Ministry of Higher Education
And Scientific Research
AL- Nahrain University
College of Science
Department of Biotechnology

Effect of anti-lysozyme and IgA antibodies of tear film on microorganisms causing eye corneal infection

A Thesis

Submitted to the Council of Science College /AL-Nahrain University as a partial fulfillment of the requirements for the Degree of Master of Science in Biotechnology

By

Marwa Adel Hussein Al-Akaidi

B.Sc. Biotechnology/College of Science/Al-Nahrain University (2012)

Supervised by

Dr. Abdulwahid B. Al-Shaibani (Professor)

Dr. Faiz A. Al-Shaqarchi (Assist. Professor)

September 2014 Dhu al-Qi'dah 1435

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ{١} خَلَقَ الْإِنسَانَ مِنْ عَلَقٍ{٢} اقْرَأْ وَرَبُّكَ الْأَكْرَمُ{٣} الَّذِي عَلَّمَ بِالْقَلَمِ{٤} عَلَّمَ الْإِنسَانَ مَا لَمْ يَعْلَمْ{٥}

سورة العلق

من الاية ١- ٥

Supervisors Certification

We, certify that this thesis entitled "**Effect of anti-lysozyme and IgA antibodies of tear film on microorganisms causing eye corneal infection**" was prepared by **"Marwa A. Hussein"** under our supervision at College of Science/AL- Nahrain University in partial fulfillment of the requirements for the Degree of Master of Science in Biotechnology.

Signature:

Name: Dr. Abdulwahid B. Al-Shaibani
Scientific degree: Professor
Date: 22 / 01 / 2015

Signature:

Name: Dr. Faiz A. Al Shaqarchi
Scientific degree: Assist. Prof.
Date: ١٩/ ٠١ / 2015

In view of the available recommendations, I forward this thesis for debate by the examinating committee.

Signature:

Name: Dr. Hameed M. Jasim

Scientific degree: Professor

Title: Head of Biotechnology Department

Date: 22 / 01 / 2015

Committee Certification

We, the examining committee certify that we have read this thesis entitled **"Effect of anti-lysozyme and IgA antibodies of tear film on microorganisms causing eye corneal infection"** and examined the student **"Marwa A. Hussein"** in its contents and that in our opinion it is accepted for the Degree of Master of Science in Biotechnology.

Signature:
Name: Dr. Hameed M. Jasim
Scientific Degree: Professor
Date: ٢٢ / ٠١ / 2015
(Chairman)

Signature:
Name: Dr. Shahla M. Salih
Scientific degree: Assist Professor
Date: ٢٢/٠١ / 2015
(Member)

Signature:
Name: Dr. Nawras K. AL- Mudaffar
Scientific degree: Ophthalmologist
Date: ١٧/٠١ / 2015
(Member)

Signature:
Name: Dr. Abdulwahid B. Al-Shaibani
Scientific degree: Professor
Date: ٢٢/٠١ / 2015
(Member and Supervisor)

Signature:
Name: Dr. Faiz A. Al-Shaqarchi
Scientific Degree: Assist Professor
Date: ١٩/ ٠١ / 2015
(Member and Supervisor)

I, hereby certify upon the decision of the examinating committee.

Signature:
Name: Dr. Hadi M. A. Abood
Scientific degree: Assist Professor
Title: Dean of College of Science
Date: / / 2015

Dedication

To the one who taught me that life is a tender symbol of loyalty, to whose light is the path for me.

Father

To who inspired me the power, kindness and patience, to the source of my life.

Mother

To whom which I never forget, the smile of my life.

Brother & sisters

I dedicate this work

Marwa

Acknowledgment

At the beginning, thanks to great Allah, Lord of the whole creation who gave me the faith and strength to accomplish this work.

My special thanks with respect and appreciation to my supervisors Prof. Dr. Abdulwahid B. Al-Shaibani and Consultant Surgeon Assist. Prof. Dr. Faiz I. Al-Shaqarchi for their continuous efforts, guidance and useful advises during this work.

