SUMMARY

This study included collection of (103) swabs samples taken from patients suffering from nasolacrimal duct obstruction (NLDO) in Ibn Al- Haetham Teaching Eye Hospital /Baghdad. When these samples were cultured on special media, ninety samples gave positive results for bacteria, while no isolate was belonged to the yeast and fungi.

Positive samples were identified by using cultural, microscopical and biochemical examinations for diagnosis. After confirming the final diagnosis by using (API) system specified for each type of bacteria, the following types and percentages of bacteria were detected:-

Staphylococcus epidermidis (28.9%), *Staphylococcus aureus*(18.9%), *Streptococcus pneumoniae* (11.1%), *Streptococcus pyogenes* (10%), *Corynebacterium diphtheriae* (6.8%), *Pseudomonas aeruginosa* (5.6%), *Escherichia coli* (4.4%) , *Propionibacterium spp.* (4.4%), *Haemophilus influenzae* (3.3%) , *Listeria grayi* (2.2%) and *Morganella morganii* (1.1%).

According to such results *Staphylococcus epidermidis* was the predominant bacteria among sample taken from patients suffering from nasolacrimal duct obstruction, followed by *Staphylococcus aureus*.

Accordingly, the ratio of (NLDO) cases in children under 10 years was (48.5%) comparing with those other aged groups, and according to the gender the percentage in females were (59.2%) and in males (40.8%).

Results of antibiotic sensitivity test for (12) different antibiotics revealed that ciprofloxacin, vancomycin and neomycin were the most effective against the isolates, while other antibiotics varied in their effect. However, most isolated bacteria were resistant to tetracycline.

After culturing *Lactobacillus plantarum* in liquid and on solid growth media to test its ability to exhibit inhibitory action against bacterial isolates of (NLDO) patients, it was noticed that it had good inhibitory action when cultured on solid growth media ,especially against gram positive bacteria. Also the inhibitory action increased to a large extent against all types of bacterial isolates with increasing concentration of bacterial filtrate.

The results of propagating both *Saccharomyces boulardii* and *Saccharomyces cerevisiae* in a number of culture media (tea, sabouraud and sabouraud enriched with yeast extract) showed that the yeasts grown in sabouraud enriched media possessed best inhibitory action against (NLDO) bacterial isolates especially after increasing the concentration of yeast filtrate for several times.

When filtrates of each isolate *Lactobacillus plantarum* and yeasts *Saccharomyces boulardii* and *Saccharomyces cerevisiae* with ampicillin antibiotic (each one separately), results showed higher inhibitory action against *Staphylococcus epidermidis* (the common isolated bacteria from (NLDO)) compared with the using the filtrates of lactic acid bacteria and yeasts or antibiotic alone.

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Very special thanks are due to my husband Ali,and my family and everyone gave me a hand to complete this work.

Heba

الخلاصة

شملت الدراسة جمع (١٠٣) عينة اخذت كمسحات من مرضى يعانون من انسداد مجرى الدمع (Nasolacrimal duct obstruction / NLDO) في مستشفى ابن الهيثم للعيون بمدينة بغداد. ولدى زرع العينات على الاوساط الزرعية ذات العلاقة ، اعطت (٩٠) عينة منها نتيجة موجبة لتواجد العزلات البكتيرية فيما لم تظهر اية عزلة تعود للاعفان والخمائر.

تم تشخيص العينات الموجبة من خلال هذة الفحوصات الزرعية،والمجهرية ، والكيموحياتية اللازمة للتشخيص وبعد ان تم التأكد من التشخيص النهائي باستخدام عُدة api الخاصة بكل نوع من البكتيريا،حددت انواع البكترياالاتية ونسبها:

Staphylococcus epidermidis (%28.9), *Staphylococcus aureus* (%18.9), *Streptococcus pneumoniae* (%11.1), *Streptococcus pyogenes* (%10), *Cornynebacterium diphtheriae* (%6,8), *Pseudomonas aeruginosa* (%5.6), *Escherichia coli* (%4.4) , *Propionibacterium spp.* (%4,4), *Haemophilus influenzae* (%3.3) , *Listeria grayi* (%2.2), *Morganella morganii* (%1.1).

بذلك تكون عزلات بكتريا *Staphylococcus epidermidis* هي السائدة في العينات المأخوذة من مرضى انسداد مجرى الدمع، تليها في ذلك عزلات بكتيريا *Staphylococcus aureus*.

وكانت النسبة المئوية لحدوث حالات انسداد مجرى الدمع في الاطفال دون العاشرة ٤٨,٥% بالمقارنة مع بقية الفئات العمرية، وتبعا للجنس كانت النسبة المئوية في الاناث ٥٩,٢% وفي الذكور ٤٠,٨%.

اظهرت اختبارات الحساسية لأثني عشر مضاد حيوي ضد العزلات البكتيرية التي تم الحصول عليها. ان مضادات السبروفلوكساسين والفانكومايسين والنيومايسين كانت الاكثر تأثيراً، بينما بقية المضادات اختلفت بالتاثير ولكن اغلب العزلات اظهرت مقاومتها لمضاد التتراسايكلين.

بعد تنمية عزلة *Lactobacillus plantarum* في اوساط زرعية سائلة وصلبة لاختبار قدرتها على اظهار فعالية تثبيطية ضد عزلات البكتيريا المرضية المعزولة من المرضى الذين يعانون من الانسداد، لوحظ امتلاك هذه العزلة فعالية تثبيطية جيدة عند نموها في الوسط الصلب ولا سيما ضد البكتيريا الموجبة لصبغة غرام. كذلك فان الفعالية التثبيطية تزداد لمدى واسع ضد كل انواع البكتيريا المعزولة مع زيادة تركيز الراشح البكتيري.

كما واظهرت نتائج تنمية كل من خميرتي *Saccharomyces boulardii*, *Saccharomyces cerevisiae* في عدد من الاوساط الزرعية (الشاي ، السابروید، السابروید المدعم بمستخلص الخميره) امتلاك الخمائر المنماة في وسط السابروید المدعم بمستخلص الخميرة لافضل فعالية تثبيطية ضد البكتيريا المرضية ولا سيما بعد زيادة تراكيز رواشح الخميرة.

اظهرت نتائج مزج رواشح كل من بكتيريا حامض اللاكتيك *Lactobacillus plantarum* وخميرتي *Saccharomyces cerevisiae* و *Saccharomyces boulardii*مع المضاد الحيوي Ampicillin (كلاً على حدة) الحصول على فعالية تثبيطية عالية ضد *Staphylococcus epidermidis*(البكتيريا الاكثر عزلا من اصابات انسداد مجرى الدمع) مقارنة باستخدام كل من رواشح عزلة بكتيريا حامض اللاكتيك، او عزلة الخمائر او المضاد كلاً على حدة.

Supervisors Certification

We, certify that this thesis entitled " Study of the Causative Agents of the Infection of Lacrimal Drainage System" was prepared by " Heba Mansour Naser Al- Taee" under our supervision in the Department of Biotechnology/ College of Science/ Al- Nahrain University as a partial requirement for the degree of Master of Science in Biotechnology.

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We, the examining committee, certify that we have read this thesis and examined the student "Heba Mansour Naser Al-Taee" in its contents, and according to our opinion, is accepted for the degree of Master of Science in Biotechnology.

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Al-Nahrain University
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Study of the Causative Agents of the Infection of Lacrimal Drainage System

A Thesis
Submitted to the College of Science/ Al- Nahrain University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biotechnology.

By

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B. Sc., Biotechnology, College of Science, Al- Nahrain University.

September **2005**

بِسمِ اللهِ الرّحْمَنِ الرَحِيم

قَالُواْ سُبحَانَكَ لا عِلمَ لَنَا إلا مَا عَلَّمتَنَآ إنَّكَ أنتَ ألعَليمُ الحَكِيمُ

صَدقَ اللهُ العَظيم
سورة البقرة
(آية ٣١)

وزارة التعليم العالي
والبحث العلمـي
جامعة النهرين
كلية العلوم

دراسة المسببات الجرثومية لالتهاب

مجرى الدمع

رسالة
مقدمة الى كلية العلوم في جامعة النهرين
وهي جزء من متطلبات نيل درجة الماجستيرعلوم في علوم التقانةالاحيائية

من قبل

هبه منصور ناصر الطائي
بكلوريوس تقانة أحيائية- كلية العلوم- ٢٠٠٢

ايلول ٢٠٠٥

1.1 Introduction:

The lacrimal drainage system is miniature drainage network which prevents tear from continuously rolling down the cheeks (website 1). Obstruction of nasolacrimal duct is a common clinical problem (Paul and Shepherd, 1994), which is common in infants and elderly people (Munro and Compbell, 2000). The obstruction of nasolacrimal duct makes lacrimal sac easily infected by several microorganisms, and *Staphylococci* are the most predominant (Hartikianen *et al*, 1997).

Obstruction problem is increasing by appearance of bacterial strains resistant to antibiotics due to their over use. However new born penicillin drops can be applied (Stephen, 1984), moreover, topical antibiotics should be reserved for superadded bacterial infection (Kanski, 2003).

Recently a great interest is made to use microorganisms or their metabolites in treatment of some diseases. Bacteria and yeasts are considered as the two type of microorganisms most commonly used in this approach. Among bacterial groups, is *Lactobacillus plantarum*, which has a great role as probiotic, due to its presence in mucosal membrane of the intestine and digestive tract of human as normal flora(Bernet *et al*.,1994). It is safe when used in foods and possess ability to produce inhibitory materials .

Yeast such *Saccharomyces cerevisiae* has probiotic effect to inhibit the growth of undesired microorganisms (Jespersen, 2003),while *Saccharomyces boulardii* is another safe yeast used for

recurrent *Clostridium difficile* colitis, as well as against antibiotic associated diarrhea (Qamar *et al.*, 2001). For such purposes mixed antibiotics with probiotics(experimentally) were used despite that treatment of nasolacrimal duct obstruction infection is performed usually by antibiotics.

This study was aimed to :

1. Isolation of bacteria and other microorganisms (if presents) from infected lacrimal drainage system.
2. Identify the isolated microorganisms.
3. Detect the most predominant microorganisms during infective period.
4. Determine the prevalence of microorganism according to the age groups.
5. Determining the most effective antibiotics in treatment of lacrimal sac infections.
6. Study in vitro the inhibitory effect of some probiotics against isolated bacteria.

1.2 Review of Literature

1.2.1 Anatomy of Lacrimal System:

The lacrimal apparatus is composed of two parts, the secretory (lacrimal glands and ducts) and excretory includes (puncta, canaliculi, lacrimal sac and nasolacrimal duct) (Berens and Saunders, 1950) ,as figure in appendix (5).

Secretory apparatus consist of:

- The main lacrimal gland:

Is an exocrine gland located in the superior lateral quadrant of the orbit within lacrimal fossa (Liesegang *et al.*, 2002). It consists of larger orbital part and a smaller palpebral part (Last, 1984). The superior one about the size of small almond (Stephen, 1984).

- Accessory lacrimal glands:

Include gland of Krause and Wolfring (Liesegang *et al.*, 2002). Both main and accessory glands are serous (Last, 1984).

- Lacrimal ducts:

There are 10-12 ducts pass from lacrimal gland to open upon upper surface of the conjunctiva at the outer part of the upper fornix (Stephen, 1984).

Excretory apparatus consist of:

- Puncta:

Are located at the posterior edge of the lid margin about 6mm lateral to the inner canaliculus (kanski, 2003). Each lid has one punctum and one canaliculus (Stephen , 1984) .

- Canaliculus:

Passes from the punctum to the lacrimal sac (Stephen, 1984). The superior and inferior canaliculi most often unite to form common canaliculus which opens to the lateral wall of lacrimal sac

(kanski, 2003). There is a fold of mucosa called sinus of Maier that prevents tear reflux from lacrimal sac back into the canaliculus (Liesegang *et al.*, 2002).

- Lacrimal sac:

Lies in lacrimal fossa between anterior and posterior lacrimal crests (kanski, 2003). The lower end is narrowed as it opens into nasolacrimal duct (Stephen, 1984).

The lacrimal sac subjected to inflammation, either acute or chronic (Berens and Saunders, 1950).

- Nasolacrimal duct:

It's about 12 mm long and it is the inferior continuation of the lacrimal sac (kanski, 2003). It passes downward and backward to open at the anterior part of inferior meatus of nose (Stephen, 1984).

1.2.2 Physiology of Lacrimal System:

The lacrimal secretion is slightly alkaline fluid containing sodium chloride as its chief constituent (Stephen, 1984). The tear courses from the lacrimal gland in the upper portion of each orbit across the surface of the eye to empty via the lacrimal duct into the nose, (Ganong, 1991). The ordinary amount of tear secreted is just sufficient to moisten the eye ball, and is lost by evaporation (Stephen, 1984). Blinking keep the cornea moist (Ganong, 1991). So, the tears evaporate or pass into the nose as fast as they produced (Tortora *et al.*, 1986).

Tear film consists of (Liesegang *et al.*, 2002):

- Inner layer (mucin) provided by goblet cell within conjunctiva.
- Intermediate aqueous layer secreted by lacrimal gland.
- Oily outer layer produced by Meibomian gland.

1.2.3 Nasolacrimal Duct Obstruction:

Is an annoying and sometimes an eye threatening ophthalmic problem (Hartikainen *et al.*, 1997).

The major causes:

A- Congenital:

Is a common problem in infant (Robb, 2001) due to failure of canalization of the lower end of the duct (kanski, 2003),and may be present in several children in same family (Traquair, 1941). It usually resolves spontaneously, epiphora may be constant or intermittent, and the pressure over lacrimal sac causes reflux of purulent discharge (kanski, 2003).

The treatment was done by massage of lacrimal sac by placing the index finger over the common canaliculus to block the reflux through the punctum and then massage firmly downward or by probing the lacrimal system, which should be delayed until the age 12 months, because spontaneous canalization may occur in 95% (kanski, 2003), the passage should be syringed with normal saline after probing gently (Traquair, 1941).

B-Acquired:

1- Idiopathic:

In majority of cases the etiology is not known (Miller, 1982).

2- Obstruction due to intranasal diseases:

Such as infiltration by nasopharyngeal tumor (kanski, 2003).

3- Due to neighboring bone injury (Traquair, 1941).

The nasolacrimal duct obstruction convert the lacrimal sac into a stagnant pool which become infected (Dacryocystitis) (Hartikainen *et al.*, 1997).

1.2.4 Dacryocystitis:

It is inflammation of lacrimal sac. It is either acute or chronic infection and both forms are usually unilateral in nature (McEwen, 1997).

Acute Dacryocystitis:

Presented with pain, tenderness, swelling, erythema, in the area of lacrimal sac and tearing (epiphora)(Behrman and Kliegman,1994). Pressure over the swelling cause fluid to regurgitates through the puncta or more rarely through the nose (Stephen, 1984).

Treatment (kanski, 2003):

- Application of local warmth and oral antibiotic such as fluxacillin.
- Incision and drainage if there is abcess in the lacrimal sac.

Chronic dacryocystitis:

Presented with epiphora and painless swelling, pressure over it cause reflux of mucopurulent material through canaliculi (kanski,2003).

Treatment:

- Dacryocystoplasty by using catheter with ballon diameter 3 mm (Janssen *et al.*, 1997).
- Dacryocystorhinostomy (DCR): by making of permanent opening between nose and sac (Berens and Saunders, 1950).

1.2.5 The Ocular Defense Mechanisms:

The eye has a number of defense mechanisms. The eye lashes prevent entry of foreign material into the eye. The lids blink 15 to 20 times per minute, during which time secretions of the lacrimal glands and goblet cells wash away bacteria and foreign bodies (Forbes *et al.*, 2002). Also the eye resists microbial attack through a variety of innate (non specific) and adaptive (specific) immune responses (Friedlaender, 1993).

