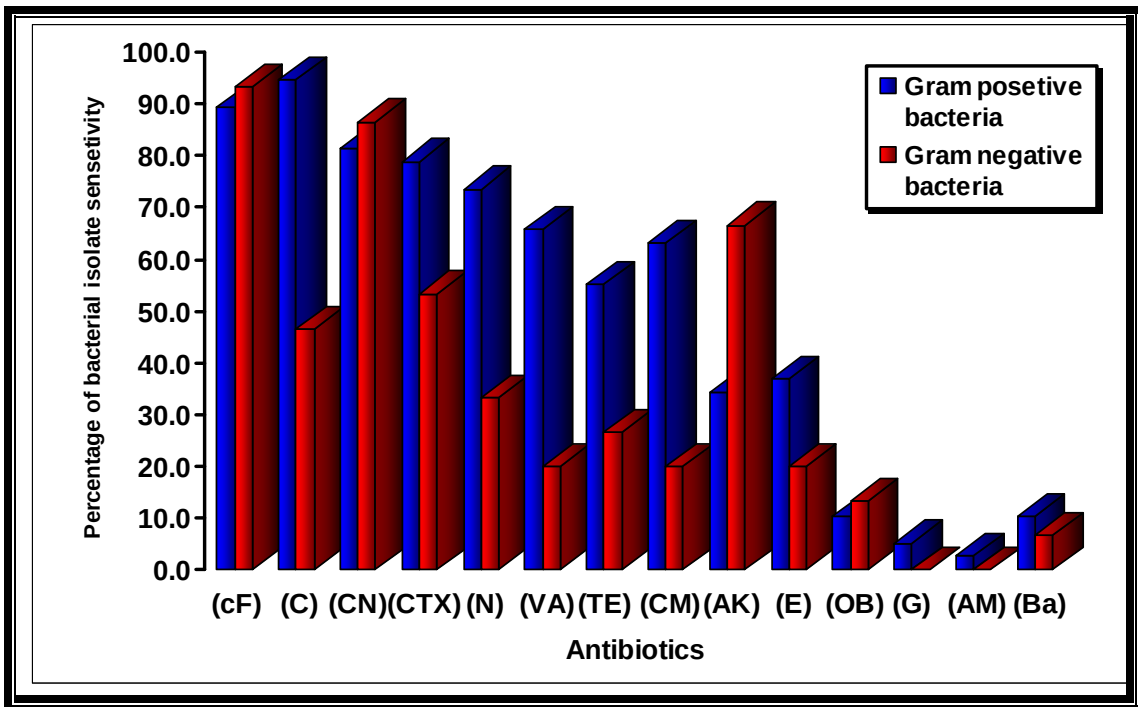

Figure (3-5): Percentage of Gram Positive and Gram Negative Bacterial Isolates From DES Sensitivity for Antibiotics.

From the above results, it may be concluded that fluoroquinolones and aminoglycosides groups of antibiotics had remarkable effect on bacteria isolates of patients suffering from dry eye Syndrome. This came almost in agreement with that of Power *et al.,* (1993) who found that there were no significant differences between effects of ciprofloxacin and chloramphenicol against 57 positive-cultures of patients suffering from eye dry Syndrome.

Ampicillin recorded lower percentage of sensitivity (2.6%) for Gram positive bacteria and no sensitivity by any bacteria of gram negative group and this belonging to their common using in treatment.

Gram negative isolates also were completely (100%) resistant to pencillin G. This may be related to the fact the outer membrane of gram negative bacteria usually prevents the entry of penicillin as long as its mode of action is through penetrating bacterial cell wall and interfering with its synthesis (Nester *et al.,* 2001). Cooper (1998) mention that taking the antibiotics even for short time and in small dose lead to increase the development of resistant isolates.

Eady and Cove (1990) suggested that an ideal topical antibiotic should not be related to an oral drug and should be restricted to topical use only. It should not select for cross-resistance or, more importantly, for multiple-resistance to unrelated antibiotics. Moreover, it should have a broad enough spectrum of effect to be used as a single agent. Unfortunately, none of the currently available topical ocular antibiotics fulfills all of these criteria.

The aim of antimicrobial therapy is to achieve a concentration of the antimicrobial agent at the site of infection high enough to kill or stop the growth of the infecting organism.

3.6: Inhibitory Effect of LAB Isolates on Pathogenic Bacteria:

3.6.1: On Solid Medium

Ability of the LAB isolates to produce inhibition effect on pathogenic bacteria was tested by growing the isolate on MRS agar medium. In this regard, AL Kassab and AL Khafaji (1992) recommended the use of MRS agar medium to grow LAB isolates under anaerobic conditions in order to obtain reasonable result.

Figures (3-7) show the inhibitory effect of *Lactobacillus acidophilus* and *Lactobacillus plantarum* grown on MRS agar against *Staphylococcus aureus* isolates from patients suffering of dry eye syndrome at three different incubation periods.

Results show that *L. acidophilus* and *L. plantarum* exhibited noticeable inhibitory effect against all pathogenic bacteria tested at most of the periods of incubation used. But *L. plantarum* gave more inhibitory effect rather than *L. acidophilus* against pathogenic bacteria as illustered in tables (3-5) and (3-6), also the incubation period for 48 and 72 hr for both bacteria gave more inhibitory effect than the incubation for 24 hr. These results indicated that the inhibitory effect increase with increasing the incubation periods. So, agreed with those obtained by Al-Yas (2006) and Al-Dulemy (2005) who found that the inhibitory effect increased after 48 hr. While obtained results was disagreed with those obtained by Al-Jebory (2005) who found that increasing incubation periods to 48hr and 72hr were unable to increase the inhibitory effect, instead less effect was recorded. Aktypis *et al*., (1998) referred that such differences in the inhibitory effect at different incubation periods may be related to the nature of LAB isolates used against test bacteria.

Depending on the results mentioned in table (3-5) and (3-6), it may be concluded that 72hr incubation period was chosen as the preferred period for the *L. acidophilus* and *L. plantarum* on MRS agar medium in order to exhibit their inhibitory effect against all species of pathogenic bacterial isolated from dry eye infection patients.

As we mentioned above *L. acidophilus* and *L. plantarum* exhibited noticeable inhibitory effect against all pathogenic bacteria tested, but it had more effect on gram positive bacteria than on gram negative bacteria, the difference on the above results of LAB effect the pathogenic bacteria was related to the type of bacteria, type of inhibitory substances, its quantities and its ability for distribution in the media (Egorov, 1985).