Grateful thanks are conveyed to the staff of Biotechnology Department / College of Science / Al-Nahrain University, especially Dr. Asma'a Ali, Dr. Nedhal Suhail, and Miss Noor.

My thanks are introduced to the surgery and laboratory staff at "Ibn Al-Haitham Teaching Eye Hospital" in Baghdad for all kinds of help and facilities they offered to accomplish this work.

Most of all I am sincerely grateful to my father, mother, brother, sisters and relatives for their help and support throughout my study.

Marwa

Summary

The study aimed to investigate the effect of anti-lysozyme and IgA antibodies in tear film on the occurrence and growth of microorganisms (bacteria and/or fungi) causing eye keratitis infection. For this purpose (105) corneal scraping samples were taken from patients suffering of microbial keratitis referred to Ibn Al-Haitham Eye Teaching Hospital in Baghdad. After culturing on the related selective media, results showed that 73 of the samples were positive for occurrence of microorganisms; of these, 68 (64.76%) were belonged to bacterial and 5(4.76%) to fungi. After the bacterial isolates were identified by cultural, microscopic and biochemical examinations, the following species and percentages were recorded: *Pseudomonas aeruginosa 29 (39.73%)*, *Staphylococcus aureus 22 (30.14%) and Staphylococcus epidermidis 17 (23.28%).* While the fungal isolates were belonged to *Aspergillus niger 3 (4.12 %) and Alternaria spp. 2 (2.73 %)* according to their identification through the cultural and microscopic examinations, addition to the types and shapes of fungal spores. Upon such findings, P.aeruginosa was the predominant bacteria and A.niger the prevalent fungi. Regarding to the patients gender, it was found that cases of microbial keratitis in the samples were more abundant in males 41 (56.16%) than in females 32 (42.85%). Patients group of age (41-59) years was the most microbial keratitis infected group with a percentage of (30.20 %), while the lowest infected group was (≥ 60) year with a percentage of (17.80 %). The IgA levels were found to be increased with the corneal ulcer infection. The mean IgA levels were significantly higher than the matched controls ($t = 2.04$ for 40 df ,$P< 0.05$) in the age group of ≤ 20 years. In the group age (21-40), the mean IgA levels were also significantly higher than those of the matched controls ($t = 4.46$ for 54 df, $P<0.001$).

Moreover, in the age groups (41-59) and (≥ 60) years significantly higher means of IgA levels (t = 4.04 for 42 df, P<0.001,and t = 2.58 for 59 df, P<0.001) were recorded in comparison to respective matched controls. Low levels of tear anti-lysozyme were observed in patients with infective corneal ulcers, when compared with the controls. The decrease in the level of lysozyme in tears for age group ≤ 20 to (21–40) years was statistically significant (P<0.001). Again, the fall in the level of tear lysozyme from the age group (41-59) to ≥ 60 years was statistically significant (P<0.001). Regarding gender factor, there were no significant differences detected among the two sexes.

List of Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviation	Full name
Agr	Accessory gene regulator
B-Toxin	Beta toxin
df	Degree of freedom
ExoU	Exotoxin U
FpvA I-III	Ferri-pyoverdine complex (type's I–III)
IL-1	Interleukin- 1
IL-4	Interleukin- 4
LASIK	Laser-assisted insitu keratomileusis
MICs	Minimum inhibitory
sIgA	Secretary immunoglobulin A
t	T-test
p	P-value
TLR5-NF-kB	Toll-like receptor 5-mediated- Necrosis factor -kappa B activation
TNF-α	Tumor necrosis factor- Alfa
TSI	Triple sugar iron agar

Chapter one

Introduction and Literature Review

1- Introduction and Literatures Review

1.1- Introduction:

Microbial keratitis is a potentially sight threatening disorder and considered to be the leading cause of monocular blindness worldwide. Infectious keratitis is characterized by a defect of the corneal epithelium (hence the terms ‘infectious keratitis’ and "ulcerative keratitis" are frequently used interchangeably) with inflammation of the underlying corneal stroma caused by replicating organisms including bacteria, viruses, fungi and protozoa (Keay, 2006).