The ocular innate immune system is composed of anatomic

barriers (bone orbit), physical barriers (mucous membranes), chemical barrier (lysozyme and lactoferrin), blood proteins (complement), and phagocytic cells, which include neutrophils and macrophages (Giese and Mondino, 2001). Inflammatory cells normally found in the conjunctiva take part in phagocytosis and in processing antigen for its elimination for the individual's immunologic memory (Friedlaender, 1993). The tears contain lysozyme, lactoferrin, and antibodies. Lysozyme, which attacks gram-positive bacteria, is a cationic low-molecular weight enzyme that reduce the local concentration of susceptible bacteria by assaulting the mucopeptides of their cell walls (Friedlaender, 1993). Lactoferrin is another major tear protein, it is iron-binding protein, affects bacterial growth by sequestering essential iron for bacterial growth (Micallef and Cuschieri, 2001). Lysozyme and lactoferrin act synergistically causing bacteriolysis in gram-negative bacteria (Micallef and Cuschieri, 2001).

While the specific immune response is composed of humoral immunity and cell mediated immunity. The humoral response is characterized by the production of antibodies (Giese and Mondino, 2001). Antibodies may be directed against gram-negative cell wall antigens and causing cell lysis. And this type requires the presence of complement. Against gram positive bacteria antibodies usually act as opsonins, coating the microorganisms and making them susceptible to phagocytosis (Friedlaender, 1993). The cell-mediated response is mediated by antigen specific T lymphocytes and various other non-specific immune cells (Giese and Mondino, 2001).

1.2.6 Microorganisms of Eye Infection:

The eye and its associates are uniquely predisposed to infection by various microorganisms, if barriers of defense mechanisms are broken by a penetrating injury or ulceration, infection may occur.

Infection can also reach the eye via the bloodstream from another site of infection.

The microorganisms infect the eye through many parts and cause diseases such as conjunctivitis, keratitis, endophthalmitis or other diseases of lacrimal apparatus. Number of bacteria can infect the eye, largely through conjunctiva (Tortora *et al.*, 1986). The major part in this study is infection in eye's lacrimal system. So many type of microorganisms can infect it such *Propionibacterium , Staphylococcus aureus , Staphylococcus epidermidis , Streptococcus pneumoniae , Streptococcus pyogenes , Corynebacterium diphtheriae , Haemophilus influenzae, Escherichia coli , Pseudomonas aeruginosa , Klebsiella pneumoniae, Proteus spp.*, and other , in addition to fungi such *Candida albicans* and *Curvulania spp. .*

Major related microorganisms to eye infection will be discussed in some detail below.

1.2.6.1 Genus *Staphylococci*:

Staphylococci are gram-positive cocci that occur in single, pairs, tetrads, short chains, and irregular "grape like" clusters. They belong to family Micrococcaceae (Kloos and Jorgensen, 1985). Members of this genus are facultative anaerobic (Harley and Prescott, 1996). They are non motile, non spore forming, catalase positive and are unencapsulated or have limited capsule (Kloos and Bannerman, 1999). They are divided into two groups depending on their ability to produce coagulase enzyme, coagulase positive and coagulase negative staphylococci (Pinna *et al.*, 1999). They can cause intraocular infection either by contamination of the intraocular lens or direct intraocular inoculation.

Infections are associated with the production of virulent factors (Giese and Mondino, 2001). Their cell surface proteins (such as

protein A, collagen, fibronectin, and fibrinogen-binding proteins) promote adhesion to host cell (O'gara and Humphrey, 2001), while Joklik *et al*., (1992) described that proteases , lipases , esterases , and lyases aid in the establishment of the organism to the skin and mucous membranes of the host. Then Brooks *et al.,* (1998) mentioned that polysaccharides , peptidoglycan , and teichoic acids are their important virulent factors.

1.2.6.1.1 *Staphylococcus aureus:*

It is major cause of dacryocystitis and dacryoadenitis, conjunctivitis and Keratitis (Baron *et al.*, 1994). Fermentation of mannitol is the reaction that differentiates it from *Staphylococcus epidermidis*. The most convenient and reliable property for diagnostic purposes is the production of coagulase enzymes that causes the coagulation of plasma (Joklik *et al.*, 1992). While Forbes *et al*., (1998) pointed out that it could produce two types of coagulase, referred to as bound and free. This organism secretes alpha, beta, gamma, and delta toxins that attack host cell membranes and mediate cell destruction (Baron *et al*., 1994).

1.2.6.1.2 *Staphylococcus epidermidis:*

It causes conjunctivitis, blepharitis, and dacryocystitis. It is positive for catalase and coagulase (Baron *et al.*, 1994). Virulent factors are the capsular polysaccharide and biofilm, which is a multilayered cell cluster, embedded in an extra cellular slime substance (Giese and Mondino, 2001). The exopolysaccharide enhances organism adhesion and provides mechanical barrier to antibiotics and host defense mechanisms. Propensity to acquire and disseminate antimicrobial resistance allows for survival in hospital setting (Forbes *et al.*, 2002).

1.2.6.2 Genus *Streptococci*:

The organism is gram positive, and catalase negative. It is most commonly encounted in infecting human (Forbes *et al.*, 2002). When these organism gains access to normally sterile site, it can cause life threatening infection.

Streptococci producing a wide variety of secondary metabolites. Many of these compounds have an important application in human medicine as antibacterial-antitumor agents. Two species belonged to streptococcus are important in nasolacrimal duct obstruction infection they are:

1.2.6.2.1 *Streptococcus pneumoniae*:

It is found in conjunctiva and infects the eye (Prescott *et al.*, 1990), it causes keratitis, conjunctivitis, orbital cellulitis (Baron *et al.*, 1994). It is a primary cause of bacterial pneumonia, meningitis and otitis media.

It is alpha - hemolysis and grows as pairs of cocci cells. It is gram positive, catalase negative and facultative aerobic or anaerobic in presence of CO_2 (Talaro and Talaro, 1996). Virulent factor of *Streptococcus pneumoniae* is capsular polysaccharide which play a major role in the establishment of infection, also it produce other factors that play a role in virulence include pneumolysin 0 , an oxygen - sensitive toxin that is cytolytic for cells, and aneuraminidase is an enzyme that degrades surface structures of host tissue. (Baron *et al.*, 1994).

Streptococcus pneumoniae diseases frequently follow a viral infection .While in the tissue *Streptococcus pneumoniae* multiplies and spread throughout the lymphatic or direct extension from the local site of infection or through blood stream causing bacteremia (Oski *et al.*, 1994).

1.2.6.2.2 *Streptococcus pyogenes:*

It causes eye infection and found in conjunctiva (Prescott *et al.*, 1990), and also causes orbital cellulitis (Baron *et al.*, 1994). It may also infect the lacrimal sac causing dacryocystitis (Hartikainen *et al.*, 1997).

Members of this species give beta-hemolytic. It produces various extracellular products, that probably enhances virulence such hyaluronidase which breaks down host cell connective tissue, streptokinase that dissolves clots, NADase , proteinases and others enzymes, while mucoid strains possess hyalurnic acid capsule that acts to inhibit phagocytosis.

1.2.6.3 *Corynebacterium diphtheriae:*

It is found in conjunctiva of the eye (Prescott *et al.*, 1990). The term diphtheroid has been used in medical bacteriology for gram positive rods that resemble and may be confused with *Corynebacterium diphtheriae*, it is presumably a species of the genus *Corynebacterium* (Coyle *et al.*, 1985). It is fastidious and grows slowly on enriched media (Rollins, 2000), they do not grow on MacConkey agar (Funke and Bernard, 1999). It is oxidase negative but catalase positive, and grows better at 37C^{o} (Brandenburg *et al.*, 1996).

Joussen *et al*., (2000) found that diphtheriod had been regarded as causative agents of serious ocular diseases.

Virulence factors of it characterized by producing diphtheria toxin, a potent exotoxin that destroy host cells (Baron *et al.*, 1994)

1.2.6.4 *Escherichia coli:*

It is found in conjunctiva of the eye (Prescott *et al.*, 1990), also it infects the lacrimal sac causing dacryocystitis (Shah *et al.*, 2001).It was isolated from feces in 1885, and named by German

bacteriologist T.Escherich. *Escherichia coli* is a common inhabitant of the large intestine of human and mammals .It also found in the guts of birds, reptiles, amphibians and insects. The bacteria are excreted in great number with feces (Pyatkin and Krivoshein,1987; Manges *et al.*,2001). It occurs singly or in pairs, capsules or microcapsules in many strains, gram negative, small rods, motile by peritrichous flagella or non motile, facultativly anaerobe, chemoorganotrophe, has both respiratory and a fermentative type of metabolism. It is catalase and methyl red positive, but negative to oxidase, vogas - proskauer and citrate. It reduces nitrate to nitrite (Harley and Prescott, 1996).

Virulence factors including production of endotoxin, capsule and pili that mediate attachment to host cell. (Baron *et al.*, 1994)

1.2.6.5 *Haemophilus influenzae:*

It causes conjunctivitis, endophthalmitis, cellulitis (Baron *et al.*, 1994) and dacryocystitis (Hartikainen *et al.*, 1997). It appears as coccobacillary or small rods, positive for (x) and (v) factors, non beta - hemolytic on rabbit blood agar , grows in presence 5-10 CO_2, and it can grow only when hemin (x factor) , NAD (V factor), thiamine , pantothenic acid , uracil, and cystene are provided by another organism or growth factor.

The mode of transmission is person to person spread by contaminated respiratory droplet.

The virulence factor for encapsulated strains, the capsule is antiphagocytic, other cell envelope factors also facilitate attachment to host cells. While noncapsulated strains, pilli and other cell surface factors, not fully understood, play a role in attachment to host cells (Forbes *et al.*, 2002)

1.2.6.6 *Listeria grayi:*

It is catalase positive, gram positive non spore forming rods, it usually grows on blood, chocolate , nutrient agar and thioglycollate broth. It appears that virulence is mediated by action of at least three components. A phospholipase, hemolysin and lipopolysaccharide. (Baron *et al.*, 1994). Also cytotoxic toxin may allow for survival within phagocytes (Forbes *et al.*, 2002).

1.2.6.7 *Propionibacterium spp.:*

It causes canaliculitis, lacrimal duct infection (Baron *et al.*, 1994) and dacryocystitis (Hartikainen *et al.*, 1997). It is anaerobic diphtheroids, irregular bacilli, pleomorphic, often club shaped and catalase positive, non motile, gram positive.

Some species produce lipase which causes release of free fatty acids from sebum triglycerides, these are irritating and comedogenic, while other produces several enzymes including hyaluronidase, chondroitin sulfatase, neuraminidase and a cylneuraminic acid (Baron *et al.*, 1994).

1.2.6.8 *Morganella morganii:*

It causes dacryocystitis (Hartikainen *et al.*, 1997), it is gram negative rods that are common as normal stool flora, and also isolated from blood, urine of infected patients. It is positive to indole, urease and methyl red but negative to gelatinase and simmon citrate (Baron *et al.*, 1994)

Virulence factor is the cell wall lipopolysaccharide endotoxin, biological activity of this component, released during growth and breakdown of gram negative bacterial cell (Baron *et al.*, 1994).

1.2.6.9 *Pseudomonas aeruginosa*:

It infects the eye causing many diseases such as keratitis, endophthalmitis (Baron *et al.*, 1994) and dacryocystitis (Hartikainen

et al., 1997). It is one of the most common microorganism encounted in hospital infection. It was found in 1966 that 24% of patients carry *Pseudomonas aeruginosa* and 38%become carriers during their stay in hospitals. It is gram and indole negative, but simmon citrate, urease and methyl red positive.

It produces many virulence factors such exotoxin A that inhibits protein synthesis, production of several proteolytic enzymes, hemolysins that destroy cells and tissue, Pilli may mediate attachment to host cells, while some strains produce alginate, a polysaccharide polymer that inhibits phagocytosis.

Pseudomonas aeruginosa remains an opportunistic pathogen that requires compromised host defenses to establish infection. (Forbes *et al.*, 2002). Causes several diseases and infections such as severe epidemic diarrhea of infants, ocular infections, burn infection, folliculitis, urinary tract infections and others (Tortora *et al.*, 1986).

1.2.6.10 *Proteus mirabilis:*

Proteus is one of the important medical genera which retains to the family Enterobacteriaceae (Swierzko *et al.*, 2000). It infects the eye and found in conjunctiva (Prescott *et al.*, 1990). It also causes infection of the eye lid (Baron *et al.*, 1994), and may cause post-keratoplasty endophthalmitis(Lam *et al.*, 1998). It is gram negative rod, motile by peritrichus flagella, facultativly anaerobe, non-spore formers, non-capsulated, its odor is very strong (Cruickshank *et al.*, 1975). The most important feature which differentiated *Proteus* from other genera in the Enterobacteriaceae is swarming phenomena (Mobley and Belas, 1995). Most isolates are sensitive to aminoglycoside antibiotics (Myrvik and Weiser, 1988).Virulence factors including endotoxins, capsules adhesion proteins and resistance to multiple antimicrobial agents (Baron *et al.*,1994).

Table (1-1): Description of important bacterial species infecting eye (Kloss and Bannerman, 1999; Forbes *et al.*, 2002).

Bacterial species	Description
Staphylococcus epidermidis	Small to medium, translucent, gray-white colonies, most colonies nonhemolytic, slime producing strain are extremely sticky and adhere to the agar surface.
Staphylococcus aureus	Medium to large, smooth, entire, slightly raised, translucent, most colonies pigmented creamy yellow, most colonies beta-hemolytic.
Streptococcus pneumoniae	Small, gray, glistening, colonies tend to be dipdown in the center and resemble a doughnut (umbilicated) as the edge.
Streptococcus pyogenes	Grayish white, transparent to translucent matte or glossy, large zone of beta-hemolysis, large colony.
Corynebacterium diphtheriae	Ranging from small, gray, and translucent to medium, white and opaque.
Listeria grayi	Small, white, smooth, translucent, moist, beta-hemolytic.
Escherichia coli	Flat, dry, pink colonies, with a surrounding darker pink area of precipitated bile salts on MacConkey medium.
Proteus spp.	May swarm depending on the amount of agar in the medium, characteristic foul smell that on MacConkey agar.
Haemophilus influenzae	Uncapsulated strains are small, smooth and translucent, while encapsulated strain form larger, more mucoid colonies, mouse nest odor, nonhemolytic on rabbit or horse blood agar.
Pseudomonas aeruginosa	Spreading and flat, serrated edges, confluent growth, often shows metallic sheen, bluish-green, red or brown pigmentation, colonies often beta-hemolytic, grapelike or corn-taco like odor, mucoid colonies that on blood agar.

1.2.7 Antibiotics:

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

Tetracycline is a bacteriostatic agent which inhibits the growth of a wide range of gram positive and gram negative bacteria. Its effect is on the protein synthesis by binding to the 30 S ribosomal subunit and interfering with aminoacyl tRNA binding (Prescott *et al.*, 1990). All tetracyclines are readily absorbed from intestinal tract and distributed widely in tissue (Jawetz *et al.*, 1998).

Chopra (1985) found that the wide use of tetracycline resulted in the spread of bacterial resistance of most Enterobacteriaceae members. Bacterial resistance is due to decreasing cell permeability, altered ribosomal target site, and production of modifying enzymes that inactivate the drug (Joklik *et al.*, 1992).

The aminoglycosidic aminocyclitol antibiotics are bactericidal drugs. They are effective against many aerobic gram negative bacilli and some gram positive germs. Anaerobic organisms are resistant to aminoglycosides (Goth, 1984). They show some toxic effect when they are administered to patients (Goth, 1978).

Gentamicin is active against gram negative bacilli include *Pseudomonas aeruginosa*. It is also effective against pencillin resistant *Staphylococci* but inactive against anaerobic and *Streptococci* (Edwards *et al.*, 1995). It inhibits protein synthesis by binding to the 30 S ribosome subunit and cause misreading of mRNA (Prescott *et al.*, 1990).