The view of investigators about the inhibition effect of *L. acidophilus* and *L. plantarum* against pathogenic bacteria is different from one to another, for instance, Jimenez-Diaz *et al*. (1993) say that it effect was only on gram positive bacteria, while Nigatu and Gash (1994) mentioned its effect on gram negative bacteria such as *Pseudomonas*, *Proteus, E. coli* and *Salmonella.* Also the explanation for its effect may due to production of inhibitory substance. In this regard, Sander and Klaenhammer (2001) found that the inhibitory effect of *L. acidophilus* is attributed to acidophilin produced, while Ocana and Elena (2004) reported that the lactocidin and plantaracin found in *Lactobacillus plantarum* had good ability to encounter pathogenic bacteria.

Table (3-5): Diameters of Inhibition Zones (mm) Caused by ***Lactobacillus acidophilus*** Propagating on MRS Solid Medium Against Bacterial Isolates of Dry Eye Syndrome Patients.

Bacterial Isolate	Diameter of Inhibition Zones (mm) Incubation Period (hr)		
	24 hr.	48 hr.	72 hr.
Staphylococcus epidermidis	**12**	**16**	**18**
Staphylococcus aureus	**12**	**16**	**16**
Streptococcus pneumoniae	**–**	**10**	**14**
Streptococcus pyogens	**–**	**9**	**13**
Corynebacterium diphtheriae	**10**	**18**	**22**
Hemophilus influenzae	**–**	**17**	**15**
Klebsiella pneumoniae	**10**	**18**	**16**
Pseudomonas aeruginosa	**10**	**18**	**15**
Proteus mirabilis	**–**	**–**	**10**
Neisseria gonorrhoeae	**10**	**22**	**20**
Escherichia coli	**11**	**14**	**18**

(□) No inhibition zone.

Table (3-6): Diameters of Inhibition Zones (mm) Caused by ***Lactobacillus plantarum*** propagating on MRS Solid Medium Against Bacterial Isolates of Dry Eye Syndrome Patients.

Bacterial Isolate	Diameter of Inhibition Zones (mm) Incubation Period (hr)		
	24 hr.	48 hr.	72 hr.
Staphylococcus epidermidis	**16**	**22**	**20**
Staphylococcus aureus	**8**	**19**	**26**
Streptococcus pneumoniae	**–**	**–**	**12**
Streptococcus pyogens	**–**	**10**	**10**
Corynebacterium diphtheriae	**12**	**22**	**26**
Hemophilus influenzae	**20**	**23**	**27**
Klebsiella pneumoniae	**20**	**21**	**20**
Pseudomonas aeruginosa	**15**	**21**	**24**
Proteus mirabilis	**–**	**8**	**11**
Neisseria gonorrhoeae	**16**	**17**	**21**
Escherichia coli	**11**	**15**	**20**

(□) No inhibition zone.

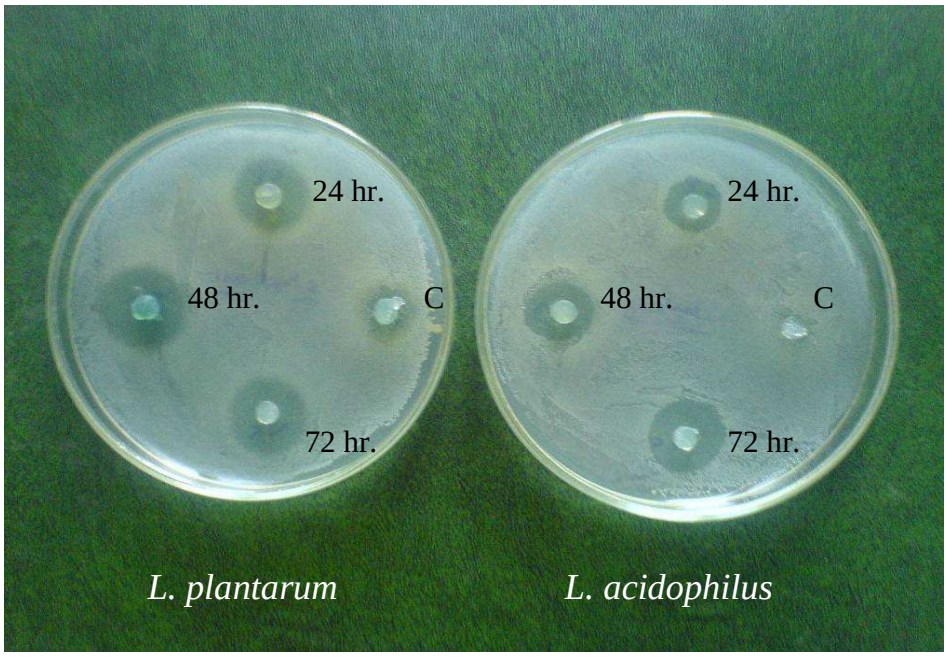

Figure 3-7: Inhibitory Effect of *L. acidophilus* and *L. plantarum* Against *Staphylococcus aureus* After Propagating in Solid Medium (MRS agar) for (24, 48 and 72 hr.). C = control.

3.6.2: In Liquid Medium

Well diffusion method has been used to determine the inhibitory effect of *L. acidophilus* and *L. plantarum* filtrates propagated for different incubation periods against the causative bacterial isolates of dry eye infection.

Results showed that no inhibition zones were recorded by the unconcentrated filtrares of both LAB isolates after incubation with the pathogenic bacterial isolates for all three periods of incubation. Such results came different from those obtained on solid medium (item 3.6.1) when some inhibition zones were produced. This may be related to the fact that LAB filtrates were diluted with the liquid medium, a state which not occurs with the solid medium. However, results of the liquid medium were totally

changed when the filtrates of the lactic isolates were concentrated. Tables (3-7) and (3-8) show that inhibitory effect of the concentrated filtrates of *L. acidophilus* and *L. plantarum* was sharply increased against all dry eye infection causative bacterial isolates with increasing the concentration of filtrate. In this regard, Barefoot and Klaenhammer (1983) stated that inhibitory substances of lactic bacteria increase upon increasing concentration of its filtrates, and death of pathogenic bacteria increases with increasing the inhibitory substances like bacteriocin, acidophilin and plantaracin of LAB.

Inhibitory effect of *L. acidophilus* and *L. plantarum* concentrated filtrates was varies according to the bacterial species and filtrate concentration. In table (3-7), the inhibitory effect was highly observable by the third concentration of *L. acidophilus* against *Staphylococcus epidermidis, Staphylococcus aureus, Hemophilus influenzae, Neisseria gonorrhoeae* and *Pseudomonas aeruginosa*, ranging between (20-25)mm, while *Streptococcus pneumoniae*, *Streptococcus pyogenes, Corynebacterium diphtheriae, Klebsiella pneumoniae* and *Escherichia coli* with inhibition zones ranging between (12-18) mm, In addition to that little effect 7mm was recorded against *Proteus mirabilis.*

Inhibitory effect against bacterial isolates decreased when the second concentration filtrate was used except for *Pseudomonas aeruginosa* which appear increasing in inhibition zones 28 mm at second concentration, but the highest effect of that concentration appeared clearly against *Staphylococcus epidermidis, Staphylococcus aureus, Hemophilus influenzae, Neisseria gonorrhoeae* and *Escherichia coli* with inhibition zones between (16-20)mm until reaching to the less sensitive isolate of *Streptococcus pyogenes* with inhibition zones 10mm at same concentration.