The presentation is acute with patients often in significant pain and distress. Infectious keratitis is a medical emergency; rapid initiation of aggressive treatment is needed to halt the disease process and limit the extent of corneal scarring and loss of vision. Risk factors for infectious keratitis due to non-viral pathogens include trauma to the eye, overnight or extended wear of conventional contact lenses or chronic ocular surface disease (including blepharitis), prior ocular surgery, systemic diseases (diabetes mellitus, rheumatoid arthritis) and use of topical corticosteroids or traditional eye medicines. Around 10 % of individuals with infectious keratitis may not exhibit any risk factors (Thomas and Geraldine, 2007). The leading predisposing factors for superlative microbial keratitis in Iraq were corneal abrasions, and ocular surface disorders (dry eye, trichiasis, old scars, or exposure keratitis) most of them were sequels of cicatricial trachoma (Al-Shakarchi, 2003).

Itahashi *et al*., (2010) published that corneal ulcer including bacterial keratitis, fungal keratitis, can cause corneal opacity, deteriorated visual acuity, or even leads to some lifelong complications. Bacterial culture and smear examination using corneal scrapings is the conventional method to detect causative pathogens of corneal ulcer.

In the light of what is mentioned above and due to the very limited studies about using conventional methods in the identification of the microbial keratitis causatives agent and studying the activity of some tear antimicrobial agents in controlling eye keratitis infection.

The aim of this study was to :

- Investigate the effect of anti-lysozyme and IgA antibodies of tear film on the occurrence and growth of microorganisms (bacterial and/or fungal) causing keratitis infection. For this purpose, microorganisms causing eye keratitis infection were isolated and identified.

1.2- Literature Review:

1.2.1- Microbial keratitis:-

The unique structure of the human eye and exposing it directly to the environment refers its vulnerable to a number of uncommon infectious diseases caused by bacteria, fungi, viruses and parasites. Host defenses directed against these microorganisms, once anatomical barriers are breached, are often insufficient to prevent infection which may lead to the loss of vision (Hazlett,2002).

Snell and Lemp (1998) stated that the eyeball is made up of two main segments: the anterior and the posterior segments. The anterior and posterior chambers of the eye are filled with a clear fluid called aqueous humor. This fluid contains glucose, amino acids, ascorbic acid and dissolved gases, which helps to provide the metabolic needs of the vascular lens and cornea. The cornea is a transparent structure at front of the eye (Figure 1.1). Dome–shaped surface measuring about 0.5 mm thick in the central and 1.1 mm in the peripheral region. The cornea serves two specialized functions: **first** it provides a protective barrier between the external environment and the internal milieu, and **second** it constitutes the main refractive element of the visual system. The barrier function of the cornea largely relies on the epithelial layer; the epithelial layer like other mucosal epithelial linings in the body constitutes the first line of defense against microbial pathogens and possesses the ability to detect their presence (Zhang *et al*., 2005). The cornea constitutes the outermost part of the eye and is in constant contact with potentially pathogenic microbes. Under normal conditions, the cornea is highly resistant to microbial invasions. However, once the epithelial integrity is breached, pathogen may invade the cornea leading to microbial infection of the cornea, commonly termed infective keratitis, which is the leading cause of loss of vision in both developed and

developing countries. In recent years the incidence of microbial keratitis has been significantly increased probably due to the increase in extended contact lens wear and Lasik surgery (Ladage, 2004).