Tobramycin is more active than gentamicin against *Pseudomonas aeruginosa* (Edwards *et al.*, 1995).

Amikacin has less intrinsic antibacterial activity than gentamicin (Edwards *et al.*, 1995). Amikacin is also used for gram negative

bacillary infection (Talaro and Talaro, 1996). It should be reserved for treatment of infection caused by these organisms (Edwards *et al.*, 1995). It is a chemically modified semi synthetic antibiotic and this chemical modification provides such antibiotic with resistance to the inactivating enzymes that are engaged in the destruction of the activity of gentamicin and tobramycin (Goth, 1984). This explains its broad spectrum of activity against organisms resistant to gentamicin and tobramycin (Joklik *et al.*, 1992).

Ampicillin is a semi synthetic pencillin which has a bactericidal action against both gram positive and gram negative. It affects the cell wall synthesis by inhibiting transpeptidation enzymes involved in the cross – linking of the polysaccharide chains of the bacterial cell wall peptidoglycan activate cell wall lytic enzymes (Prescott *et al.*, 1990). It is susceptible to degradation by Beta-lactamases and not well absorbed with food. There are a number of ampicillin esters including bacampicillin and pivampicillin, they are better absorbed and produce higher blood levels of ampicillin (Edwards *et al.*, 1995).

Vancomycin are glycopeptide bacterocidial antibiotic (Edwards *et al.*, 1995). It is a narrow-spectrum bactericidal antibiotic active against aerobic and anaerobic gram positive organism. Gram negative organisms are not completely affected by this drug (Yao and Moellering, 1999). It's most effective in the treatment of staphylococcal infection in cases of pencillin and methicllin resistance or in patients with the allergy to penicillins. It has also been chosen to treat *Clostridium* infections in children and endocarditis caused by *Enterococcus faecalis* since it is very toxic and hard to administer.

Vancomycin should be used only in the most serious life threatening conditions (Talaro and Talaro, 1996). It prevents cell wall formation at a site different from Beta-lactam antibiotics by acting at the second stage of cell wall synthesis, which is the

polymerization of the peptidoglycan polymer (Laurence *et al.*, 1997; Goth, 1984). Resistance emerges as a result of the binding affinity with this agent (Yao and Moellering, 1999).

Ciprofloxacin is most important of new fluroquinolones. It has a relatively broad spectrum with particularly high activity against aerobic gram negative bacilli including *Salmonella*, *Shigellae*, *Compylobacter* and *Pseudomonas*, it is also active against *Chlamydia* and *Mycoplasmas* but not against anaerobic bacteria. Gram positive bacteria are sensitive to it. It diffuses readily into infected tissues and cells (Edwards *et al.*, 1995).

These drugs exhibit several ideal treatment include potency and broad spectrum even in the minimal concentration. They are readily absorbed from the intestine and less subjected to the microbial resistance than other drug (Talaro and Talaro, 1996). It inhibits bacterial DNA thereby induce the formation of a relaxation complex analogue instead of super coiled one (Joklik *et al.*, 1992; Laurence *et al.*, 1997). Yao and Moellering (1999) attribute the resistance of this drug to alter DNA or to change the outer membrane porin protein which decreases permeability of this drug through the cell wall.

Chloramphenicol, which is originally isolated in the late 1940 from *Streptomyces venezuelae*, (Brooks *et al.*, 1998) is a potent broad spectrum antibiotic with a unique nitrobenzene structure (Talaro and Talaro, 1996). It is more effective than tetracycline against *Haemophilus influenzae*. It is principally a bacteristatic antibiotic which is effective against many gram positive and gram negative bacteria (Laurence *et al.*, 1997). It inhibits the protein synthesis by binding to 50 S ribosomal subunits, thereby tRNA molecules are not attached to the aminoacyl and peptidyl binding sites of the ribosome (Atlas, 1995). Consequently, Yao and Moellering, (1999) stated that transpeptidation process of peptide chain elongation is prevented. The resistance to this drug is

attributed to the production of acetyl transferase enzyme that can inactivate drug. (Atlas, 1995)

1.2.8 Probiotic:

Probiotic are living microorganisms that when ingested, have beneficial therapeutic effect (Surawicz, 2003).

The word probiotic has been known since the beginning of the nineties, and originally used by Lilley and Stillwell in (1965) for substance(s) that stimulates other microorganism growth. Several terms such as “friendly” “beneficial” “healthy” bacteria are also known for probiotics.

Available probiotics are characterized as non pathogenic, but even being microorganisms can infect when a patient is severely immunosuppressed, it has demonstrated an ability to prevent and treat some infections, effective use of probiotics could decrease patients exposure to antimicrobials (Elmer, 2001).

The probiotic mechanism of action may include receptor competition effects on mucine secretion or probiotics immunomodulation of gut associated lymphoid tissue (Madsen, 2001).

Probiotics compounds supplied by oral administration has been demonstrated to be well tolerated and safe (Madsen, 2001), and several *Lactobacillus spp.* are given in yogurt or as tablet or suppository forms have shown clinical efficacy as a treatment (Elmer, 2001).

Probiotic can influence intestinal physiology either directly or indirectly through modulation of the immune system, the result shown (Marteau *et al.*, 2001):

a- Good tolerance of yogurt compared with milk in subjects with primary and secondary lactose maldigestion.

b- Use of *Saccharomyces boulardii* and *Enterococcus faecium* to

prevent further recurrence of *Clostridium difficile* associated diarrhea.

c- Use of fermented milk containing *Lactobacillus rhamnosus* to shorten the duration of diarrhea in infants with rotavirus enteritis.

Effects that are otherwise suggested for diverse probiotics include alleviation of diarrhea of miscellaneous causes prophylaxis of gastrointestinal infections, which includes traveler's diarrhea and immunomodulation (Marteau *et al.*, 2001). Also probiotic reported to enhance the digestion and absorption of protein, fat, calcium and phosphorus.

1.2.9 Important Probiotic Microorganisms:

Microbial probiotics have a beneficial effect and not harmful to the host. All strains must have been studied comprehensively prior to use in humans or animals and thus are given GRAS (Generally Regarding As Safe).

The microorganisms characterized by pure, viable culture of a well identified microbial species (Casas and Dobrogosz, 2000), production of metabolites to inhibit or kill pathogen and efficient adherence to intestinal epithelial cells to reduce or prevent colonization of pathogens (Ried *et al.*, 1993).

Table (1-2) Summarized probiotics microorganisms and their safety status (Donohue and Salminen, 1996).

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

Major Probiotic microorganisms used:

1.2.9.1 Bacteria:

1.2.9.1.1 *Lactobacillus:*

This lactic acid bacterium can be used to potentially protect against pathogenic microorganisms (Gangemi *et al.*, 2001). It has common properties of gram positive, non spore forms, catalase negative, anaerobic or aerotolerant, fastidious bacteria and produces lactic acid as the main product of fermentation process (Holzapfel *et al.*, 1998).

It plays an essential role in the majority of food fermentation, and a wide variety of strains are routinely employed as starter cultures in the manufacture of dairy, meat, vegetable and bakery

products. It represents an original and attractive approach, as they are safe organisms that are used in food starters and probiotics (Grangette *et al.*, 2001). Lactobacilli can be used as adjuncts for flavor formation in cheese manufacture (Amarita *et al.*, 2001).

Lactobacilli are either homofermentative organism, convert glucose into 95% of lactic acid with minimum amount of carbon dioxide or heterofermentative bacteria produce 50% Lactic acid and other organic acids (Chakraborty, 1996). *Lactobacilli*, and specifically *Lactobacillus plantarum*, are an important group of microorganisms in ovine cheeses, even though they are not ordinarily included in the starter cultures added (Oneca *et al.*, 2003). As well as growth of spoilage and pathogenic bacteria in foods, is inhibited due to competition for nutrients and the presence of starter derived inhibitors such as lactic acid, hydrogen peroxide and bacteriocins (Abee *et al.*, 1995). The bacteriocins, is a heterogeneous group of proteinaceous antibiotics that often display a high degree of target specificity, although many have a very wide spectrum of activity (kalmokoff *et al.*, 1996). The mode of action was identified as bactericidal (Messi *et al.*, 2001). And the peptide bacteriocins are usually divided into lantibiotics and non lantibiotics (Eijsink *et al.*, 2002).

1.2.9.2 Yeasts:

They are unicellular, spherical, ellipsoidal or oval in shape, and usually (with the exception of candida) do not form hypha, that are about 5-10 times larger than bacteria (Harley and Prescott, 1996). They reproduce either asexually by budding and transverse division, or sexually through spore formation (Prescott *et al.*, 1990). They are eukaryotes and generally recognized as save status have made *Saccharomyces cerevisiae* a popular host for large scale industrial production of hetrologous proteins (Burden and Eveleigh,

1990; Romanos *et al.*, 1992). The yeast *Saccharomyces cerevisiae* grows well in a medium containing glucose as the sole source of carbon and energy and ammonia as the sole source of nitrogen (Magasanik, 2003). They involved in basically three groups of indigenous fermented products: non-alcholic starchy foods, alcholic beverages, and fermented milk (Jespersen, 2003). *Saccharomyces cerevisiae* is used for many diverse purposes and it has become one of the economically most important microorganism that are used in large scale biotechnological processes (Filkweert, 1999), it has bean used for centuries for the production of fermented food such as acider, wine, beer and bread (Hennequin *et al.*, 2001). *Saccharomyces cerevisiae* is mainly related to stimulation of lactic acid bacteria, improvement of nutritional value, probiotic effects, inhibition of undesired microorganisms and production of tissue – degrading enzymes may also be observed (Jespersen, 2003).

While *Saccharomyces boulardii*, now considered as strain of *Saccharomyces cerevisiae* (McCullough *et al.*, 1998), it is a nonpathogenic yeast that protects against antibiotic associated diarrhea and recurrent *Clostridium difficile* colitis (Qamar *et al.*, 2001), or it is associated with enteral or parenteral nutrition (Mcfarland *et al.*, 1996).

1.2.10 Synergistic Effect of Probiotics and Antibiotic:

Probiotics are defined as mono or mixed culture of live microbes that when applied to animal or human possess beneficial effect on health of the host, these beneficial effects include diseases treatment and prevention as well as improvement of nutrients digestion and absorption (Fuller, 1991). It assists in the establishment of intestinal population which is beneficial to animal and antagonist to harmful microbes (Green and Sainsbury, 2001).

While antibiotics which are used in the treatment but associated

with some side effect (Scultz *et al.*, 2003; Bergogne and Bereziu, 1995) such as toxicity, allergy, cancer, drug resistance and residues in food. Also the intensive antibiotics treatment notices a lack of poise of intestinal pathogenic flora in quantity and mostly in quality (Campeanu *et al.*, 2001).

Further clinical uses of probiotic were to hasten recovery from acute gastroenteritis in children and the prevention of antibiotic – associated diarrhea (Hatakka *et al.*, 2001).

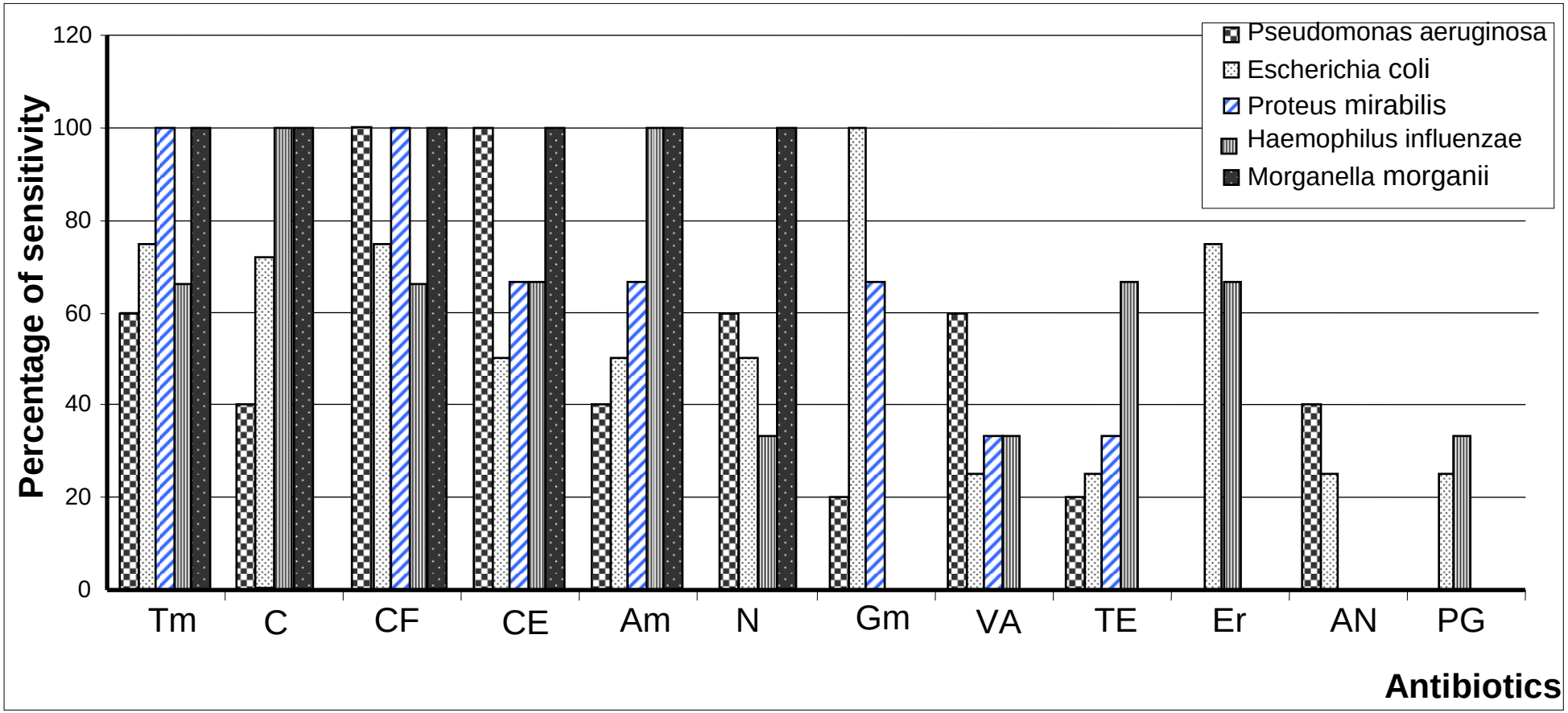


Figure (3-5): Susceptibility of Gram-negative Bacterial Isolates to Antibiotics.

Tm: Tobramycin.	N: Neomycin.	AN: Amikacin.
C: Chloramphenicol.	GM: Gentamicin.	PG: Pencillin G.
CF: Ciprofloxacin.	VA: Vancomycin.	
CE: Cefotaxime.	TE: Tetracycline.	
Am: Ampicillin.	Er: Erythromycin.	

3.1 Nasolacrimal Duct Obstruction (NLDO) Patients:

3.1.1 Distribution of (NLDO) Patients According to Gender:

From a total of 103 specimens suffering of nasolacrimal duct obstruction (NLDO), there were 61 (59.2%) female and 42 (40.8%) male. Bacterial growth was detected in 58 (95.1%) of female specimens, while only 2 (3.3%) showed no growth , in addition to one specimen (1.6%) was considered as contaminant. Regarding male specimens, bacterial growth appeared in 32 (76.2%) specimens, no growth in 6 (14.3%) specimens and 4(9.5%) were counted as contaminants table(3-1).

The relatively high percentage of female (NLDO) patients as compared with that of male patients may be due to contamination that occurs by using some types of eye cosmetics. In this regard, shah *et al.*, (2000) found that the higher incidence of eye infection which was detected in old females had been correlated with the excessive use of cosmetics.