While inhibitory effect against all bacterial isolates was low with the first concentration, more effect was excreted by this concentration against *Staphylococcus epidermidis, Staphylococcus aureus, Hemophilus influenzae, Neisseria gonorrhoeae* and *Pseudomonas aeruginosa* when inhibition zones reached between 14-21mm.

Adversely, filtrates of the first and second concentration had no effect against *Proteus mirabilis*. While *Streptococcus pyogenes* was not affected by the first concentration as shown in table (3-7).

Table (3-7): Diameters of Inhibition Zones (mm) Caused by Concentrated Filtrates of ***Lactobacillus acidophilus*** Propagating in MRS Broth Against Bacterial Isolates of dry Eye Syndrome Patients.

Bacterial Isolate	Diameter of Inhibition Zones (mm)		
	One fold (50 ml)	Two fold (25 ml)	Three Fold (12.5 ml)
Staphylococcus epidermidis	**17**	**20**	**20**
Staphylococcus aureus	**14**	**17**	**25**
Streptococcus pneumoniae	**9**	**13**	**15**
Streptococcus pyogens	**–**	**10**	**12**
Corynebacterium diphtheriae	**9**	**11**	**12**
Hemophilus influenzae	**13**	**16**	**21**
Klebsiella pneumoniae	**10**	**13**	**14**
Pseudomonas aeruginosa	**21**	**28**	**24**
Proteus mirabilis	**–**	**–**	**7**
Neisseria gonorrhoeae	**15**	**18**	**20**
Escherichia coli	**13**	**17**	**18**

(□) No inhibition zone.

Result of table (3-8) declared that *Lactobacillus plantarum* had different inhibitory effect on bacterial isolates. The inhibitory effect was highly observable by the third concentration against *Staphylococcus epidermidis* and *Staphylococcus aureus* which giving inhibition zones 30 mm and 27 mm respectively, While moderate effect were appeared against *Streptococcus pneumoniae, Hemophilus influenzae, Klebsiella pneumoniae, Pseudomonas aeruginosa, Neisseria gonorrhoeae* and *E. coli* with inhibition zones ranging from (18-23) mm, but little effect were exhibited against *Streptococcus pyogenes, Corynebacterium diphtheriae* and *Proteus mirabilis* when the inhibition zones between 10-13 mm.

Inhibitory effect decreased for all bacterial isolates with the second concentration compared to the third concentration when the second concentration resulted more effect against *Staphylococcus epidermidis, Staphylococcus aureus, Hemophilus influenzae* and *Pseudomonas aeruginosa* with ranging between (24-28) mm inhibition zones.

Decreasing the inhibitory effect is associated with decreases concentration, and this was obvious with the first concentration when the highest effect recorded 12mm in diameter against *Escherichia coli* and only 10mm against *Corynebacterium diphtheriae,* while no effect was recorded against *Proteus mirabilis* and *Streptococcus pyogenes.*

Table (3-8): Diameters of Inhibition Zones (mm) Caused by Concentrate Filtrate of ***Lactobacillus plantarum*** Propagating in MRS Broth Against Bacterial Isolates of Dry Eye Syndrome Patients.

Bacterial Isolate	Diameter of Inhibition Zones (mm)		
	One fold (50 ml)	Two fold (25 ml)	Three fold (12.5 ml)
Staphylococcus epidermidis	**23**	**28**	**30**
Staphylococcus aureus	**20**	**25**	**27**
Streptococcus pneumoniae	**16**	**19**	**20**
Streptococcus pyogens	**–**	**–**	**13**
Corynebacterium diphtheriae	**10**	**10**	**12**
Hemophilus influenzae	**17**	**24**	**20**
Klebsiella pneumoniae	**13**	**15**	**18**
Pseudomonas aeruginosa	**20**	**25**	**22**
Proteus mirabilis	**–**	**8**	**10**
Neisseria gonorrhoeae	**16**	**20**	**23**
Escherichia coli	**12**	**16**	**19**

(□) No inhibition zone.

Results show that illustrated that filtrates concentrated to three folds gave high effect against *Staphylococcus epidermidis, Staphylococcus aureus***,** *Hemophilus influenzae, Neisseria gonorrhaeae* and *Escherichia coli,* but lower against *Streptococcus pneumoniae, Streptococcus pyogenes, Pseudomonas aeruginosa* and *Corynebacterium diphtheriae* and very little effect on *Klebsiella pneumoniae.*

In general, inhibitory effect of the concentrated filtrates of *L. acidophilus* and *L. plantarum* against gram positive was more than on gram negative bacterial isolates.

Thornton and Troyer (2006) reported that eating yoghurt, as well as applying it in a compress to the eyes, will help eliminate bacterial infection of dry eye due to the compact action of *Lactobacillus acidophilus* in yoghurt on bacteria causing this disease. Another report (Meadows, 2005) found that eating half a cup of yoghurt (containing live cultures) three times a day is important to promote health in all epithelial tissues, including the conjunctiva.

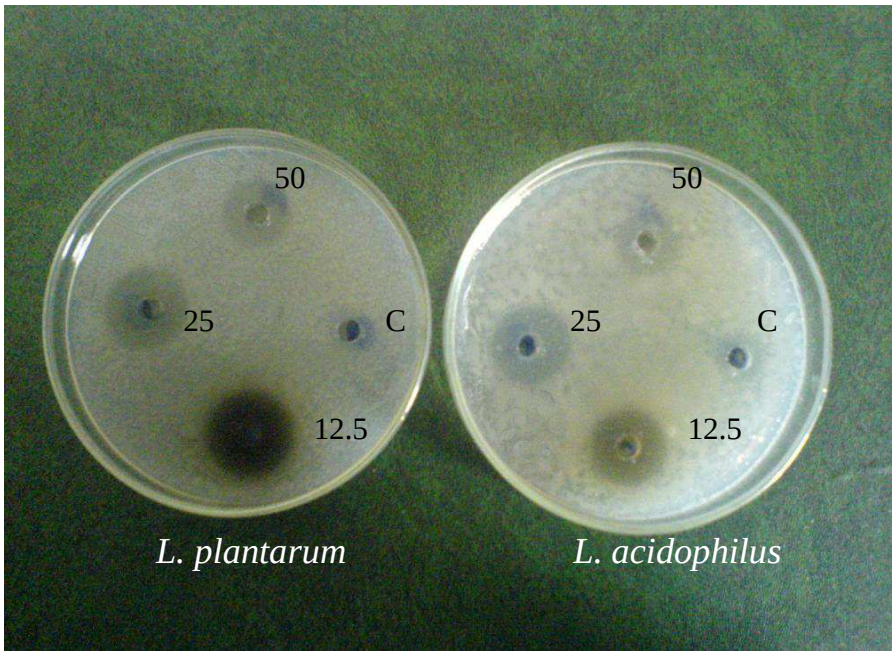

Figure 3-15: Inhibitory Effect of Concentrated Filtrate of *L. acidophilus* and *L. plantarum* Against *Staphylococcus aureus* After Propagating in Liquid Medium (MRS broth) for 24 hr.