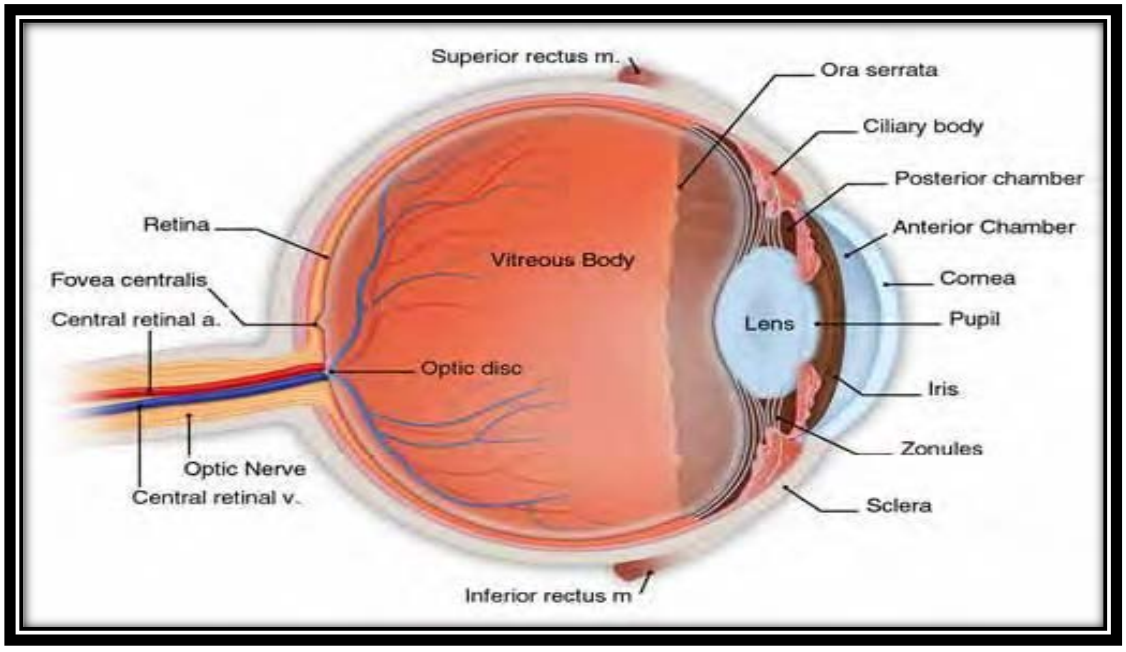


Figure1.1: Anatomy of the eyeball (Gao, 2009).

Hall *et al*., (1999) elucidated that keratitis is an inflammation of the cornea, it is a potentially sight threatening disorder and the leading cause of monocular blindness worldwide. Migration of inflammatory cells into the mammalian cornea may result in the disruption of the critical condition that maintains transparency, resulting in corneal pacifications or complete blindness.

Pearlman *et al*., (1997) mentioned that the cornea is normally avascular; recruitment of inflammatory cells into this site initially occurs via limbic vessel in periphery and migrates to the site of trauma. The number of inflammatory cells present in the cornea determines the severity of keratitis, and that recruitment of inflammatory cells, notably eosinophil's, is regulated by IL-4. Keratitis can be caused by bacterial, viral, or fungal pathogens, dry eyes resulting from swelling of the eyelid or diminished ability to form tears,

exposure to very bright light, foreign objects that injure or become lodged in the eye, it can also cause by sensitivity or allergic reactions to eye makeup, dust, pollen, pollution, or other irritants. The symptoms of keratitis include tearing, pain, sensitivity to light, inflammation of the eyelid, decrease in vision and redness.

There are many types and causes of keratitis that occurs in both children and adults. Organisms cannot generally invade an intact, healthy cornea. However, certain conditions can allow an infection to occur. For example, a scratch can leave the cornea prone to infection. A very dry eye can also decrease the corneas protective mechanisms (Shukla *et al*., 2008; Sethi *et al*., 2010).

1.2.2-Types of microbial keratitis:

Following are some common types of keratitis:

1.2.2.1- Bacterial keratitis:

Bacterial keratitis is a sight-threatening process. A particular feature of bacterial keratitis is its rapid progression; corneal destruction may be complete in 24-48 hours with some of the more virulent bacteria. Corneal ulceration, stromal abscess formation, surrounding corneal edema and anterior segment inflammation are characteristic of this disease (Keay, 2006).