3.1.2 Distribution of (NLDO) Patients According to the Age:

Results tabulated in table (3-1) show that most patients were infants and children of ages between 0-9 years with a percentage of 48.5% and the majority of them were under the age of 2 years. Moreover, nasolacrimal duct infection of this group recorded the highest percentage among all of other groups where it reached 52.2%. Such results are almost agreed with these of Robb (2001) who found that congenital nasolacrimal duct obstruction was a common problem among infants.

Adversely, few cases of (NLDO) were recorded in the age group

of 20 – 29 years. Moreover, age groups of 50 – 59, 40 – 49, 30 – 39 and 10 – 19 years come next to the 0 – 9 year group when the infection percentages by (NLDO) were27.8, 8.9, 5.6, and 3.3% respectively. In infants and young children, failure of canalization of the lower end of nasolacrimal duct is the causative factor of (NLDO) with secondary infection. While, weakness which is a property accompanied to elderly patients may be the cause of such high percentages of infection due to the weakness of muscles pump (Kanski ,2003).

Table (3-1): Distribution of (NLDO) patients according to gender, age and infection by nasolacrimal duct obstruction (NLDO):

Age group	Gender		Total No. of patients	Percentage of group (%)	Infected Patients		
	Male	Female			Number	percentage* (%)	percentage** (%)
(0 – 9)	27	23	50	48.5	47	52.2	94
(10 – 19)	2	3	5	4.9	3	3.3	60
(20 – 29)	2	2	4	3.9	2	2.2	50
(30 – 39)	4	3	7	6.8	5	5.6	71
(40 – 49)	5	4	9	8.7	8	8.9	88.8
(50 – 59)	2	26	28	27.2	25	27.8	89.2
Total	42 +	61	103	100	90	-	-

No.: Number.

* Percentages of infected patients were calculated according to the total number of infected patients.

** Percentages of infected patients were calculated according to the total number of patients at same age group.

3.2 Isolation and Identification of Bacterial Isolates:

Swabs specimens from eyes of patients suffering from nasolacrimal duct obstruction (NLDO) were cultivated on the most common and selective media specialized for each suspected bacterial genus and species. Then the bacterial isolates were primary identified according to their cultural and microscopically characteristics, while the final identification was performed throughout the biochemical tests table (3-2). Api system was also used to ensure identification of the isolates.

3.2.1 *Staphylococci spp.:*

When the NLDO specimen was cultured on blood agar incubated at 37C^{o} 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 considered 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 characteristic, gram staining showed gram positive cocci all grouped mainly in clusters which are the properties of *Staphylococcus*.

Results of biochemical tests performed on the isolates of *Staphylococci* showed that suspected *Staphylococcus aureus* isolates were positive to coagulase and catalase, but negative to oxidase test.

Furthermore, they were able to change the color of mannitol salt agar from red to yellow. *Staphylococcus epidermidis* isolates, on the other hand, were unable to change such color but negative to the coagulase test such biochemical characterization agreed with those stated by Harley and Prescott (1996).

Results of api Staph kit which are shown in the appendix (1) confirmed the previous conventional identification.

3.2.2 *Streptococci spp.:*

Microscopical examination of the gram stain smear taken from a suspected colony of *Streptococcus* grown on blood agar was gray and 2mm in diameter illustrated that cells of the isolate were gram positive cocci grouped mainly as short or long chain, however some colonies gave beta-hemolysis on the blood agar, which were suspected to be belonging to *Streptococcus pyogenes*, while other gave alpha-hemolytic which may belong to *Streptococcus peumoniae.*

Biochemical characteristics of all suspected *Streptococcus* isolates were catalase and oxidase negative. *Streptococcus pyogenes* was differentiated from *Streptococcus pneumoniae* where growth of the latter was inhibited by the optachin disc (optachin sensitivity), while the former *Streptococcus pyogenes* was unaffected.

Also they were subjected to api 20 Strept kit to be identified and ensure from it as in appendix (2)

3.2.3 *Haemophilus influenzae:*

Cultural examination showed that colonies of this species, after incubation on chocolate agar at 37C^{o} for 24 hrs, were small, smooth and moist. Furthermore, on the blood agar they did not exhibit any hemolytic types and did not grow on MacConkey agar.

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

The biochemical tests of the suspected isolates were oxidase positive and positive result for special requirement X, V factors.

3.2.4 *Escherichia coli:*

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

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

Final identification of *Escherichia coli* was achieved by the biochemical tests. It was negative to oxidase, urease, citrate utilization and gelatin liquefaction, but gave positive result for

catalase, indole, 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 kit. As shown in appendix (3)

3.2.5 *Proteus mirabilis*

On blood agar, colonies produced were characterized by swarming phenomena , while on MacConkey agar, they were small in size 1 mm in diameter and pale in color. Gram staining of the suspected isolates showing that cells were small gram negative rods found separately and near to each other. Forbes *et al.*, (2002) declared that such morphological and cultural properties were similar to those characterizations of *Proteus spp.*

In order to identify *Proteus mirabilis*, biochemical tests were performed. Results of such tests indicated that the suspected isolates were negative to oxidase, indole, citrate utilization, and ability to grow on (TSI) medium changing its surface and bottom color to yellow (Acid reaction) and produced CO_2 gas ,H_2S , while results were positive to urease, gelatin liquefaction.

Moreover, api 20E kits was also used to ensure the identification. As shown in the appendix (3).

3.2.6 *Morganella morganii:*

After incubation on blood agar, colonies were small in size, pale color.

Microscopical examination of suspected colony were gram negative small rod distribution on the slide .While the biochemical tests of the suspected isolates were positive to urease, indole, catalase and negative for oxidase, gelatin liquefaction, citrate utilization. Such cultural and microscopical characteristics agreed with those of Prescott *et al.*, (1990).

Also api 20E kit used to ensure their diagnosis as shown in appendix (3).

3.2.7 *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 size (3-5) mm in diameter.

Microscopical examination of suspected colony illustrated cells were small gram negative rods found separately but closed to each other. Such cultural and morphological characteristics are similar to those of *Pseudomonas aeruginosa* (Brooks *et al.*, 1998).

Biochemical characterizations of the suspected isolates shown that they were positive to oxidase, catalase, gelatin liquefaction, citrate utilization and able to grow on (TSI) fermenting its surface and changing its color from red to yellow. They were, on the other hand, negative to indole as well as CO_2 and H_2S production. Such biochemical results are the characterization of *Pseudomonas aeruginosa* (Collee *et al.*, 1996) api 20E kit system used to confirm its results shown in the appendix (3).

3.2.8 *Corynebacterium diphtheriae:*

After culturing on blood agar colonies appeared gray in color, size (0.5-1) mm in diameter, while unable to grow on MacConkey agar.

Microscopical examination of suspected colony appeared small,club shaped ends, gram positive and arrangement as Chinese letter and also show distinctive granules. The isolates of *Corynebacterium diphtheriae* were subjected to various biochemical tests and gave negative result for oxidase, gelatin liquefaction, urease but positive for catalase.

The suspected isolate subjected to api Coryne kit system, results shown in the appendix (4).

3.2.9 *Listeria grayi :*

After the incubation period, colony on the blood agar appeared small, white and producing alpha-hemolysis.

While microscopical examination of the suspected isolates showing that cells were slender, rod shaped, bacilli with narrow end, gram positive and occurring singly and in chains. Such cultural and morphological characteristic are similar to those of *Listeria grayi* that agreed with Cruickshank *et al.*, (1975).

Biochemical tests done to identify the suspected isolates, shown that they were positive for catalase and negative for urease and gelatin liquefaction. Also the suspected isolates subjected to api Coryne system, as shown in appendix (4).

3.2.10 *Propionibacterium spp.:*

Cultural examination shown that colonies of this species, after incubation on blood agar were small (1-2) mm in diameter, convex, creamy to yellow in color. Furthermore produce alpha-hemolytic on blood agar. Moreover, under the oil immersion of compound light microscope, smear of the suspected species appeared as club shape with narrow end and gram positive similar to *Corynebacterium.*

The suspected isolates subjected to various biochemical tests, that they were positive for catalase, oxidase, indole and gelatin liquefaction, but negative to urease.

Table (3-2) Biochemical characterizations of the bacterial isolates obtained form patients suffering from nasolacrimal duct obstruction (NLDO).

Species	Catalase	Oxidase	Urease`	Indole	Gelatin liquefacti-on	Citrate utilization	TSI	CO_2	H_2S	Mannitol	Coagulase	X	V	Optachin
Staphylococc-us epidermidis	+	–	N	N	N	N	N	N	N	+	–	N	N	N
Staphylococ-cus aureus	+	–	N	N	N	N	N	N	N	+	+	N	N	N
Streptococcu-s pneumoniae	–	–	N	N	N	N	N	N	N	N	N	N	N	+
Streptococ-cus pyogenes	–	–	N	N	N	N	N	N	N	N	N	N	N	–
Escherichia coli	+	–	–	+	–	–	A/A	+	–	N	N	N	N	N
Proteus mirabilis	+	–	+	–	+	–	A/A	+	+	N	N	N	N	N
Morganella morganii	+	–	+	+	–	–	A1K/A	+	–	N	N	N	N	N
Pseudomon-as aeruginosa	+	+	+	–	+	+	A1K/A	–	–	N	N	N	N	N
Corynebact-erium diphtheriae	+	–	–	N	–	N	N	N	N	N	N	N	N	N
Listeria grayi	+	–	–	N	–	N	N	N	N	N	N	N	N	N
Propionibac-teruim spp.	+	+	-	+	+	N	N	N	N	N	N	N	N	N
Haemophilus influenzae	N	+	N	N	N	N	N	N	N	N	N	+	+	N

N : no test

(+): positive result (-) : negative result
(ALK): alkaline reaction (A): acid reaction

3.3 Occurrence of Bacterial Isolates in NLDO Patients:

Results of the isolated bacterial species in table (3-3), showed that *Staphylococcus epidermidis* was the most frequent bacteria isolated from patients suffering from (NLDO), with percentage of isolation (28.9%). Several investigator found almost similar findings, Todres and Fugate (1996) mentioned that this bacteria presents at any where infant found in contact with as mother, nurses and towels, Hartikainen *et al.*, (1997) stated that most frequent cultured bacteria of NLDO is belonged to *Staphylococcus epidermidis* . This bacterium was found also by Tortora *et al.*, (1986) as the most common bacteria associated with eye infection. Infection of this bacteria caused by 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).

The table also shows that *Staphylococcus aureus* was the second predominant bacteria with a percentage of 18.9%. It is found usually in the environment of new borns where tears has no longer possess immunological defense against this microorganisms. In addition, periorbital lymphatic tissue is not developed completely to be effective against this bacterium (Friedlaender, 1993).

From the above results, genus *Staphylococcus* which is represented by *Staphylococcus epidermidis* and *Staphylococcus aureus* was represented by highest percentage 47.8% of total bacterial isolates obtained from patients of nasolacrimal duct obstruction included in this study.

On the other hand, *Streptococcus pneumoniae* came as the third bacteria when its occurrence in the eye patients formed 11.1%. However, such result was different from that obtained by Kuchar *et al.*, (2000) who found that this bacterium was the most frequently cultured bacteria associated with the infection of nasolacrimal duct. Another species of *Streptococcus* are *Streptococcus pyogenes* was isolated in a percentage of 10%.

The total occurrence of both species of *Streptococcus* genus (*Streptococcus pneumoniae* and *Streptococcus pyogenes*) was reached 21.1% making the isolates of such genus just after the most frequent genus *Staphylococci*.

Regarding *Corynebacterium diphtheriae* , only 6 isolates were obtained which form 6.8%, such percentage is nearly from that obtained by Brooks and Frazier (1998) when they found 6.7% of total isolate from NLDO patients.

Other bacterial species (*Pseudomonas aeruginosa*, *Escherichia coli, Propionibacterium spp., Proteus mirabilis, Haemophilus influenzae, Listeria grayi* and *Morganella morganii*) were represented by lower percentages 5.6, 4.4, 4.4, 3.3, 3.3, 2.2, and 1.1%, respectively. The previously mentioned bacterial distributed in all age groups. On the other hand, 8 patients' specimens were free of any bacterial growth. Such finding may be related to the use of antibiotics by the patients, or due to the epiphora without obstruction that attributes to malposition of eye lid making the puncta not parallel to the direction of tear drainage without infection, and that agreed with Stephen (1984).The remaining 5 specimens were contaminated which were avoided.

Table (3-3): Numbers and percentages of bacterial isolates detected in 103 patients of nasolacrimal duct obstruction (NLDO).

Bacterial species	Number	Percentage(%)
Staphylococcus epidermidis	26	28.9
Staphylococcus aureus	17	18.9
Streptococcus pneumoniae	10	11.1
Streptococcus pyogenes	9	10
Corynebacterium diphtheriae	6	6.8
Pseudomonas aeruginosa	5	5.6
Escherichia coli	4	4.4
Propionibacterium spp.	4	4.4
Proteus mirabilis	3	3.3
Haemophilus influenzae	3	3.3
Listeria grayi	2	2.2
Morganella morganii	1	1.1
Total	90	100
No growth	8	
Contaminated	5	
Total specimens	103	

3.4 Antibiotics Sensitivity of (NLDO) Bacterial Isolates:

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

Standard discs diffusion method was used to detect the susceptibility of pathogenic bacteria for antibiotic.

3.4.1 Susceptibility of *Staphylococcus:*

Results in figure (3-1) declared that the 43 isolates of *Staphylococci spp.* (26 *Staphylococcus epidermidis*, 17 *Staphylococcus aureus*) were sensitive to most of the 12 antibiotics used in the study. *Staphylococcus epidermidis* isolates were highly sensitive to each of vancomycin (92.3%), ciprofloxacin (88.5%), neomycin, amikacin (84.6%) for each and chloramphenicol (80.8%). Other antibiotics were less effective against *Staphylococcus epidermidis* when the percentages of sensitivity ranged from 57.7%for cephotaxime to 7.7% for ampicillin: which also means that most of isolates of this species were highly resistant to ampicillin. Such high percentage of resistance may be related to the extensive use or misuse of antibiotics.

Isolates of *Staphylococcus aureus* were also sensitive to each of vancomycin with a percentage of 94.1%, ciprofloxacin 82.4%, neomycin 82.4%, and chloramphenicol 76.5%. Percentages of

sensitivity were decreased for this species isolates toward the remaining antibiotics used. However, most of *Staphylococcus aureus* isolates were high resistant for both pencillin G and ampicillin when the percentages of sensitivity were only 10% and 5.9% , respectively.

Results in above declared that such two species of *Staphylococcus* could be treated efficiently by antibiotics : Vancomycin, ciprofloxacin, amikacin, neomycin, and chloramphenicol.