50 = 1 fold, 25 = 2 folds, 12.5 = 3 folds, C = control.

3.7: Minimum Inhibitory Concentration (MIC) of LAB Concentrated Filtrates:

3.7.1: **For *Lactobacillus acidophilus***:

To determine MICs of the *L. acidophilus* concentrated filtrates required to inhibit bacterial growth of dry eye infection, different ratios were prepared from the three-fold concentrated filtrate of *L. acidophilus*, as previously mentioned in item (2.2.11).

Table (3-9) contains MICs of the concentrated filtrate of *L. acidophilus* propagated in MRS broth. Results of the table declared that the first two ratios (1:9 and 2:8) had no observed effect against all bacteria when heavy growth of the pathogenic bacteria was noticed after incubation. Increasing the ratio to (3:7) led to decrease growth of *Staphylococcus aureus* and *Staphylococcus epidermidis* to the moderate level, while at ratio (4:6) other bacteria: *Streptococcus pneumoniae*, *Hemophilus influenzae*, *Pseudomonas aeruginosa* and *Proteus mirabilis*, were affected.

Sharpe decreases in growth to the light level were recorded for *Staphylococcus aureus* and *Staphylococcus epidermidis* after treatment with ratio (4:6), and with ratio (5:5) for others. The last four ratios of *L. acidophilus* (6:4, 7:3, 8:2, 9:1) were quite enough to retard any growth of all the test bacteria.

From the above results, it may be concluded that filtrate ratio of (4:6) is the MIC value for *Staphylococcus aureus* and *Staphylococcus epidermidis*, and (5:5) ratio for *Streptococcus pneumonia*, *Hemophilus influenzae*, *Pseudomonas aeruginosa* and *Proteus mirabilis*.

Table (3-9). Minimum Inhibitory Concentrations (MICs) of Different Ratios Prepared from the Three-fold Concentrated Filtrate of ***L. acidophilus*** Propagating in MRS Broth Against Dry Eye Bacterial Isolates.

Isolate	Ratios (Concentrated Filtrate: Brain Heart Infusion Broth)								
	1:9	**2:8**	**3:7**	**4:6**	**5:5**	**6:4**	**7:3**	**8:2**	**9:1**
Staphylococcus epidermidis	+++	+++	++	+	-	-	-	-	-
Staphylococcus aureus	+++	+++	++	+	-	-	-	-	-
Streptococcus pneumoniae	+++	+++	+++	++	+	-	-	-	-
Hemophilus influenzae	+++	+++	+++	++	+	-	-	-	-
Pseudomonas aeruginosa	+++	+++	+++	++	+	-	-	-	-
Proteus mirabilis	+++	+++	+++	++	+	-	-	-	-

Heavy Growth = +++ **Medium Growth = ++**

Light Growth = + **No Growth = -**

3.7.2: For *Lactobacillus plantarum*

Results in table (3-10) also show that first two ratios (1:9 and 2:8) had no effect on bacterial when clear growth of these test bacteria was observed. Increasing the ratio to 3:7 led to minimize growth of *Staphylococcus aureus* and *Staphylococcus epidermidis* to the moderate level, while at ratio (4:6) other bacteria: *Streptococcus pneumoniae, Hemophilus influenzae, Pseudomonas aeruginosa* and *Proteus mirabilis*, were affected.

Sharpe decreases in growth to the light level were recorded for *Staphylococcus aureus, Staphylococcus epidermidis* and *Hemophilus influenzae* after treatment with ratio (5:5), and with ratio (6:4) for others.

Also, filtrate ratio for *L. plantarum* (7:3) and above caused total inhibition for the test bacteria.

Depending on the just mentioned findings, 5:5 filtrate ratio of *Lactobacillus plantarum* is recorded and selected as the MIC value for *Staphylococcus aureus, Staphylococcus epidermidis,* and *Hemophilus influenzae.* While it is 6:4 for *Streptococcus pneumoniae, Pseudomonas aeruginosa* and *Proteus mirabilis.* Such results agreed with those of obtained by Al- Jeboury (2005) who found that the MIC of *Lactobacillus plantarum* concentrate filtrates were (50%) and (60%) that completely inhibit the growth of test bacteria.

Table (3-10): Minimum Inhibitory Concentrations (MICs) of Different Ratios Prepared from the Three-fold Concentrated Filtrate of ***L. plantarum*** Propagating in MRS Broth Against Dry Eye Bacterial Isolates.

Isolate	Ratios (Concentrated Filtrate: Brain Heart Infusion Broth)								
	1:9	**2:8**	**3:7**	**4:6**	**5:5**	**6:4**	**7:3**	**8:2**	**9:1**
Staphylococcus epidermidis	+++	+++	++	++	+	-	-	-	-
Staphylococcus aureus	+++	+++	++	++	+	-	-	-	-
Streptococcus pneumoniae	+++	+++	+++	++	++	+	-	-	-
Hemophilus influenzae	+++	+++	+++	++	+	-	-	-	-
Pseudomonas aeruginosa	+++	+++	+++	++	++	+	-	-	-
Proteus mirabilis	+++	+++	+++	++	++	+	-	-	-

Heavy Growth = +++ **Medium Growth = ++**

Light Growth = + **No Growth = -**

2.1 Materials

2.1.1 Equipment and Apparatus:

The following equipment and apparatus were used during this study:

Equipment	**Company(Origin)**
Autoclave	Tomy (Japan)
Balance	Ohans (France)
Compound light microscope	Olympus (Japan)
Distillator	GFL (Germany)
Electrical Oven	Sanyo (Japan)
Candle jar	Rod well (England)
Incubator	Termaks (England)
Lyophilizer	Virtis (U.S.A)
Micropipette	Witey (Germany)
Millipore filter paper unit	Millipore corp (U.S.A)
pH-meter	Mettler GmbH-Toledo (U.K)
Refrigerated centrifuge	MSE (UK)
Sensitive balance	Delta Range (Switzerland)
Water bath	GFL (Germany)