Tang *et al*., (2009) stated that interruption of an intact corneal epithelium and/or abnormal tear film permits entrance of micro-organisms into the corneal stroma, where they may proliferate and cause ulceration. Virulence factors may initiate microbial invasion, or secondary effector molecules may assist in the infective process. Many bacteria display several adhesions on fimbriated and non-fimbriated structures that may aid in their adherence to host corneal cells. During the initial stages, the epithelium and stroma in the area of injury and infection swell and undergo necrosis. Acute inflammatory cells (mainly neutrophils) surround the beginning ulcer and cause necrosis of the stromal

lamellae. Diffusion of inflammatory products including cytokines posteriorly elicits an outpouring of inflammatory cells into the anterior chamber and may create a hypopyon. Different bacterial toxins and enzymes (including elastase and alkaline protease) may be produced during corneal infection contributing to the destruction of corneal substance.

Local predisposing causes of keratitis include trauma, contact lens wear and ocular surface disorders. Trauma may be mechanical or non-mechanical, all types of contact lenses have been implicated, and the ocular surface lesions that predispose to corneal infection include dry eye syndromes and other disorders that result in conjunctival scarring such as trachoma. Persons who have altered host defenses such as reduced blinking, decreased tear flow, lowered tear lysozyme, chronic blepharitis, and reduced corneal sensation are also all predisposed to keratitis. The immune state of the host probably determines the severity but not the susceptibility to corneal infection, which in the immunocompromised has a fulminant and prolonged course. Symptoms of corneal infection include foreign body sensation, pain, photophobia, and blepharospasm. Red swollen lids are accompanied by a purulent discharge. Clinical examination of the cornea reveals suppuration, usually centrally, with an overlying epithelial defect and adherent mucopurulent or purulent exudates. Intraocular involvement results in hypopyon, possibly secondary glaucoma and a mild verities (Harry and Misson, 2001).

Hosted bacteria may infect the cornea, while others are found on the lid margin as part of the normal flora. Some of the bacteria responsible for corneal infection are: *Pseudomonas aeruginosa*, *Staphylococcus aureus, Staphylococcus epidermidis, and Streptococcus pneumoniae*. The conjunctiva and cornea are protected against infection by blinking, washing away of the debris by the flow of the tears, entrapment of the foreign particle by mucus, the antibacterial property of the tears and the barrier function of the corneal

epithelium (James *et al.*, 2007). Gram-positive cocci (*Staphylococcus* and *Streptococcus* species), *Pseudomonas* (Gram negative), and fungi species were the predominating causative agents responsible for suppurative microbial keratitis in Iraq (Al-Shakarchi, 2007).