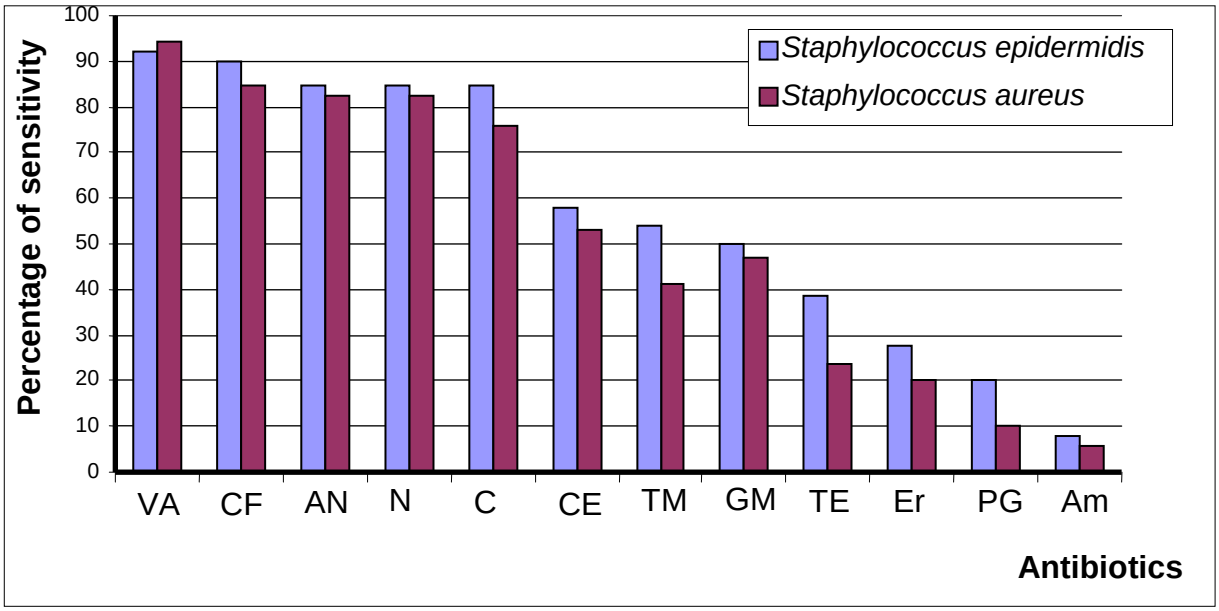


Figure (3-1) : Susceptibility of *Staphylococcus spp.* Isolated from Nasolacrimal Duct Obstruction patients to Antibiotics.

VA: Vancomycin	CE: Cephotaxime	PG: Pencillin G
CF: Ciprofloxacin	TM: Tobramycin	AM: Ampicillin
AN: Amikacin	GM: Gentamicin	
N: Neomycin	TE: Tetracycline	
C: Chloramphenicol	ER: Erythromycin	

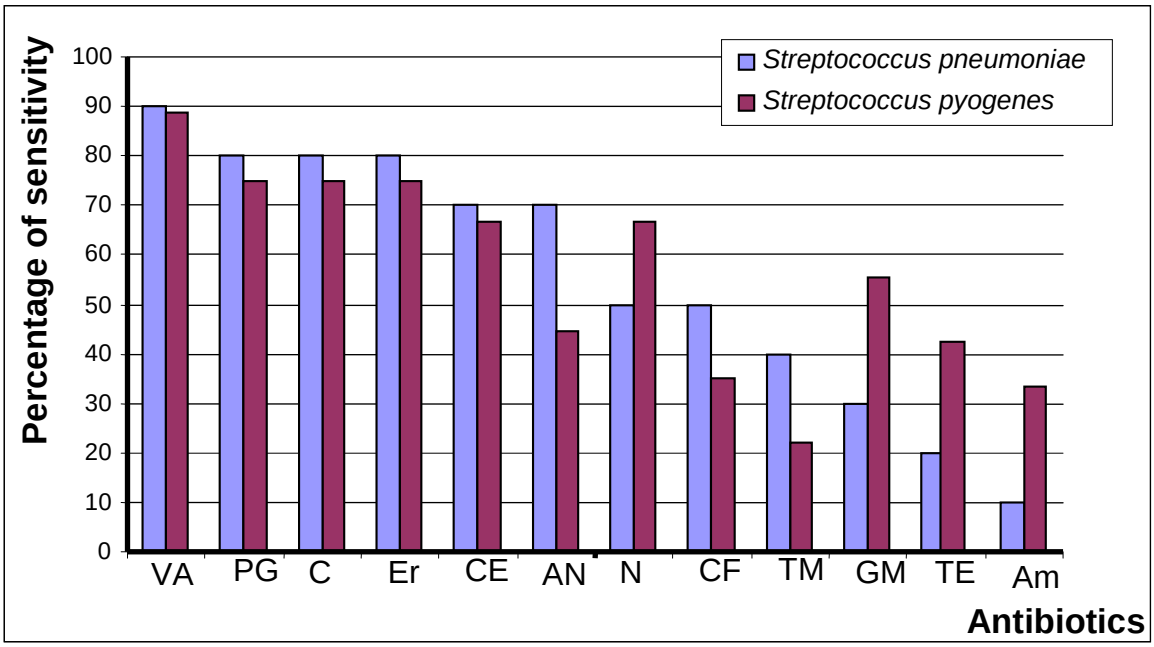
3.4.2 Susceptibility of *Streptococcus*:

Figure (3-2) shows the result of susceptibility of 19 isolates of *Streptococcus spp.* (10 *Streptococcus pneumoniae.* And 9 *Streptococcus pyogenes*) to the antibiotics used.

Vancomycin was the most effective antibiotics against both species of *Streptococcus*. When the sensitivity percentages reached 90% of the *Streptococcus pneumoniae* isolates and 88.9% of *Streptococcus pyogenes* isolates. Pencillin G, erythromycin , and cephotaxime come next, respectively, to vancomycin in their effects on the isolates of both *Streptococci* species, while most of the antibiotics used in the study had more effects on isolates of *Streptococcus pneumoniae,* neomycin, gentamicin, tetracycline and ampicillin were more effective on *Streptococcus pneumoniae* when percentage of isolates sensitivity were 50, 30, 20 and 10% respectively compared to 66.7, 55.6, 44.4 and 33.3% of *Streptococcus pyogenes* isolates, respectively.

Also from the above figure (3-2) it could be concluded that *Streptococcus pneumoniae* was the most resistant *Streptococci* isolates to ampicillin 10% and *Streptococcus pyogenes* isolates were mostly resistant to tobramycin 22.2 %.

Depending on such finding, vancomycin, pencillin G, chloramphenicol, erythromycin and cephotaxime had a similar pattern of effect on the isolates of both species of *Streptococcus*, and may be considered as the medicine of choice for such bacteria.



Figure(3-2): Susceptibility of *Streptococcus spp.* Isolated from Nasolacrimal Duct Obstruction to Antibiotics.

VA: Vancomycin	AN: Amikacin	TE: Tetracycline
PG: Pencillin G	N: Neomycin	AM: Ampicillin
C: Chloramphenicol	CF: Ciprofloxacin	
ER: Erythromycin	TM: Tobramycin	
CE: Cephotaxime	GM: Gentamicin	

3.4.3 Susceptibility of *Corynebacterium diphtheriae* and *Listeria grayi*:

Results of antibiotic susceptibility of 6 isolates of *Corynebacterium diphtheriae* indicate that most of the isolates were sensitive totally (100%) to both vancomycin and cephotaxime figure(3-3). Other antibiotics were less effective against *Corynebacterium diphtheriae* isolates coming next to the previous two antibiotics, where tobramycin and amikacin with percentages of isolates sensitivity of 83.3% and 83.3%. Other antibiotics were

less in their effects on the *Corynebacterium diphtheriae*, with isolates sensitivity percentages ranging from 66.7% for tetracycline down to only 33.3% for pencillin G.

Regarding *Listeria grayi*, Ciprofloxacin were the most effective antibiotics when all isolates were killed, which mean (100%) sensitivity. However, lowest sensitivity percentage for *Listeria grayi* isolates was 0% which recorded toward erythromycin.

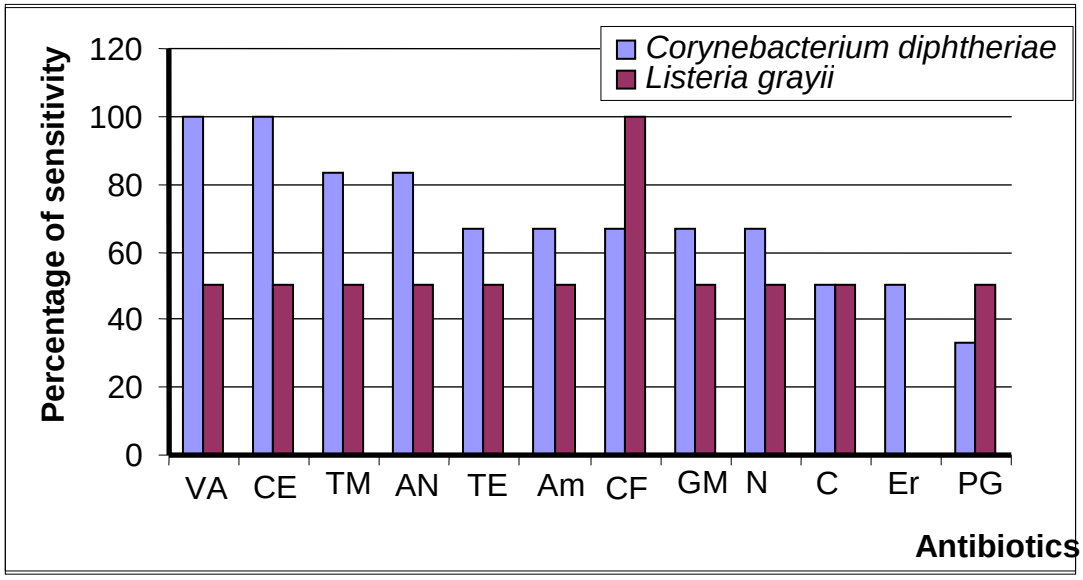


Figure (3-3):Susceptibility of *Corynebacterium diphtheriae* and *Listeria grayi* Isolated from Nasolacrimal Duct Obstruction to Antibiotics.

VA: Vancomycin	AM: Ampicillin	Er: Erythromycin
CE: Cephotaxime	GM: Gentamicin	PG: Pencillin G
TM: Tobramycin	CF: Ciprofloxacin	
AN: Amikacin	N: Neomycin	
TE: Tetracycline	C: Chloramphenicol	

3.4.4 Susceptibility of *Propionibacterium spp*.:

Results in figure (3-4) illustrate the susceptibility of 4 isolates of *Propionibacterium spp.* to the 12 antibiotics used in this study. All isolates were sensitive to neomycin (100%), while they varied against other 11 antibiotics. However, the isolates were highly sensitive toward vancomycin with a percentage of 75% and each of cephotaxime, amikacin, ciprofloxacin, chloramphenicol and erythromycin also with percentages of 75% for each one.

Adversely, the isolates of *Propionibacterium spp.* were mainly resistant to ampicillin, gentamicin, and pencillin G with percentage of sensitivity 25% for each of the 3 antibiotics.

This resistance may belong to the randomly using of these three antibiotics by patients.

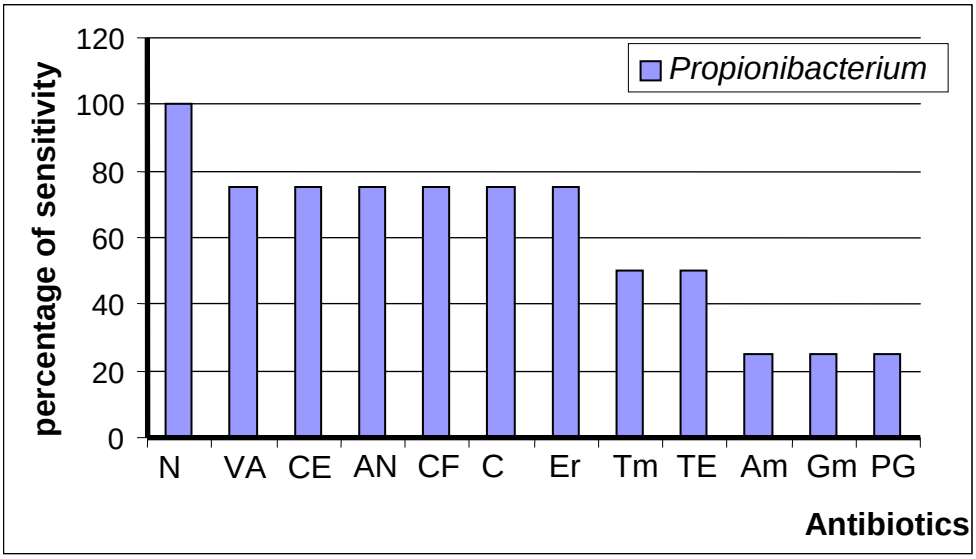


Figure (3-4): Susceptibility of *Propionibacterium spp.* isolated from Nasolacrimal Duct Obstruction to Antibiotics:

N: Neomycin	C: Chloramphenicol	GM: Gentamicin
VA: Vancomycin	ER: Erythromycin	PG: Pencillin G
CE: Cephotaxime	TE: Tetracycline	
AN: Amikacin	TM: Tobramycin	
CF: Ciprofloxacin	AM: Ampicillin	

3.4.5 Susceptibility of Gram-negative Bacteria:

Figure (3-5) shows susceptibility of bacterial isolates belonging to the species of *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Haemophilus influenzae* and *Morganella morganii* to antibiotics.

Pseudomonas aeruginosa was sensitive to cephotaxime when none of its 5 isolates were able to resist this antibiotic, this bacteria was also sensitive (but in less degree) to antibiotics vancomycin, neomycin, and tobramycin with percentages of 60% for each. On the other side, all *Pseudomonas aeruginosa* isolates totally resisted (100%) to antibiotics pencillin G and erythromycin.

Regarding, *Escherichia coli*, all of its 4 isolates, were sensitive to gentamicin, while 3 of them (75%) were sensitive to each of tobramycin, chloramphenicol, ciprofloxacin, and erythromycin. Susceptibility of the *Escherichia coli* isolates against other seven antibiotics varied until only one isolates (25%) was sensitive to each of amikacin, tetracycline, vancomycin, and pencillin G.

As noticed in *Proteus mirabilis*, that all isolates were sensitive to each of tobramycin, ciprofloxacin at percentage 100% for each. While 2 isolates of them (66.6%) were sensitive to each of ampicillin, gentamicin and cephotaxime. On the other hand, all isolates were totally (100%) resistant to antibiotics such amikacin, neomycin, chloramphenicol, pencillin G, and erythromycin.

Results of antibiotics susceptibility of *Haemophilus influenzae*, appeared all of its 3 isolates of *Haemophilus influenzae*, were sensitive to chloramphenicol and ampicillin. While 2 of them (66.6%) were sensitive to each of tetracycline, tobramycin,

cephotaxime, ciprofloxacin, and erythromycin. Susceptibility of the *Haemophilus influenzae* isolates against other antibiotics varied until only 1 isolates (33.3%) was sensitive to each of vancomycin, neomycin and pencillin G, until reached to all isolates of *Haemophilus influenzae* was totally (100%) resistant to antibiotics amikacin and gentamicin.

While antibiotics sensitivity of *Morganella morganii*, noticed that its 1 isolate was sensitive (100%) to each of ciprofloxacin, chloramphenicol, cephotaxime, tobramycin, neomycin and ampicillin. On the other hand this isolate was not affected by other remaining antibiotics.

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Depending on the previous results regarding susceptibility of nasolacrimal duct obstruction. (NLDO) bacterial isolates to the 12 antibiotics used, ciprofloxacin was the most effective antibiotic among them. Ciprofloxacin is known to possess good and readily diffusing ability into infected cells and tissues (Edwards *et al.*, 1995). Results also declared that the antibiotics were effective against both gram negative and gram positive bacteria especially isolates of *Streptococcus pneumonia*.

Adversely, the NLDO isolates were more resistance to antibiotics tetracycline, pencillin G, with a highest resistance appears against tetracycline, due to its common and extensive use. Resistance of a bacterial strain to tetracycline may belong to the presence of B-lactam ring making such strain an active to the tetracycline group. (Weinrich and Bene, 1976; Nishine and Nakazawa, 1976; Epstein *et al.*, 1997; Hoiby *et al.*, 2000). Moreover, presence of resistant plasmid and its ability to transfer from one bacterium to another may attribute for making sensitive bacteria a resistance one, which lead finally to difficulty in treating such resistance (Watanakunakorn, 1982; Lyon and Skurray, 1987; Karawaya and Bobo, 1990; Falace *et al.*, 1999).

Edwards *et al.*, (1995) contributed the resistance of some bacterial isolates to ampicillin due to degradation of the B-lactam ring by the enzyme B-lactamase of such isolate.