2.1.2: Chemicals

The following chemicals were used in this study:

Chemical	**Company(origin)**
Sodium hydroxide , Urea, Glycerol, Peptone, Glucose, Sodium acetate trihydrate, Triammonium citrate	BDH-England
Meat extract, Yeast extract, Agar, Gelatin, Skim milk	Biolife-Italy
Hydrogen peroxide	Difco-USA
Tetramethyl-p-pheneylene diamine dihydrochloride, Crystal violate, Safranine, Iodine, $MnSO_4.4H_2O$, $MgSO_4.7H_2O$	Fluka-Switzerland
Ethanol	Local market-Iraq
Tween-80	Sigma-USA

2.1.3 Stains:

Stain	Company (origin)
Crystal violet	Fluka(Germany)
Safranin	Fluka

2.1.4 Antibiotics:

The following antibiotics discs were used in this study:

Antibiotics	Abbreviations	Concentration (μg)	Company(origin)
Amikacin	AK	30	AL-Razzi (Iraq)
Ampicillin	AM	10	AL-Razzi (Iraq)
Bacitracin	Ba	10	AL-Nadear (Iraq)
Cefotaxime	CTX	30	AL-Nadear (Iraq)
Chloramphenicol	C	30	AL-Razzi (Iraq)
Ciprofloxacin	CF	5	AL-Nadear (Iraq)
Clindamycin	CM	2	AL-Razzi (Iraq)
Cloxacillin	OB	5	AL-Nadear (Iraq)
Erythromycin	E	15	AL-Razzi (Iraq)
Gentamicin	CN	10	AL-Razzi (Iraq)
Neomycin	N	30	AL-Razzi (Iraq)
Penicillin G	G	10(U)	AL-Nadear (Iraq)
Tetracycline	TE	30	AL-Nadear (Iraq)
Vancomycin	VA	30	AL-Nadear (Iraq)

2.1.5 Microorganisms:

Lactobacillus acidophilus and *L. plantarum* were obtained from the Department of Biotechnology / College of Science, Al- Nahrain University, Baghdad from local collection.

2.1.6: Culture Media

2.1.6.1: Ready to Use (Powdered) Media:

Medium	**Company (Origin)**
Nutrient agar	Oxoid (England)
Nutrient broth	Oxoid
Brain heart infusion broth	Oxoid
MacConkey agar	Oxoid
Urea agar Base	Oxoid
Mannitol salt agar	Difco(USA)
Triple sugar Iron (TSI) agar	Oxoid
Muller-Hinton agar	Oxoid
Litmus milk broth	Qxoid

2.1.6.2: Laboratory-Prepared Media:

Medium
Blood Agar Base
Chocolate Agar
Man-Rogoza-Sharpe (MRS) Broth
Man-Rogoza-Sharpe (MRS) Agar

2.1.7: API System Kits (API-Bio merieux, Lyon, France)

Four types of API -System kits were used including:

2.1.7.1: API 20E

It consists of:

Gallery contains 20 microtubes having dehydrate substrate.

Reagents: TDA, IND, VP, OX.

2.1.7.2: API Staph

It consists of:

Gallery contains 20 microtubes having dehydrate substrate.

Reagents: VP1, VP2, N I T1, N I T2, ZYMA, ZYMB.

2.1.7.3: API 20 Strep:

It consists of:

Gallery contains 20 microtubes having dehydrate substrate

Reagents: VP1, VP2, TDA, N I T1, N I T2.

2.1.7.4: API Coryne

It consists of:

Gallery contains 20 microtubes having dehydrate substrate

Reagents: N I T1, N I T2, ZYMA, ZYMB, PYZ.

2.2: Methods

2.2.1: Preparation of Culture Media:

2.2.1.1: Ready to Use Media:

Media listed in table (2.1.6.1) were prepared according to the manufactures instructions fixed on their containers. After adjusting pH, they were sterilized in the autoclaved at 121°C for 15 minutes.

2.2.1.2: Laboratory Prepared Media:

2.2.1.2.1: Blood Agar Medium

It was used for cultivated aerobic cocci, and prepared according to (Atlas *et al*., 1995), by autoclaving blood agar base after adjusting pH to 7.0, cooled to 45°C, then 5% human blood was added and mixed well.

2.2.1.2.2: Chocolate Agar Medium

It was used for cultivation of fastidious bacteria, and prepared as in item (**2.2.1.2.1**). Then heating it until it turned to characteristic brown color. (Atlas *et al*., 1995).

2.2.1.2.3: Gelatin Medium

It was used for gelatinase production bacterial isolates, and prepared according to Stolp and Gadkari, (1984) by adding 12% (w/v) gelatin to nutrient broth. Then sterilized by autoclave.

2.2.1.2.4: Urea Agar Medium

It was used for urease production bacterial isolates, and prepared according to Baron *et al.*, (1994), by preparing 950 ml of urea agar base as recommended by manufacturing company, then sterilized by autoclave, and cooled to 50°C, then 50 ml of 40% filtered - sterilized urea was added.

2.2.1.2.5: Litmus Milk Medium:

It was prepared by dissolving 100g of powder skim milk and 5g of litmus medium in 1L of D.W. then sterilized by autoclave for 10 min, it was used for identification of LAB (Baily *et al.,* 1990).

2.2.1.2.6: Mans-Rogoza- Sharpe (MRS) Broth

This media was used for enrichment and cultivation of lactic acid bacteria, and prepared according to Harrigin and MacCance, (1976) by dissolving:

Ingredient	Quantity (%)
Peptone	10 g
Meat extract	10 g
Yeast	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 pH was adjust to 6.0-6.5, the medium was autoclaved.

2.2.1.2.7: Mans-Rogoza- Sharpe (MRS) Agar:

It was used for cultivation of lactic acid bacteria, and prepared as in item (**2.2.1.2.6)** with the addition of 20 g agar.

2.2.2: Sterilization

2.2.2.1: Moist-Heat Sterilization:

Media and solutions (unless otherwise different) were sterilized in the autoclave at 121 °C (15 Ib/ in) for 15 min.

2.2.2.2: Dry – Heat Sterilization:

Electric oven was used to sterilize the glass wares at 160-180°C for 2-3 hrs.

2.2.2.3: Membrane Sterilization:

Millipore filter unit (0.44 mm) was used to sterilize the filtrates of *Lactobacillus acidophilus* and *Lactobacillus plantarum.*

2.2.3 Specimens Collection

Specimens were collected from patients who suffering from dry eye syndrome. The specimens were taken from patients in Ibn Al-Haetham Teaching Eye Hospital in Baghdad during the period between Novembers- July, 2006.