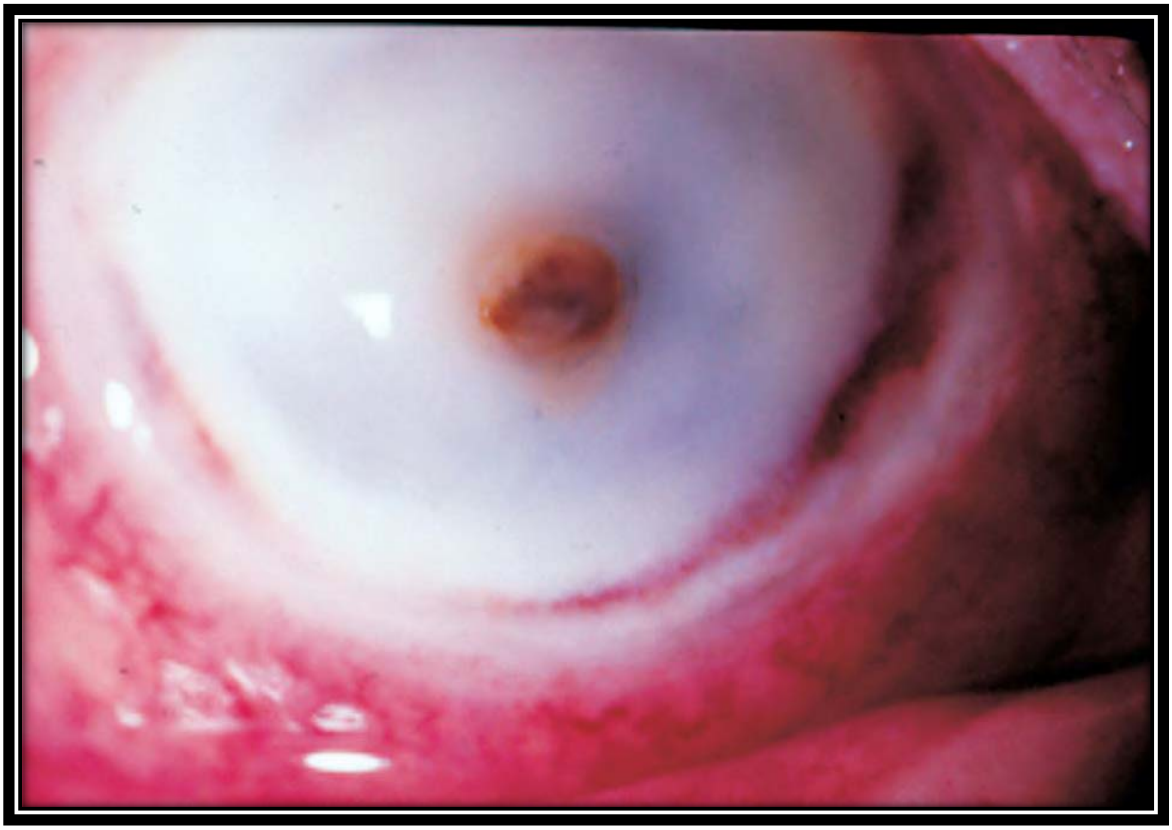


Figure (1- 2): Eye suffering from *Pseudomonas* keratitis (Kordic *et al*., 2008).

1.2.2.1.1- Bacteria causing keratitis:

Pathogens occur mostly as either bacteria or viruses. Others occur as fungi, protozoans or parasitic worms. These cause disease symptoms in a variety of ways. When these pathogens invade the host's tissue, they interfere with the normal functions of the body and in some other cases they destroy cells and tissues of the host's organ, the eye inclusive (Ikonne and Odozor, 2009). Various types of bacteria are able to cause keratitis. Following are the common species:

1.2.2.1.1.1- *Pseudomonas aeruginosa*:

Pseudomonas aeruginosa is a Gram-negative non-spore forming, straight or slightly curved rods. They are typically motile by means of one or more polar flagella, aerobic rod belonging to the family Pseudomonadaceae (Lomholt *et al*., 2001).

Ryan and Ray (2004) reported that the simplest medium for growth of *P.aeruginosa* consists of acetate for carbon and ammonium for nitrogen. With an optimum temperature for growth of 37 ◦C despite its ability to grow at temperatures as high as 42◦C in the laboratory. It is resistant to high concentration of salts and dyes, weak antiseptic and many commonly used antibiotics. *P.aeruginosa* secretes a variety of pigments, including pyoverdine (yellow-green and fluorescent), pyocyanin (blue-green) and pyorubin (red-brown). These natural properties of the bacterium, certainly, contribute to its ecological success as an opportunistic pathogen (Collee *et al*., 1996).

Vermon *et al*., (2006) mentioned that *P.aeruginosa* causes far reaching deleterious infection in the human eye. It is one of the most common causes of bacterial keratitis and has been isolated as the etiologic agent of neonatal ophthalmia. *Pseudomonas* can colonize the ocular epithelium by means of a fimbrial attachment to sialic acid (a component of mucinous coatings the epithelial cells which's responsible for the increase in salivary aggregation of *Pseudomonas aeruginosa)* receptors . If the defenses of the environment are compromised in any way, the bacterium can proliferate rapidly and through the production of enzymes such as elastase, alkaline protease and exotoxin A, causes a rapidly destructive infection that can lead to the loss of entire globe.