3.5 Inhibitory Effect of LAB against (NLDO) Bacterial Isolates:

3.5.1 On Solid Medium:

Inhibitory activity of *Lactobacillus plantarum* grown on MRS agar medium was tested against bacterial isolated from (NLDO) patients.

Result show that this bacterium possessed highly inhibitory activity against all pathogenic bacterial isolates, especially gram positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Corynebacterium diphtheriae* …) then gram negative bacteria. In this regard, Jimenez-Diaz *et al.*, (1993) found that *Lactobacillus plantarum* excreted good inhibitory effect only on gram-positive bacteria such as *Clostridium*, while Lewus *et al.*, (1991) found this LAB bacterium effective on *Listeria*. On the other side, Nitagu and Gashe (1994) pointed out that all isolates of *Lactobacillus plantarum* obtained from fermented foods had inhibitory effect on gram-negative bacteria, including *Pseudomonas*, *Escherichia coli*, *Proteus* and *Salmonella*.

3.5.2 In Liquid Medium:

Inhibitory activity of *Lactobacillus plantarum* grown in MRS broth medium was tested against bacterial isolates from (NLDO) patients.

Results of table (3-4) show that the inhibitory effect of *Lactobacillus plantarum* filtrates was different against (NLDO) bacterial species. The highly affected bacteria for all concentration

were *Listeria grayi*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Corynebacterium diphtheriae*. While other bacteria were only affected by the second and third concentration they were *Propionibacterium spp.*, *Streptococcus pneumoniae* and *Streptococcus pyogenes*. The other remaining bacteria (*Escherichia coli*, *Proteus mirabilis*, *Haemophilus influenzae* and *Morganella morganii*) have been affected by the third concentration only.

Such results agreed with those of Gupta *et al.*, (1998) who found that the filtrate of lactic acid bacteria grown in MRS broth medium had high inhibitory effect against both gram positive and gram negative bacteria studied. Al-Kassab and Al-Khfaji (1992) pointed out that production of inhibitory substance from lactic acid bacteria is attributed to its growth in MRS medium. Also the production of inhibited materials by *lactobacillus* is dependent on the medium used for growth, also that tween 80 induced the production of protein by increasing the production activity of bacteria.

Table (3-4):Inhibition zones (mm) caused by filtrate of *Lactobacillus plantarum* grown in MRS broth medium against bacterial isolates of (NLDO) patients.

Bacterial isolate	Inhibition zones (mm) of LAB filtrates		
	First concentration 0.36 mg/ml	Second concentration 1.10 mg/ml	Third concentration 3.33 mg/ml
Staphylococcus epidermidis	12	14	18
Staphylococcus aureus	10	19	20
Streptococcus pneumoniae	-	13	15
Streptococcus pyogenes	-	10	12
Escherichia coli	-	-	10
Proteus mirabilis	-	-	8
Corynebacterium diphtheriae	9	14	18
Haemophilus influenzae	-	-	6
Listeria grayi	17	20	23
Pseudomonas aeruginosa	10	17	19
Morganella morganii	-	-	7
Propionibacterium spp.	-	12	16

(-) No inhibition zone.

3.6 Inhibitory Effect of Yeasts against (NLDO) Bacterial Isolates:

3.6.1 On Solid Medium:

Inhibitory effect of yeasts (*Saccharomyces cerevisiae and Saccharomyces boulardii*) grown on sabouraud agar was tested against bacterial isolates of (NLDO) patients.

Results of table (3-5) declare that inhibitory activity of yeasts against bacterial isolates was different, considerable effect recorded by both yeasts (*Saccharomyces cerevisiae and Saccharomyces boulardii*) against *Listeria grayi*, *Proteus mirabilis* and *Staphylococcus epidermidis* bacterial isolates. Other bacterial species were also affected but depending on the type of yeast, for instance, *Saccharomyces cerevisiae* exhibited good inhibitory effect against *Haemophilus influenzae* and *Staphylococcus aureus*, while *Escherichia coli*, *Propionibacterium spp.* and *Streptococcus pneumoniae* were affected only by filtrate of *Saccharomyces boulardii*. Other bacteria (*Pseudomonas aeruginosa* and *Morganella morganii*) were not affected by any type of the both yeasts.

From the above findings, it may be concluded that production of inhibitory substance was against (NLDO) bacterial isolate.

Table (3-5): Inhibition zone diameter (mm) given by yeasts grown on sabouraud agar against bacterial isolates from (NLDO) patients on solid medium.

Bacterial isolate	*Saccharomyces cerevisiae*	*Saccharomyces boulardii*
Staphylococcus epidermidis	4	7
Staphylococcus aureus	7	-
Streptococcus pneumoniae	-	3
Streptococcus pyogenes	6	-
Pseudomonas aeruginosa	-	-
Escherichia coli	-	5
Proteus mirabilis	6	6
Propionibacterium spp.	-	7
Haemophilus influenzae	6	-
Listeria grayi	8	9
Morganella morganii	-	-

(-) no inhibition zone.

3.6.2 In Liquid Medium:

3.6.2.1 For *Saccharomyces cerevisiae*:

3.6.2.1.1 In Tea Medium:

Inhibitory effect of filtrate of *Saccharomyces cerevisiae* grown in tea medium was tested against bacterial isolates of (NLDO) patients,while there was no effect show when used tea medium alone.

Results of table (3-6) show that the inhibitory effect of *Saccharomyces cerevisiae* varied with the bacterial species and concentration of filtrate. The inhibitory effect was highly observable by the third concentration against *Propionibacterium spp.*, *Haemophilus influenzae*, *Staphylococcus aureus* and *Escherichia coli*, ranging between (20-21) mm, while *Streptococcus pneumoniae*, *Proteus mirabilis*, *Listeria grayi*, *Staphylococcus epidermidis* and *Streptococcus pyogenes* with inhibition zones ranging between (19-15) mm.

Inhibitory activity against bacterial isolates decreased when the second concentration filtrate was used, but the highest effect of that concentration appeared clearly against *Staphylococcus aureus* and *Streptococcus pneumoniae* 18 mm inhibitory zone, until reaching to the less sensitive isolate of *Staphylococcus epidermidis* with inhibition zones 8 mm 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 aureus* , *Listeria grayi* , *Haemophilus influenzae* , *Pseudomonas aeruginosa* and *Escherichia coli* when inhibition zones reached between 13-10 mm.

Adversely, filtrates of the first and second concentration had no effect against *Corynebacterium diphtheriae* and *Morganella morganii*. While *Staphylococcus epidermidis*, *Streptococcus pyogenes* and *Proteus mirabilis* were not affected only by the first concentration as shown in table (3-6).

Table (3-6): Inhibition zones diameter (mm) of yeast filtrate of *Saccharomyces cerevisiae* grown in tea against bacterial isolates from (NLDO) patients.

Bacterial isolate	Inhibition zone diameter (mm) of		
	First concentration 4.81 mg/ml	Second concentration 14.44 mg/ml	Third concentration 43. 33 mg/ml
Staphylococcus aureus	13	18	20
Staphylococcus epidermidis	-	8	15
Streptococcus pneumoniae	6	18	19
Streptococcus pyogenes	-	13	15
Proteus mirabilis	-	13	19
Listeria grayi	12	14	19
Propionibacterium spp.	12	15	21
Haemophilus influenzae	11	13	20
Pseudomonas aeruginosa	10	13	14
Escherichia coli	10	11	20
Corynebacterium diphtheriae	-	-	10
Morganella morganii	-	-	8

(-) no inhibition zones.

3.6.2.1.2 In Sabouraud Medium:

Filtrate of *Saccharomyces cerevisiae* grown in sabouraud medium was tested for their inhibitory effect against bacterial isolates of (NLDO) patients.

Effect of *Saccharomyces cerevisiae* was different depending

on bacterial species and concentration of filtrate. Third concentration was highly effective against *Staphylococcus aureus*, *Propionibacterium spp.*, *Proteus mirabilis Streptococcus pneumoniae* and *Listeria grayi* with inhibition zones of (20-17) mm, table (3-7).

Inhibitory activity decreased against all bacterial isolates by using the second concentration of filtrate. When the inhibition zones ranged between (18-8) mm. However, this concentration was more effective against *Staphylococcus aureus* and *Streptococcus pneumoniae* when the diameter of inhibition zone18, 16 mm for each one, compared to its effect against *Pseudomonas aeruginosa*, *Haemophilus influenzae*, then *Proteus mirabilis* with inhibition zones ranged between (13-12) mm. While no effect was recorded against *Morganella morganii*, *Escherichia coli* and *Corynebacterium diphtheriae*.Table(3-7).

Inhibitory effect against all bacterial isolates decreased for the first concentration of the yeast filtrate when that concentration excreted noticeable effect against *Staphylococcus aureus*, *Listeria grayi* and *Haemophilus influenzae* with inhibition zones (13-11) mm, while little effect 6 mm was recorded against *Streptococcus pneumoniae*. However, no effect was detected against many other bacteria used in this study, show table (3-7).

Table (3-7): Inhibition zones diameter (mm) of yeast filtrates of *Saccharomyces cerevisiae* grown in sabouraud medium against bacterial isolates from (NLDO) patients:

Bacterial isolate	Inhibition zone diameter (mm) of		
	First concentration 4.44 mg/ml	Second concentration 13.33 mg/ml	Third concentration 40 mg/ml
Staphylococcus epidermidis	-	8	15
Staphylococcus aureus	13	18	20
Streptococcus pneumoniae	6	16	18
Streptococcus pyogenes	-	13	15
Proteus mirabilis	-	12	18
Listeria grayi	12	14	17
Propionibacterium spp.	12	13	19
Haemophilus influenzae	11	12	13
Pseudomonas aeruginosa	10	13	14
Escherichia coli	-	-	16
Corynebacterium diphtheriae	-	-	10
Morganella morganii	-	-	8

(-) no inhibition zone

3.6.2.1.3 In Sabouraud Containing (10g) Yeast Extract:

Inhibitory effect of filtrate of *Saccharomyces cerevisiae* grown in sabouraud medium containing yeast extract was tested against bacterial isolates of (NLDO) patients.

Result of table (3-8) declare that *Saccharomyces cerevisiae* had different inhibitory effect on which varied according to species and concentration of the filtrate. The inhibitory effect was highly observed by third concentration against *Staphylococcus aureus, Propionibacterium spp., Streptococcus pyogenes* and *Staphylococcus epidermidis* giving inhibition zones ranging between (22-18) mm, but little effect was exhibited against *Morganella morganii* with only 12 mm diameter of inhibition zone.

Inhibitory activity decreased for all bacterial isolates with the second concentration compared to the third concentration when the second concentration resulted in more effect against *Staphylococcus aureus, Streptococcus pyogenes, Listeria grayi* and *Escherichia coli* with ranging between (19-14) mm inhibition zone.

Decreasing the inhibitory activity is associated with the decreases concentration, and this was obvious with the first concentration when the highest effect recorded was 13 mm in diameter against *Staphylococcus aureus* and only 9 mm against *Pseudomonas aeruginosa* and *Streptococcus pyogenes*, while no effect was recorded against *Proteus mirabilis* and *Escherichia coli.*

Table (3-8): Inhibition zones (mm) of filtrate of yeast *Saccharomyces cerevisiae* grown in sabouraud medium containing yeast extract against bacterial isolates from (NLDO) patients:

Bacterial isolate	Inhibition zone diameter (mm) of		
	First concentration 9.40 mg/ml	Second concentration 28.22 mg/ml	Third concentration 84.66 mg/ml
Staphylococcus epidermidis	7	11	18
Staphylococcus aureus	13	19	22
Streptococcus pneumoniae	7	10	15
Streptococcus pyogenes	9	15	19
Proteus mirabilis	-	14	17
Listeria grayi	6	15	18
Propionibacterium spp.	9	13	19
Haemophilus influenzae	8	9	14
Pseudomonas aeruginosa	9	12	16
Escherichia coli	-	14	16
Corynebacterium diphtheriae	6	11	17
Morganella morganii	8	11	12

(-) no inhibition zone.

3.6.2.2 For *Saccharomyces boulardii*:

3.6.2.2.1 In Tea Medium:

Filtrate of *Saccharomyces boulardii* grown in tea medium was tested for their effects against isolates of (NLDO) patients,while thereis no effect recorded when used tea medium without yeast

against bacterial isolate.

Table (3-9) shows different inhibitory effect of filtrates of *Saccharomyces boulardii*. Highly inhibitory effect was recorded by the third concentration of filtrate against *Staphylococcus aureus* 24 mm, *Staphylococcus epidermidis* 18 mm, and simple effect was exhibited by this concentration against *Morganella morganii* 6mm.

Inhibitory activity decreased for all bacterial isolates by using second concentration, but more effect was detected at such concentration against *Staphylococcus aureus* 20 mm, *Staphylococcus epidermidis* 13 mm, and simple effect against *Listeria grayi* 5 mm.

With the first concentration, the inhibitory effect decreased against all bacterial species, but more effect was noticed against *Staphylococcus aureus* 14 mm.

Some bacterial species were only affected by the third concentration, they were *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Morganella morganii*, while others were affected by both second and third concentration ,they were *Escherichia coli*, *Haemophilus influenzae*, *Listeria grayi* and *Propionibacterium spp.*, as shown in table (3-9).

Table (3-9): Inhibition zones (mm) of *Saccharomyces boulardii* grown in tea medium against bacterial isolates from (NLDO) patients:

Bacterial isolate	Inhibition zone diameter (mm) of		
	First concentration 4.74 mg/ml	Second concentration 14.22 mg/ml	Third concentration 42.66 mg/ml
Staphylococcus epidermidis	9	13	18
Staphylococcus aureus	14	20	24
Streptococcus pneumoniae	-	-	-
Streptococcus pyogenes	-	-	10
Proteus mirabilis	9	11	17
Listeria grayi	-	5	7
Propionibacterium spp.	-	16	8
Haemophilus influenzae	-	7	9
Pseudomonas aeruginosa	-	-	7
Escherichia coli	-	9	11
Corynebacterium diphtheriae	9	17	21
Morganella morganii	-	-	6

(-) no inhibition zone

3.6.2.2.2 In Sabouraud Medium:

Inhibitory effect of filtrate of *Saccharomyces boulardii* grown in sabouraud medium was tested against bacterial isolates of

(NLDO) patients ,while there was no observable effect show when used tea medium alone against bacterial isolates.

Results of table (3-10) show that the inhibitory effect of *Saccharomyces boulardii* was differed with bacterial species and filtrate concentration. The third concentration was highly active against *Corynebacterium diphtheriae* 19 mm, *Staphylococcus epidermidis* 18mm and *Staphylococcus aureus* 16 mm inhibition zones. At that time the inhibitory activity was decreasing for all bacterial isolates by using second concentration, such concentration had high effect against *Staphylococcus epidermidis* with inhibition zone 16 mm but little against *Propionibacterium spp.*6 mm .

As concentration of the yeast filtrates decreases to the first concentration, all bacterial isolates were less affected, especially *Staphylococcus epidermidis* when only 8 mm diameter of in concentration was recorded. Moreover, no effect was detected by such concentration against many bacterial isolates.

Some bacterial species were affected only by the third concentration, such as *Streptococcus pyogenes* and *Listeria grayi*. While other species, *Morganella morganii*, was not affected by any filtrate concentration .