After making schirmer test type I (with out anesthesia) as shown in figure (2-1) for 60 patients suffering from dry eye syndrome. Then each specimen was obtained by direct application of sterile moistened cotton swab. After patient was requested to look up, the lower eye lid was pulled down and moistened swab be rubbed over the lower conjunctiva sac from medial to lateral

side and back again, All specimens were soon brought to the laboratory, then swab was directly streaked separately on blood agar, Chocolate agar and MacConkey agar.

The same procedure was done for twenty normal eyes individuals from both gender and different age group, in order to make them as control.

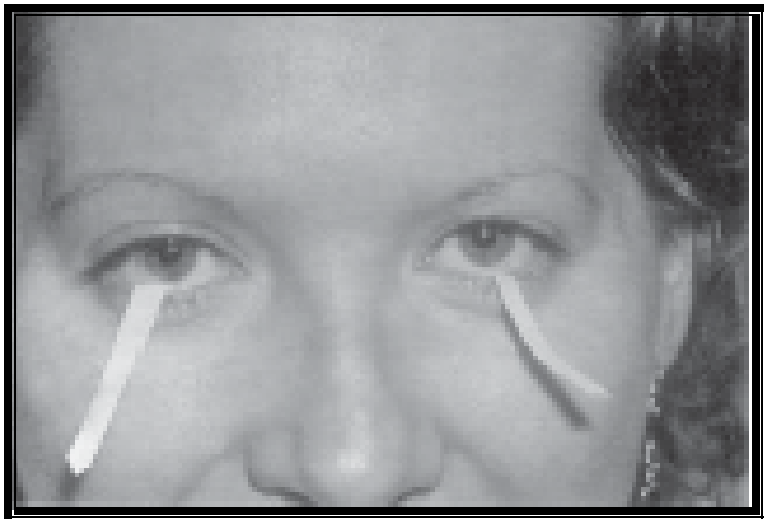

Figure (2-1): Schirmer test for patients suffering from dry eye syndrome (Stein, 2004)

2.2.4: Culturing:

All specimens that taken from dry eye patients were inoculated on:

- Blood agar: For isolation of aerobic cocci.

-Chocolate agar: For cultivation of *Hemophilus influenzae.*

-MacConky agar: For cultivation of Gram negative bacteria

Then cultures of isolates grown on these media were characterized by microscopical examination and biochemical tests. Presumptive identification of isolates was made on basis of the above criteria. Identification of any growth was confirmed by using Api-system.

2.2.5: Maintaining Bacterial Isolates:-

Maintenance of bacterial isolates was performed according to (Atlas *et al.,* 1995), as the following:

2.2.5.1: Short-Term Storage:

Colonies of bacterial isolates were maintained for two weeks on the surface of agar medium (Nutrient agar). The plates were tightly warped with Parafilm and stored at 4°C.

2.2.5.2: Medium-Term Storage:

Bacterial isolates were maintained in stab cultures for longer periods (few months). Cultures were prepared in screw-capped vials containing (5-8) ml of nutrient agar and stored at 4°C.

2.2.5.3: long-Term Storage:

15-20% glycerol were added to each screw-capped tubes containing 10 ml of nutrient broth. After autoclaving, they were inoculated with bacteria and incubated at 37°C for 24 hr., then kept in the freezer(-18 C), bacteria can be stored for many years in this medium without significant loss of viability.

2.2.6 Identification of Bacterial Isolates (Holt *et al*., 1994; Atlas *et al.*, 1995):

2.2.6.1 Morphological and Cultural Characteristics:

Morphology of colonies were studied on brain heart infusion agar (Collee et *al*., 1996). Color, shape, size, edge of colonies and type of lysis were recorded after 24 hr. of incubation at 37°C.

2.2.6.2: Microscopical Examination:

Gram stain method was used to describe cells morphology. The method was done according to (Harley and Prescott, 1996).

2.2.6.3: Biochemical Tests:

2.2.6.3.1: Catalase Production (Brook *et al*., 1998):

This test was performed by adding (2-3) drops of 3% hydrogen peroxide on a cleaned slide, and a single colony of bacterial growth was fixed on it, Appearance of bubbles was regarded as a positive result.

2.2.6.3.2: Oxidase Production (Harely and Prescott, 1996):

Filter paper was saturated with the substrate (tetramethyle-p-phneylene-diamine-dihydrochloride); colony of bacterial isolate to be tested was rubbed on the filter paper with a sterile wooden applicator stick. An immediate color change to deep blue indicates a positive result.

2.2.6.3.3: Coagulase Production by Tube Method (Kloss and Jorgensen, 1985):

A large well isolate colony was transferred into a test tube containing 0.5ml reconstituted plasma and incubated at 37°C for 4 hrs, if conversion of the plasma into a soft gel was observed, this indicate positive result. Also tubes that showed negative results were left at room temperature overnight and reexamined.

2.2.6.3.4: Hemolysis Patterns on Blood Agar (Atlas *et al.*, 1995):

A single colony of overnight growth culture was streaked on blood agar. After incubation, the type of hemolysis produced by the growing colonies was observed and recorded.

2.2.6.3.5: Aerobic Mannitol Fermentation Test (Atlas *et al.*, 1995):

Bacterial isolate was streaked on the plate containing mannitol salt agar, and the plate was incubated at 37°C for overnight. The change in color from deep pink to yellow gives a positive indication of mannitol fermentation.

2.2.6.3.6: Urease Test (Atlas *et al.*, 1995):

Urease activity was detected by inoculating the surface of urea agar slant tubes with the bacterial growth, and then incubated at 37°C for 24 hrs. Appearance of red-violet color indicates a positive result, while yellow indicates the negative one.

2.2.6.3.7: Triple Sugar Iron (TSI) Test (Atlas *et al.*, 1995):

Bacterial isolate was stapped in and/or streaked on the (TSI) agar slant, then incubated at 37°C for 24 hr. If the color of the medium changed from red to yellow, this indicates ability of fermentation and acid formation. Appearance of black precipitate indicates ferric sulfate formation, while pushing the agar to the top indicates CO_2 formation.

2.2.6.3.8: Gelatin Test (Harely and Prescott, 1996):

It detects gelatinase production and was done by inoculating a test tube containing gelatin medium with the isolate by stapping inside 3/4 of the medium toward the bottom, then incubated at 37°C for 24 hr or longer (incubation time usually depends on the species of bacteria). After that the tube was placed in the refrigerator (at 4°C) for 30min, the surface of the medium was noticed weather it was aqueous which indicate a positive result, or solid as negative result.

2.2.6.3.9: Optachin Susceptibility Test:

Commercially available optachin were applied to a quarter of blood agar plate that had been streaked with a few colonies of the organisms. After overnight incubation at 35°C in anaerobic jar, inhibition zones were measured. Zones equal to and greater than 14 mm with 6 mm disk were indicative of inhibition, and the isolate was identified as *Streptococcus pneumoniae*, while inhibition zones smaller than 14 mm may belonged to other species of *Streptococcus pyogenes* (Rouff *et al*., 1999).