Pseudomonas aeruginosa, a major opportunistic pathogen, is a common cause of bacterial keratitis, especially in the contact lens wearers. Its infection

leads to a rapid destructive keratitis and potential perforation within (2–4) days (Fleiszig and Evans, 2002).

The pathogenesis of *P. aeruginosa* keratitis consists of colonization of the cornea, induction of pro-inflammatory cytokines (TNF-α, IL-1) (Kernacki *et al*., 1998; Rudner *et al*., 2000), the migration of neutrophils into the cornea to kill and remove the pathogen and subsequent corneal damage due to the neutrophil response and bacterial factors (Pillar and Hobden, 2002).

Feldman *et al*., (1998) and Comolli *et al*., (1999) reported that flagella and pili play potential roles in the pathogenicity of *P. aeruginosa* as adhesion's factors. Type IV pili are surface appendages with a role in adherence (Hahn, 1997) or non-flagellar 'twitching' motility that are produced by many bacterial pathogens. It has been demonstrated that mutants of *P. aeruginosa* defective in twitching motility show reduced ability to colonize the cornea in a mouse model (Zolfaghar *et al*., 2003). Thus, twitching motility appears to contribute to the role of pili in corneal infection. In addition, it has been shown that flagellum assembly mutants are attenuated in their ability to invade corneal epithelial cells (Fleiszig *et al*., 2001). The flagella of *P. aeruginosa* also contribute to the inflammatory responses of corneal epithelial cells in a TLR5-NF-kB signaling a pathway-dependent manner (Zhang *et al*., 2003).

Pseudomonas aeruginosa is capable of secreting a number of proteins with potential roles in pathogenicity (Pillar and Hobden, 2002; Sato *et al*., 2003; Schulert *et al*., 2003). These include proteases (alkaline protease, staphylolytic protease, elastase, protease IV), heat-labile and heat-stable haemolysins, phospholipase C and exotoxins A, S, T, U and Y. Clinical isolates of *P. aeruginosa* that secrete type III toxins (ExoS, ExoT, ExoU and ExoY) have been associated with greater morbidity or mortality (Finck-Barbancon *et al*., 1997; Fleiszig *et al*., 1997; Roy-Burman *et al*., 2001).

Fleiszig *et al.*, (1997) reported that *P. aeruginosa* isolates generally conform to one of two pathogenicity-related phenotypes: cytotoxicity or invasion, and that these phenotypes correlate with the presence of exoU (encoding exotoxin U) or exoS (encoding exotoxin S), respectively. It has since become clear that some strains of *P. aeruginosa* can exhibit exoU-independent cytotoxicity (Dacheux *et al.*, 2000). However, a number of studies have assessed the relative distributions of these mutually exclusive type III effector genes (Fleiszig *et al*., 1997; Parsons *et al*., 2002; Berthelot *et al*., 2003; Lanotte *et al.*, 2004). Under iron-limiting conditions, *P. aeruginosa* strains secrete siderophores such as the yellow-green pyoverdines. There are at least three different pyoverdines (type's I–III) that chelate iron, with the resulting ferri-pyoverdine complex being transported back into the bacteria by specific cell-surface receptor proteins (FpvAI-III) (De Chial *et al*., 2003). Beare *et al*., (2003) found that Pyoverdines also acts as signaling molecules inducing the production of three secreted virulence factors exotoxin A, PrpL endoprotease and pyoverdine itself.

There is evidence that some *P. aeruginosa* strains are more virulent and better able to invade the cornea (Fleiszig *et al*., 1997). Such properties may be a consequence of either the combination of genes possessed by a particular strain or variations in gene expression. Epidemic clones of *P. aeruginosa* have also been reported amongst isolates from patients with keratitis (Lomholt *et al*., 2001). They were characterized by high activity of an unusually sized elastase, high alkaline protease activity and possession of the exoU gene, a recognized marker for cytotoxicity (Lee *et al*., 2003).