Table (3-10): Inhibition zones (mm) of filtrate of *Saccharomyces boulardii* grown in sabouraud medium against bacterial isolates from (NLDO) patients:

Bacterial isolate	Inhibition zone diameter (mm) of		
	First concentration 4.14 mg/ml	Second concentration 12.44 mg/ml	Third concentration 37. 33 mg/ml
Staphylococcus epidermidis	8	16	18
Staphylococcus aureus	7	15	16
Streptococcus pneumoniae	5	11	15
Streptococcus pyogenes	-	-	9
Proteus mirabilis	7	12	18
Listeria grayi	-	-	8
Propionibacterium spp.	5	6	11
Haemophilus influenzae	7	10	12
Pseudomonas aeruginosa	5	11	14
Escherichia coli	4	8	12
Corynebacterium diphtheriae	6	9	19
Morganella morganii	-	-	-

(-) no inhibition zone

3.6.2.2.3 In Sabouraud Medium Containing Yeast Extract:

Filtrate of *Saccharomyces boulardii* grown in sabouraud medium containing yeast extract was tested against bacterial isolates of (NLDO) patients. As shown in table (3-11).

Inhibitory effect of *Saccharomyces boulardii* filtrates was

different according to the bacterial species and filtrate concentration. The inhibitory effect was highly considerable when the third concentration was applied against *Corynebacterium diphtheriae* 21 mm and *Staphylococcus epidermidis* 20 mm, but it was simple against *Streptococcus pneumoniae*.

Inhibitory activity decreased against all bacterial isolates by using second concentration. But more effect was detected by this concentration against *Staphylococcus epidermidis, Staphylococcus aureus* and *Corynebacterium diphtheriae* 18 mm zone of inhibition for each one. Simple effect at that concentration was against *Morganella morganii* and *Streptococcus pneumoniae* 7 mm.

Higher effect for the first concentration of *Saccharomyces boulardii* filtrate was reached 11 mm which was against *Haemophilus influenzae*, and only simple effect 3 mm against *Streptococcus pneumoniae.*

In the present study, inhibitory effect of *Saccharomyces boulardii* and *Saccharomyces cerevisiae* was noticed against almost bacterial isolates but highest effect was recorded when yeast grown in sabouraud containing yeast extract against *Staphylococcus aureus*, while Casas and Dobrogosz (2000) found that such activity of *Saccharomyces boulardii* was detected against bacterial growth causing diarrhea such as *Shigellae* and *Salmonella*. Inhibitory activity usually varies according to the filtrate dilution and it increased when such dilution decreased, because the inhibitory substances was concentrated leading to increasing its activity.

Table (3-11): Inhibition zones (mm) of filtrates of *Saccharomyces boulardii* grown in sabouraud medium containing yeast extract against bacterial isolates from (NLDO) patients:

Bacterial isolate	Inhibition zone diameter (mm) of		
	First concentration 9. 25 mg/ml	Second concentration 27.77 mg/ml	Third concentration 83. 33 mg/ml
Staphylococcus epidermidis	8	18	20
Staphylococcus aureus	8	18	19
Streptococcus pneumoniae	3	7	9
Streptococcus pyogenes	7	12	15
Proteus mirabilis	9	11	18
Listeria grayi	10	15	19
Propionibacterium spp.	7	10	13
Haemophilus influenzae	11	15	19
Pseudomonas aeruginosa	9	11	15
Escherichia coli	7	9	12
Corynebacterium diphtheriae	8	18	21
Morganella morganii	5	7	11

Also the inhibitory activities effect of yeast filtrate were developed with the medium, and highly inhibitory activity appeared in medium were supplied with protein compounds. And Izgu and Altinbay (1997) attributed the inhibitory activity of yeast to produce killing toxin and lactic acid that play role in decreasing the pH and inhibit bacterial growth. Also from the above result show the yeast have

wide spectrum effect against gram positive and gram negative bacteria and that may attributes to ability to produce killing protein specific for degregate plasma membrane of sensitive cells.

3.7 Synergistic Effect of Probiotic (LAB and yeast) with Antibiotics:

Synergistic effect of bacterial or yeast filtrates with antibiotic against one isolate of *Staphylococcus epidermidis* was tested. As shown in table (3-12).

The inhibitory effect of mixed LAB filtrate with antibiotic against *Staphylococcus epidermidis,* it was more effective than using LAB filtrate or antibiotic alone.

Results table (3-12) show the inhibitory effect of mixing each of the two yeast filtrate with the same antibiotic was great against *Staphylococcus epidermidis* than using each of probiotic or antibiotic alone, when there is no effect show when used antibiotic alone.

Highly effect was shown with the yeast *Saccharomyces cerevisiae* grown in sabouraud containing yeast extract and LAB in MRS medium 20 mm inhibition zones at third concentration.

On the other hand, at second concentration the inhibitory effect of bacteria grown in MRS medium and yeast grown in sabouraud, tea, and sabauroud containing yeast extract media, decreased, but highest effect was recorded against *Staphylococcus epidermidis* 18 mm inhibition zone when use the filtrate of LAB and simple effect

was recorded with *Saccharomyces cerevisiae* in tea medium.

Almost all filtrates of bacterial and yeast, decreased their inhibitory activity against *Staphylococcus epidermidis* at first concentration. While no inhibitory effect of *Saccharomyces cerevisiae* grown in tea medium.

From above results show the inhibitory effect increase ,and that attribute to the increase inhibition effect of inhibitory substance with activity of antibiotic.

Table (3-12): Inhibition zones (mm) given by mixed probiotics and antibiotic in different medium against *Staphylococcus epidermidis*.

Mixes Microorganism filtrate + ampicillin	Inhibition zone diameter (mm) of			
	Medium	Third dilution	Second dilution	First dilution
Lactobacillus plantarum	MRS	10	15	20
Saccharomyces cerevisiae	Tea	-	9	16
Saccharomyces cerevisiae	Sabouraud	7	11	16
Saccharomyces cerevisiae	Sabouraud with yeast extract	9	13	20
Saccharomyces boulardii	Tea	8	12	19

(-) no inhibition zone

2.1 Materials

2.1.1 Apparatus and Equipments :

Apparatus or equipment	Company (Nation)
Incubator	Gallenkamp (England)
Autoclave	Gallenkamp
oven	Gallenkamp
Water bath	Gallenkamp
Candle jar	Rod well
Compound light microscope	Olympus(Japan)
Water Distiller	Exelo (England)
Balance (sensitive)	Delta Rang (switzer land)
pH- Meter	Metter Gmbh- Teledo (U.K)
Cooling centrifuge	Harrier (U.K)
Balance	Ohaus (France)
Lyophilizer	Christ (Germany)
Centrifuge	Hermlxe Laboratech Nik (Germany)
Micropipette	Oxford (USA)

2.1.2 Chemicals:

Chemical	Company (Nation)
Ethanol	Riedel- De Haeny (Germany)
Triammonium citrate	Riedel- De Haeny
Sodium acetate trihydrate	Riedel- De Haeny
Hydrochloric acid	BDH(England)
Urea	BDH
Glucose	BDH
Sodium hydroxide	BDH
Glycerol	BDH
Iodine	BDH
Peptone	BDH
$MnSO4.4H_2O$	Fluka (Germany)
$MgSO4.7H_2O$	Fluka
Tetramethyl-p-Pheneylene Diamine Dihydrochloride	Fluka
Tween- 80	Oxiod(England)
H2O2	Difco(USA)
Meat extract	Biolife (Italy)
Yeast extract	Biolife
Agar	Biolife
Gelatin	Biolife
Normal saline	Pharmaceutical solution industry (Saudi – Arabia)

2.1.3 Stains and Reagents:

Stain or reagent	Company (Nation)
Crystal violet	Fluka (Germany)
Safranin	Fluka
Methylene blue	BDH (England)
Kovac's reagent	Oxoid (England)

2.1.4 API System kits (API- Bio merieux, Lyon, France):

The API-system kits were used include:

2.1.4.1 API 20 E:

It consists of:

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

2.1.4.2 API Staph:

It consists of:

- Gallery contains 20 microtubes having dehydrated substrate.
- Reagents: Vp1, Vp2, N1T1, N1T2, ZYMA, ZYMB.

2.1.4.3 API 20 Strep:

It consists of:

- Gallery contains 20 microtubes having dehydrated Substrate.
- Reagents: VP1, VP2, TDA, IND, N1T1, N1T2.

2.1.4.4 API Coryne:

It consists of:

- Gallery contains 20 microtubes having dehydrated Substrate.
- Reagents: NIT1, NIT2, ZYMA, ZYMB, PYZ.

2.1.5 Culture Media:

2.1.5.1 Ready-Prepared Media:

Medium	Company (Nation)
Nutrient Agar	Mast (England)
Nutrient Broth	Mast
Brain-Heart Infusion broth	Mast
MacConkey Agar	Oxoid (England)
Urea Agar Base	Oxoid
Peptone water	Oxoid
Triple sugar Iron (TSI) Agar	Oxoid
Simmon Citrate Agar	Oxoid
Thioglycollate Broth	Oxoid
Mueller-Hinton Agar	Oxoid
Mannitol Salt Agar	Oxoid

2.1.5.2 Laboratory-Prepared Media:

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

2.1.6 Antibiotics Discs (Al-Razi company / Iraq):

Antibiotic name	Symbol	Concentration (µg)
Ampicillin	AM	10
Pencillin G	PG	10 unit
Cephotaxime	CE	30
Gentamicin	GM	10
Amikacin	AN	30
Neomycin	N	30
Tobramycin	TM	10
Chloramphenicol	C	30
Erythromycin	ER	15
Vancomycin	VA	30
Tetracycline	TE	30
Ciprofloxacin	CF	5
Optachin differential disc	OP	
X,V factor		

2.1.7 Microorganisms:

The following bacterial and yeast isolates were obtained from the Department of Biotechnology / College of science, Al-Nahrain University, Baghdad.

Microorganism
Lactobacillus plantarum
Saccharomyces cerevisiae
Saccharomyces boulardii

2.2 Methods:

2.2.1 Preparation of Culture Media:

2.2.1.1 Ready-Prepared Media:

Media listed in table (2.1.5.1) were prepared according to the instructions fixed on their containers.

All media were sterilized in the autoclave after adjusted pH.

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 dissolving 37 g of blood agar base in one litter of distilled water. Then, the medium sterilized in the autoclave. After cooling to 45C^{o}, 5% of blood was added to it, mixed and distributed in sterile petri-dishes.

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 50C^{o}, then 50 ml of 40% urea sterilized by filtration was added.

2.2.1.2.5 Man-Rogoza-Sharpe (MRS) Broth:

It was used for cultivating *lactobacillus plantarum* and prepared according to Harrigan and MacCance,(1976) as follow:

The following ingredients were dissolved in 100 ml distlled water.

Ingredient	Quantity (%)
D-glucose	2 g
Meat extract	1g
Peptone	1g
Yeast extract	1g
Triammonium citrate	0.2g
Sodium acetate trihydrate	0.5g
Tween- 80	0.1ml
$MgSO_4 . 7H_20$	0.0 2g
$MnSO_4 . 4H_20$	0.005g

After pH was adjusted to 6.0, the medium was sterilized in the autoclave.

2.2.1.2.6 Man- Rogoza-Sharpe (MRS) Agar:

It was used for cultivating *Lactobacillus plantarum*, and prepared as in item (2.2.1.2.5) with the addition of 20g agar.

2.2.1.2.7 Sabouraud Dextrose Broth:

It was used for cultivation of yeasts and molds, and prepared according to Emmons *et al.*, (1977) by dissolving 10g peptone and 20g glucose in one litter of distilled water with the aid of heat .

After pH was adjusted to 5 the medium was sterilized in the autoclave.

2.2.1.2.8 Sabouraud Dextrose Agar:

It was used for cultivation of yeasts and molds, and prepared as in item (2.2.1.2.7) with the addition of 20g agar.

2.2.1.2.9 Tea Medium:

It was used for cultivation of yeasts, and prepared by boiling 10g of dry tea from local market in one litter of distilled water for 30 min. Tea extract was obtained by filtration through filter paper, then 20g of glucose was dissolved into the filtration and volume was completed to one litter with the distilled water, then the medium was sterilized in the autoclave. After pH was adjusted to 5.

2.2.2 Sterilization:

2.2.2.1 Moist- Heat Sterilization:

All media and solutions were sterilized in the autoclave at 121C^{o} (15Ib / in^{2}) for 15 min.

2.2.2.2 Oven Sterilization:

Electric oven was used to sterilize the glass ware at 160-180$C^{o}$for 3-2 hrs .

2.2.2.3 Filtration:

Millipore filter unit 0.44 mm used to sterilize the of *Lactobacillus plantarum* filtrate and yeasts filtrate .

2.2.3 Specimens Collection:

Specimens were collected from patients who had signs of nasolacrimal duct obstructions such as epiphora (watery eye). The specimens were taken from these patients in the minor surgery theater in Ibn Al–Haetham Teaching Eye Hospital - Baghdad, by the ophthalmologist during period between January till July, 2004. Each specimen was obtained by direct application of a sterile cotton swab to the purulent secretion refluxed from the puncta after gentle pressure over the lacrimal sac or irrigating the lacrimal drainage system by syringing the lower puncta with sterile saline and take swab from the refluxed material from upper puncta.

While in children, the specimens were taken by sterile probe after passing it down the nasolacrimal duct. These procedures were done under general anaesthesia for children, while in adult it was done under local anaesthesia.

After that, the swab or probe directly streaked, separately, on blood agar, MacConkey agar, chocolate and sabouraud agar, in addition to the inoculation in thioglycollate broth. Then all specimens were soon brought to the laboratory.

2.2.4 Culturing:

In the laboratory, each specimen was separately streaked on the surface of each the above mentioned media, incubated at $37C^{O}$ for 24 hrs aerobically for the first three media, and anaerobically (in the Anaerobic jar offering 5-10% CO_2) for the thioglycollate broth medium, while the fourth one sabouraud dextrose agar, incubated at $28C^{o}$ for 3-5 days to detect molds and yeasts. On the other hand, the probiotic microorganisms cultivated by using, MRS medium, which was used for cultivation *Lactobacillus plantarum*, was incubated

anaerobically at 37C^{o} for 24 hrs. Moreover yeasts such *Saccharomyces cerevisiae* and *Saccharomyces boulardii* were also cultivated into sabouraud dextrose medium and incubated at 28C^{o} for 3-5 days.

2.2.5 Maintenance of Bacterial Isolates:

Maintenance of bacterial isolates was performed according to Maniatis *et al.*, (1982) as following:

2.2.5.1 Short-Term Storage:

Bacterial isolates (after propagated for the optimum temperatures and periods of incubation) were maintained for a few weeks on plates containing the related medium. The plates were tightly wrapped with parafilm and stored in the refrigerator (at ≈ 4C^{o}) until use .

2.2.5.2 Long-Term Storage:

Bacteria can be stored for many years in medium containing 15% glycerol. This was done by inoculating bacteria into screw-capped containing appropriate medium for each bacterial species with 15% glycerol, and then incubated at 37C^{o} for appropriate time for each bacterium. After that all isolates were stored at – 20C^{o}.

2.2.6 Identification of Bacterial Isolates:

The isolated bacteria were identified as follow:

2.2.6.1 Cultural Characterization:

Colonies grown on blood agar, chocolate agar, MacConkey agar, were described according to their shape, size, color, and type of lysis (Baron and Finegold, 1990).

2.2.6.2 Microscopical Examination:

Gram stain method was used to describe cells morphology and grouping of each suspected isolate grown on the previous culture media. Cells shape, grouping and gram reaction were among properties examined (Harley and Prescott, 1996).

2.2.6.3 Biochemical Tests:

2.2.6.3.1 Catalase Test:

A drop 3%of hydrogen peroxide solution was placed on a cleaning slide and a small amount of the bacterial growth was added to it. Formation of bubbles indicates a positive result (Brooks *et al.*, 1998)

2.2.6.3.2 Coagulase Test:

A large well isolated colony was transferred into a test tube containing 0.5 ml of reconstituted plasma and incubated at 37C^{o} for about 4 hrs, degree of clotting constituted resulted was recorded (Kloss and Jorgensen, 1985).