2.2.7: Identification of Lactic Acid bacteria (LAB):

The following tests were used:

2.2.7.1 Gram Stains (Harley and Prescott, 1996):

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

2.2.7.2: Catalase Production (Brook *et al*., 1998):

This test was performed by adding (2-3) drops of 3% hydrogen peroxide on a cleaned slide, and then a single colony of the bacterial growth was added to it, Appearance of bubbles was regarded as positive result.

2.2.7.3: Growth on Nutrient Agar:

Nutrient agar was inoculated with the suspected bacteria, then incubated at 37°C for 24 hrs. Positive result was defined by absence of growth.

2.2.7.4: Acid production and Clot formation in Litmus milk test (Kandler and wises, 1986):

Tubes containing litmus milk medium (10 ml) each were inoculated with 1% of the suspected isolate, then incubated at 37°C for 24 hr. formation of clot and change in pH indicate a positive result.

2.2.7.5: Growth at 15°C and 45 °C:

Test tubes containing MRS broth were inoculated with overnight culture of LAB isolate, incubated at 15°**C** or 45°C for 24 hrs. Bacterial growth (turbidity) was regarded as positive result.

2.2.7.6: Gelatin Test (Harely and Prescott, 1996):

It detects gelatinase production and done by inoculating a test tube containing gelatin medium with the isolate by stapping, then incubated at 37°C for 24 hr or longer (depending on the species). After that the tube was placed in the refrigerator (at 4°C) for 30min, the surface of the medium was noticed weather it was aqueous which indicate a positive result, or solid as negative result.

2.2.8: API System:

Api kits were used to ensure the identification of the bacterial isolates. The technique was done by transferring a well separated colony from each pure culture (by using sterile loop) and emulsified in 5 ml suspending medium (with sterile D.W.) by rubbing against the side of the tube and mixed with the water by flame loop.

Then 5 ml of tap water was dispensed into incubation tray to provide a humid atmosphere during incubation. After that, with a sterile Pasteur pipette, the microtubes were inoculated, and some section were completely filled with sterile mineral oil, and then incubated at 37°C for 24 hrs. Reactions not requiring reagent were recorded first, then appropriate reagents for each Api type were added to some microtubes need that, then recorded it. After that the biochemical profiles obtained were transformed into a numerical profile and compare it with those listed in index by transform all biochemical results into seven digits number by placing into group and consigning a specific value for each result.

2.2.9 Antibiotics Susceptibility Test:

A disk diffusion method was employed (Vandepitte *et al*., 1991).Muller Hinton agar was used for some species, while blood agar for *Streptococcus spp*, and chocolate agar for *Hemophilus influenzae*. A spreader was used to streak the inoculums on the plate surface. The inoculated plates were then placed at room temperature for 10 minutes to allow absorption of excess moisture. With sterile forceps the selected antibiotic disks were placed on the inoculated plates and incubated the plates at 37°C for 18 hr. in an inverted position.

After incubation, the diameter of inhibition zone were noted and measured by a ruler in mm, results were determined according to the National Committee for Clinical Laboratory Standards (NCCLS, 1994).

2.2.10 Determining of *Lactobacillus acidophilus* and *Lactobacillus plantarum* Inhibitory Effect:

-On Solid Media:

A culture of *Lactobacillus acidophilus* previously grown in MRS broth were streaked separately on MRS agar, and incubated under anaerobic conditions at 37°C for various periods of times; 24, 48 and 72 hr. (Silva *et al*., 1987).

After incubation, and with the aid of cork porer (5mm), disks of the grown culture were put reversely on the surface of BHI agar that was

previously inoculated with 0.1 ml of pathogenic bacteria by using spreader, then incubated at 37°C for 24 hr. After that, the inhibition zone around the disk was measured and estimated in (mm). The optimum incubation time that gives greater inhibition effect was determined.

Same *L. acidophilus*, Procedure was used for *Lactobacillus plantarum.*

-On liquid Media:

MRS broth was inoculated by 1 % of *Lactobacillus acidophilus* culture, then incubated an aerobically at 37°C for various periods of times (24, 48 and 72 hr) (Schillinger and Luck, 1989; Lewus *et al*., 1991).

After incubation the culture was centrifuged at 6000 rpm for 15 min to get supernatant which contained the filtrate of grown cells. After adjusting pH to 6.5 by using NaOH (1M), it was filtered through Millipore filter unit (0.44mm) Then the well diffusion method that mention by Vignolo *et al.*, (1993) was used on BHI agar. The plate was inoculated with 0.1 ml of pathogenic bacteria by using spreader. Then by the cork porer (5mm) wells were made in agar and filled by the filtrate of *L. acidophilus* and incubated at 37 °C for 24 hrs. 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.*, 1995). The filtrate was concentrate by freeze-dryer and the well diffusion method was repeated to detect the effect of concentrated filtrate against pathogenic bacteria. The control in concentrate filtrate was contains MRS broth that concentrated also.

Also the same procedure was repeated for *Lactobacillus plantarum.*

2.2.11: Determining Minimum Inhibitory Concentration (MIC) of Filtrates (Nester *et al*., 2001):

The minimum inhibitory concentration was determined by assaying the ability of bacteria to grow in broth cultures containing different concentrations of the LAB filtrates. Serial dilutions generating decreasing concentrations (1:9; 2:8; 3:7; 4:6; 5:5; 6:4; 7:3; 8:2; 9:1) as concentrated filtrate: brain heart infusion broth was prepared in test tubes. Then, a known concentration of bacteria isolate (100µl) was added to each tube. The tubes were incubated for at least 18 hours, and then examined for visible growth or turbidity and recorded as; light (+), medium (++), heavy (+++), and no growth (–). Growth was estimated by measuring optical density ($OD._{600}$) nm was read for each dilution. The lower concentration of the concentrated filtrate that prevented growth of pathogenic bacteria was considered as the minimum inhibitory concentration.

4.1: Conclusions:

- *Staphylococcus epidermidis* was the most dominant bacteria of patients suffering from dry eye syndrome, followed by *Staphylococcus aureus.*
- Ciprofloxacin and gentamicin were the most effective antibiotics against the bacteria isolates but in general, its effect on Gram negative was more than on Gram positive bacterial isolates, while almost all of the isolates were resist to ampicillin and pencillin G.
- *Lactobacillus acidophilus* and *Lactobacillus plantarum* exhibit good inhibitory activity when grown on MRS solid medium against the dry eye syndrome bacterial isolates.
- Inhibitory activity increased sharply upon concentrating lactic filtrates especially to three-fold.

4.2: Recommendations:

- Using other probiotic microorganisms to detect their inhibitory effect against bacteria causing dry eye syndrome**.**
- Trying and developing other media to propagate the probiotic microorganism to improve inhibitory effect.
- In vivo application of the probiotic filtrates in treatment of dry eye syndrome.

C.V.

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- رقم الهاتف: ٧٦٥٠٨٦٥
- حصلت على شهادة البكلوريوس سنة ٢٠٠٤ للاطروحة الموسومة:
  (Assay of *Lysium barbarum* extract on mice cell line).
- تم القبول والمباشرة بدارسة الماجستير سنة ٢٠٠٤ ـ ٢٠٠٥
- حصلت على شهادة الماجستير سنة ٢٠٠٧ للاطروحة الموسومة:
  (Effect of probiotics on bacterial isolates accompanying dry eye syndrome).

Dedication

To the light of my eyes, my Father and Mother…

To my beating Heart, my Husband…

To my Soul, the sweet angel my Aunt…

Linda

SUMMARY

This study included collection of (60) swab samples taken from patients of dry eye syndrome referred to Ibn Al-Haetham Teaching Eye Hospital in Baghdad. Results showed that (53) of these samples were positive for bacterial occurrence after culturing on related selective media.

Bacterial isolates were identified by cultural, microscopic and biochemical examinations. After confirming the conventional identification by (Api) kits specified for each type of bacteria, the following species and percentage of bacteria were recorded :-

Staphylococcus genus (45.9%), *Streptococcus* genus (17%), *Coynebacterium diphtheriae* (9.3%), *Hemophilus influenzae* (7.4%), (5.6%) to each of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, while (3.7%) to each of *Proteus mirabilis* and *Neisseria gonorrhoeae* and (1.8%) to *Escherichia coli.*

According to such results *Staphylococcus epidermidis* was the predominant bacteria among others followed by *Staphylococcus aureus.*

Regarding to the patients, high percentage (45%) of dry eye syndrome cases was recorded in the old-age group (above 60 years) compared to other groups, and according to the gender, cases of dry eye syndrome were more abundant (56.7%) in the females than in males (43.3%).

Results of antibiotic sensitivity of isolates toward (14) different antibiotics revealed that ciprofloxacin and gentamicin were the most effective against all isolates, while choloramphenicol exhibited its effect on gram positive bacterial isolates only. Despite that other antibiotics varied in their effect, most of the isolates were resistant to ampicillin and pencillin G.

After culturing *Lactobacillus acidophilus* and *L. plantarum* in liquid and on solid media for different incubation periods (24, 48,72hr) to test there inhibitory ability against bacterial isolates of dry eye syndrome patients, it was noticed that the solid medium was more efficient in exhibiting such activity after 72 hr of incubation especially against gram positive bacteria.

Despite the unconcentrated filtrates of lactic isolates showed no observable inhibitory activity on pathogenic bacteria, their effects against all bacterial isolates of dry eye syndrome increased to large extents upon increasing concentration of the filtrates.

Minimum inhibitory concentrations (MIC,s) of *Lactobacillus acidophilus* and *L. plantarum* concentrated filtrate were determined after propagated in MRS broth (each one separately). It was found that MIC values are lower with *L. acidophilus* application than with *L. plantarum*. Regarding such finding it can be concluded that the former lactic isolate was more efficient against bacterial causes of dry eye syndrome.

Examining Committee's Certification

We, the Examining Committee, certify that we read this thesis and have examined the student in its contents and that, according to our opinion; it is adequate as a thesis for the Degree of Master of Science, in Biotechnology.

Signature:
Name:
Scientific Degree:
Date:

(Chairman)

Signature:
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Scientific Degree:
Date:
(Member)

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Name:
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(Member)

Signature:
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Title:
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I here by, certify upon the decision of the examining committee

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1.1: Introduction

The continuous production and drainage of tears is important to the eye's health and this related to its essential functions: keep the eye moist, help wounds heal and protect against eye infection, so any disorder in quantity or quality of tears lead to a collection of symptoms called dry eye syndrome (Zhou *et al.*, 2004). It's a common ophthalmologic abnormality involving bilateral disruption of the precorneal tear film. It is caused either by in-adequate tears or an inability to maintain an effective tear film (Schaumberg *et al.*, 2003). Decreased tear secretion and clearance initiate an inflammatory response on the ocular surface, and Pflugfelder (2000) suggests that this inflammation plays a role in the pathogenesis of dry eye.

Dry eye syndrome can occur in any age group, but is especially prevalent in people over age 65. It estimated that about 10 to 15 percent of American in this age group has one or more symptoms of this disease (Schein 1999).

Inflammation problem is increasing due to appearance of bacterial strains resistant to over-used antibiotics. However, topical antibiotics should be reserved for superadded bacterial infection (Kanski, 2003).

Microbial cultures have been used for thousands of years in food and alcoholic fermentations. In the past century, they have undergone scientific scrutiny for their ability to prevent and cure a variety of diseases particularly in developing countries (Drisko *et al*., 2003).

Among bacteria groups, certain species of *Lactobacillus* are considered to be the most commonly used type of bacteria as probiotic, due to their presence in mucous membrane of intestine and digestive tract of human as normal microflora, safe used in food industry, enhance both the specific and nonspecific immune response, they actively produce anti-bacterial substances which kill or inactivate hostile disease-causing bacteria (Sander and Klaenhamer, 2001). For such purpose, some locally isolated *Lactobacillus* spp. were used as probiotics for treating in vitro bacteria isolated from conjunctiva of patients with dry eye syndrome.

Aims of this study are:

1. Isolation and identification of bacteria accompanying dry eye syndrome.

2. Detecting the most predominant bacteria during infective period.

3. Determining the prevalence of disease according to sex and age of patients.

4. Determining the most effective antibiotics against bacteria associated with dry eye syndrome.

5. Study the inhibitory effect of some local *Lactobacillus* isolated against isolated pathogenic bacteria, and comparing with that of commonly used antibiotics.

6. Determining the minimum inhibitory concentrations of concentrated filtrates of *Lactobacillus acidophilus* and *L. plantarum.*

List of Appendices

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Supervisor Certification

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Chapter One

Introduction

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جمهورية ألعراق
وزارة ألتعليم ألعالي وألبحث ألعلمي
جامعة ألنهرين
كلية ألعلوم
قسم ألتقانة ألأحيائية

تدثير ألمعالجة ألحيوية على عزلات البكتريا المصاحبة لمتلازمة جفاف ألعين

رسالة

مقدمة إلى كلية ألعلوم – جامعة ألنهرين

وهي جزء من متطلبات نيل درجة ماجستير علوم في ألتقانة ألأحيائية

من قبل

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آيار ٢٠٠٧

ربيع ألثاني ١٤٢٨