2.2.6.3.3 Indole Test:

It was done by inoculating peptone water with the bacteria from the bacterial culture, then incubated at 37C^{o} for 24 hrs after incubation, 0.05 ml of kovac's reagent was added and mixed gently. Positive result was reported by appearance of red ring on the surface of the tube (Collee *et al.*, 1996).

2.2.6.3.4 Oxidase Test:

This test was performed by moisturing filter paper with a few drops of freshly prepared solution of Tetramethyl – p – phenylene diamine dihydrochloride , then a champ of cells was picked-up from the slant growth with a sterile wooden stick and

smear on the moistened paper. Violet or purple color development within 10 seconds indicates a positive result (Atlas *et al.*, 1995).

2.2.6.3.5 Simmon Citrate Test:

It was done by streaking the suspected isolate on simmon citrate agar, and incubated at 37C^{o} for 24 hrs. A positive result was detected by changing color of medium from green to blue (Collee *et al.*, 1996) .

2.2.6.3.6 Triple Sugar Iron (TSI) Test:

Bacterial isolates was cultured on (TSI) agar slant by stapping and streaking on slant surface, then incubated at 37C$^{\circ}$ for 24 hrs. If the color of medium changed from red to yellow this indicates acid formation, while appearance of black precipitate indicates ferric sulfate formation, while pushing the agar to the top indicates CO_2 formation (Atlas *et al.*, 1995).

2.2.6.3.7 Urease Test:

Urease activity was detected by inoculated the surface of urea agar slants with the bacterial growth and incubated at 37C° for 24 hrs. Appearance of red - violet color indicates a positive result , while yellow indicates the negative one (Atlas *et al.*, 1995).

2.2.6.3.8 Growth in Mannitol Salt Agar:

Mannitol salt agar was used to isolate and differentiate *Staphylococci* by ability of *Staphylococcus aureus* to grow and ferment it, and cause change its color from red to yellow, while *Staphylococcus epidermidis* not ferment it (Baron *et al.*,1994).

2.2.6.3.9 Gelatin Test:

It was done for gelatinase productionby inoculating a tube containing gelatin medium with the isolate by stapping of the 3/4 medium to the bottom of the tube and incubated at 37C^{o} for 24 hrs (or longer).

The incubation time usually depends on the species of bacteria. After the tube was placed in the refrigerator (at ≈ 4C^{o}) for 30 min, the surface of the medium was noticed weather it was aqueous which indicates a positive result , or solid as negative result (Harley and Prescott, 1996).

2.2.6.3.10 Optachin Susceptibility Test:

Commercially available optachin disks were applied to a quarter of blood agar plate that had been streaked with a few colonies of the test organisms.

After overnight incubation at 35C^{o} in anaerobic jar, inhibition zones were measured. Zones equal to and greater than 14 mm with 6 mm discs were indicative of inhibition, and the isolate was identified as *Streptococcus pneumoniae*, while the inhibition zones were smaller than 14 mm the isolates may be belonged to other species of *Streptococcus*. (Rouff *et al.*, 1999).

2.2.7 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 a sterile loop) and emulsified in 5 ml suspending medium (with sterile D.W.) by rubbing against the side of the tube and mixed through 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 pasture pipette, the microtubes were inoculated, and some microtubes were completely filled. After inoculation, some sections were completely filled with sterile mineral oil, then incubated at 37Co 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.

2.2.8 Antibiotics Susceptibility Test:

The disc diffusion method was employed (Vandepitte *et al.*, 1991) Mueller-Hinton agar was used for some species, while the blood agar for *Streptococcus spp.* and chocolate agar for *Haemophilus influenzae.*

The inoculum was prepared by transferring sufficient growth from a pure culture of the isolate into a tube of normal saline solution, while the brain heart infusion for, *Haemophilus influenzae* and *Streptococcus pneumoniae* until its turbidity matched the turbidity of standard Macfarland solution (10^8) cell/ml . A sterile cotton swab was dipped into the inoculum, and excess inoculum was removed by pressing and rotating the swab firmly against the side of tube above the level of the liquid. The swab was streaked all over the surface of the medium many times with the rotation of the plate through an angle of 60 after each application. Finally, the swab was passed round the edge of the agar surface. Then, the inoculum was left to dry for a few min at room temperature with the lid closed. The antibiotic discs were placed on the inoculated plates with the aid of a sterile forceps. Each disc was gently pressed down to ensure even

contact with the medium, then the plates were incubated at 37C^{o} for 24 hrs. After that, the diameter of each zone was measured in mm. The results were compared with the national committee, for clinical laboratory standards (Vandepitte *et al.*, 1991).

But to study the antibiotics sensitivity for *Propionibacterium spp.*, broth disc method was used according to as follow:

Appropriate numbers of antibiotic discs were added into screw capped tubes containing 5 ml of thioglycollate broth .Then, the inoculated tubes were kept at room temperature for 2 hrs to allow diffusion of the antibiotic into the medium. After that, the tubes were inoculated with 0.1 ml of broth containing *Propionibacterium spp.* and screw capped tightly, they were incubated at 37C^{o} for 48–72 hrs. Controls of non – inoculated tubes and tubes without antibiotic were prepared. Interpretation done by observing the turbidity. Susceptibility was indicated when there was turbidity.

2.2.9 Determining of *Lactobacillus plantarum* Inhibitory Effect:

2.2.9.1 On Solid Medium:

A culture 1% of *Lactobacillus plantarum* was inoculated in test tube containing MRS broth , then incubated at 37C^{o} for 24 hrs. After incubation, with the aid of a sterile microbiological loop, part of the growth was streaked on the surface of MRS agar, and Incubated anaerobically at 37C^{o} for 24 hrs (Silva *et al.*, 1987). After that , discs of 5 mm were cut by a sterile cork borer and fixed, reversely, on the surface of nutrient agar previously spreaded with 0.1 ml of the test bacterial isolate. Inhibition zones in mm were measured by a ruler after incubation at 37C^{o} for 24 hrs.

2.2.9.2 In Liquid Medium:

Culture of *Lactobacillus plantarum* was prepared by propagating 2% inoculum into MRS broth at pH 6 then incubated at 73C^{o} for 24 hrs (Silva *et al.*, 1987). After incubation the culture was centrifuged at 6000 rpm for 10 min. Free cells supernatant was taken and filtrated throughout millipore filters 0.44 mm. Then 200 ml of filtrate of *Lactobacillus plantarum* was concentrated by using lyophilizer to get 2g powder from it, Later , the stock solution was prepared by dissolving 100 mg of powder in 10 ml sterile distilled water, after that serial dilution was prepared from stock solution by mixing 0.5 ml from stock solution with 1 ml of uninoculated MRS medium to obtain third concentration (5 mg/1.5 ml). After that, taking 0.5 ml from third concentration and mixed with uninoculated MRS medium to gain second concentration with concentration (1.66mg/1.5ml), also first concentration was prepared by taking 0.5 ml from second concentration and mixed with 1ml of uninoculated MRS medium to obtain first concentration with concentrate (0.55mg/1.5ml).

Then well diffusion method of Piared *et al.*, (1992) was applied to detect the inhibition activity of *Lactobacillus plantarum* by streaking 0.1 ml of the test bacterial isolated each one separately on nutrient agar with the aid of a sterile spreader, and by using a sterile cork borer, wells 5 mm in diameter were made on the surface of nutrient agar, then the wells were filled with 100 µL of LAB filtrate. After incubation at 37C^{o} for 24 hrs, inhibition zones of the filtrate were measured.

2.2.10 Determining *Saccharomyces spp.* Inhibitory Effect:

2.2.10.1 On Solid Medium:

Saccharomyces cerevisiae and *Saccharomyces boulardii* were separately propagated in sabouraud broth medium, and incubated at 28C^{o} for 24 hrs, part of the growth was streaked on the surface of sabouraud agar pH 5.5, then incubated at 28C^{o} for 48 hrs. (Kennes *et al*., 1991). With the aid of a cork borer discs of 5 mm were prepared from the previous growth, and put reversely on the surface of nutrient agar previously spreaded with 0.1 ml of test bacterial isolates. Then plates were incubated for 24 hrs at 37C^{o}, after that diameter of inhibition zones were measured in mm by a ruler.

2.2.10.2 In Liquid Medium:

Yeast culture were prepared by inoculating 2% of previously activated yeast of *Saccharomyces cerevisiae*, separately,into(sabouraud medium, tea medium, sabouraud containing yeast extract medium) respectively, with pH 5.5, and incubated at 30C^{o} for 48 hrs. After incubation, they were centrifuged at 6000 rpm for 10 min. Free cells supernatant was taken and filtrate throughout millipore filter 0.44mm. Then 200 ml of filtrate of *Saccharomyces cerevisiae* grown in (sabouraud, tea, sabouraud containing yeast extract) respectively, were concentrated separately by lyophilizer to get powder from each media weighing (24g, 26g, 50. 8g) respectively, then stock solution was prepared by dissolving (1200 mg, 1300 mg, 2540 mg) respectively in 10 ml of sterile D.W. On the other hand, serial dilution for each medium was prepared by taking 0.5 ml of stock solution from each medium and mixed with 1 ml of uninoculated medium, separately, to obtain third concentration with concentrate(60 mg/ 1.5 ml, 65 mg/1.5

ml, 127 mg/1.5 ml) respectively, then by taking 0.5 ml from third concentration of each medium and mix with 1 ml uninoculated medium gain second concentration with concentrate(20 mg/ 1.5ml, 21.66 mg/1.5 ml, 42.33 mg/1.5 ml) respectively. Also first concentration was prepared by taking 0.5 ml from second concentration and mixed with 1 ml of uninoculated medium and its concentrations (6.66 mg/1.5 ml, 7.22 mg/1.5 ml, 14.11 mg/1.5 ml) respectively.

Well diffusion method was applied to detect the inhibitory activity of yeast, by streaking each test bacterial isolates on nutrient agar with a sterile spreader. By a sterile cork borer, well 5 mm were made on the surface of nutrient agar previously streaked with test bacterial isolates, and filled with 100 μl of each dilution the incubated at $37C^{o}$ for 24 hrs. And inhibition zones measured in mm.

Then , the same previous procedure was done for *Saccharomyces boulardii*, but the powder weight obtained by lyophilizer were (22.4g, 25.6g, 50g) respectively for each medium. The stock solutions were (1120 mg/10 ml, 1280 mg/10 ml, 2500 mg/10 ml) respectively. While third concentration were (56 mg/1.5 ml, 64 mg/1.5 ml, 125 mg/1.5 ml) , respectively, and second concentration (18.66 mg/1.5 ml, 21.33 mg/1.5 ml, 41.66 mg/1.5 ml), respectively, while first concentration (6.22 mg/1.5 ml,7.11 mg/1.5 ml, 13.88 mg/1.5 ml) respectively.

2.2.11 Synergistic Inhibitory Effect of Probiotic and Ampicillin:

According to above mention, different concentration dilutions of *Lactobacillus plantarum* filtrate were prepared .Each dilution was mixed with ampicillin 100mg/ml (by dissolved 500mg ampicillin in 5

ml sterile istilled water) in equal volume. The inhibitory activity of mixing was tested against the most predominant bacteria *Staphylococcus epidermidis* by using well diffusion method as illustrated in item (2.2.10.2).

Same experiment was repeated but the mixing various dilutions from each of the two yeasts *Saccharomyces cerevisiae* and *Saccharomyces boulardii,* separately with ampicillin in equal volume. Then the inhibitory effects were tested against *Staphylococcus epidermidis* by using well diffusion method as in previous item.

4.1 Conclusions:

- *Staphylococcus epidermidis* was the most dominant bacteria of patients suffering from dacryocystitis due to nasolacrimal duct obstruction, followed by *Staphylococcus aureus*.
- Ciprofloxacin and vancomycin were the most effective antibiotics against the bacterial causative agents found in (NLDO) patients, while most of the bacterial isolates were sensitive to these two antibiotics. However, the isolates were resistant to tetracycline and erythromycin.
- Probiotic bacteria *Lactobacillus plantarum* grown in MRS medium exhibited good inhibition activity against the (NLDO) bacterial isolates.
- When yeasts *Saccharomyces cerevisiae* and *Saccharomyces boulardii* were grown in different media (Tea, sabauroud, and sabauroud supplemented with yeast extract) they excreted observable inhibitory activity against NLDO bacterial isolates especially yeast grown in sabouraud enriched with yeast extract.
- Inhibitory activity of probiotics bacteria and yeasts was increased sharply when the filtrates of such probiotics microorganism were concentrated.
- Mixing each of the filtrate of probiotic microorganisms, separately, with the ampicillin led to increase their inhibitory effect compared to using each one alone.

4.2 Recommendations:

- Other probiotic microorganisms are used to detect their inhibitory effect against bacteria of (NLDO) patients.
- Using other media to propagated the probiotic microorganism before testing their inhibitory activity, as well as changing fermentation conditions such as oxygen, pH, temperature and period of incubation.
- Ciprofloxacin and vancomycin are recommended to be used topically as drug of first choice in the treatment of lacrimal drainage infection due to (NLDO).
- In vivo application of the probiotic filtrate.
- Genetic study of the nasolacrimal duct obstruction.

List of Abbreviations

BDH	British Drug House
IND	Indole
LAB	*Lactobacillus plantarum.*
MRS	Man Rogoza Sharpe
NIT	Nitrate
NLDO	Nasolacrimal Duct Obstruction.
OX	Oxidase
PYZ	Pyrazinamidase
TDA	Treptophane Eaminase.
VP	Vogas proskaure
ZYMA	Zymin A
ZYM B	Zymin B

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Appendix (1): Results of the API Staph kit system used for diagnosis *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, MEl: 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- acetyl- glucosamine, ADH: Arginine, URE: Urea.

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

Tests	*Streptococcus pneumonia*e	*Streptococcus pyogenes*
VP	-	-
HIP	-	-
ESC	-	-
PYRA	V	+
αGAL	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, αGAL: 6- Bromo-2-naphthyl α- D-galactopyranoside, βGUR: Naphthol AS-BIβ- D- glucuronate, βGAL: 2- naphthyl- β- D- galactopyranoside, PAL: 2- naphthyl phosphate, LAP: L- Leucine-2- nphthylamide, 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, Morganella morganii* and *Pseudomonas aeruginosa.*

Tests	***Escherichia coli***	***proteus mirabilis***	***Morganella morganii***	***Pseudomonas aeruginosa***
ONPG	+	-	-	-
ADH	-	-	-	+
LDC	+	-	-	-
ODC	+	+	+	-
CIT	-	-	-	+
H2S	-	+	-	-
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, CIT: Sodium citrate, H2S: Sodium thiosulfate, URE: Urea, TDA: Tryptophane, IND: Indole, VP:Creatine sodium pyruvate, GEL: Kohn's gelatin, Glu: Glucose, MAN: Mannitol, INO: Inositol, SOP: Sorbitol, RHA: Rhamnose, SAC: Sucrose, MEL: Melibiose, AMY: Amygdalin, ARA: Arabinose, OX: Oxidas.

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

Tests	*Corynebacterium diphtheriae*	*Listeria grayi*
NIT	+	-
PYZ	-	-
PYrA	-	-
PAL	-	-
βGUR	-	-
BGAL	-	-
α GLU	+	V
β NAG	-	V
ESC	-	+
URE	-	-
GEL	-	-
O	-	-
GLU	+	+
RIB	+	+
XYL	-	-
MAN	-	+
MAL	+	+
LAC	-	+
SAC	-	-
GLYG	V	-
CAT	+	+

(+): positive results.
(-): negative results.
V: variable.
NIT: Nitrate reduction, PYZ: Pyrazinamidase, PYrA: Pyrrolidonyl Arylamidase, PAL: Alkaline phosphotase, βGUR: beta- Glucuronidase, βGAL: beta- Galactosidase, α GLU: alpha- Glucosidase, βNAG: N- Acetyl- B- Glucosaminidase, ESC: Esculin, 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.

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