1.2.2.1.1.2-*Staphylococcus aureus*

Staphylococcus aureus belongs to the group of bacteria called *staphylococci*, which are gram positive occurring in microscopic clusters

resembling grapes. Its colony is large yellow on rich medium and is often hemolytic on blood agar. It can grow at a temperature range of 15 to 45 ◦C and in Sodium chloride concentrations as high as 15% (Todar, 2004).

Almost all strains of *S. aureus* produce enzyme coagulase *S. aureus* which causes a variety of suppurative (pus-forming) infections in humans. It causes superficial skin lesions such as boils, sties, pneumonia, meningitis, urinary tract infections and deep seated infections such as osteomyelitis and endocarditis (Prescott, 2005).

Asbell and Stenson (1982) stated that *S. aureus* is the leading cause of bacterial keratitis in adults, including those who have sustained penetrating corneal injuries or are compromised by immunodeficiency. *Staphylococcus* keratitis can result in irreversible corneal scarring, leading to the loss of visual acuity or blindness. Multidrug-resistant strains of *S. aureus* further complicate the therapy of these infections (Moreira and Daum, 1995; Liesegang, 1998). Tissue damage during *Staphylococcus* keratitis results from the action of bacterial products on ocular tissues and from the host inflammatory response to infection (Chusid *et al*., 1979; Callegan *et al*., 1994). Bohach *et al*., (1997) mentioned that *S. aureus* can produce a variety of toxins, including the hemolytic exoproteins α, β, δ, and γ toxins. In the rabbit keratitis model, strains producing α toxin have been shown to cause extensive tissue damage and ocular inflammation.

Purified α toxin injected into the rabbit cornea in nanogram quantities caused corneal epithelial erosions, marked edema, and ocular inflammation (Moreau *et al*., 1997). β toxin has been shown to induce edema in rabbit eyes during keratitis and when purified toxin is injected into the cornea. However, strains producing δ toxin, but not other hemolysins, produce minimal corneal virulence, suggesting that δ toxin is not an important virulence factor in keratitis (Callaghan *et al*., 1997). When the roles of specific *staphylococcal* proteins (α-

toxin and protein A) was examined in corneal virulence of an experimental rabbit model of keratitis, results showed that proteins other than α-toxin were contributing to the ocular virulence during *S. aureus* keratitis (Callegan *et al*., 1994). The expression of multiple proteins potentially involved in virulence is controlled by the accessory gene regulator (Agr) system. Mutants defective in Agr demonstrate reduced expression of some proteins normally induced in stationary phase (e.g.,β-toxin) and do not express many other such proteins, including several hemolytic toxins and enzymes(Kornblum *et al*., 1990). Agr-defective mutants produce increased quantities of coagulase and the cell wall-associated proteins, including protein A, clumping factor, and fibronectin binding protein (Foster and McDevitt, 1994).

1.2.2.1.1.3- *Staphylococcus epidermidis:*

Staphylococcus epidermidis is a Gram-positive, coagulase negative coccus, grows best in aerobic conditions, and is a part of our normal flora. The hosts for the organism are humans and other warm-blooded animals. It is one of the leading pathogens of nosocomial infections, particularly associated with foreign body infections (Nilsson *et al*., 1998).

Staphylococcus epidermidis, a commensal of the eye, accounts for 45% of total cases of bacterial keratitis. However, the pathogenic potential of it which is responsible for such a high percentage of keratitis, has been explored only recently (Nayak and Satpathy, 2000).

Nayak *et al*., (2002) and Nayak *et al*., (2007) mentioned that a polysaccharide slime was one of the virulence factors of *S.epidermidis* in extra ocular infections and has been shown to be produced by isolates from keratitis' lesions, rather than by control organisms. The role of slime in the pathogenicity of *S.epidermidis* in bacterial keratitis is mainly due to slime mediated adherence.

Ismail *et al*., (2011) reported that one important property of *S.epidermidis* which is responsible for its persistence and opportunistic invasion in the tissue is its ability to produce slime. Slime not only helps the organism in adhesion to host cells but also protects it from phagocytosis and from the action of antibiotics. This species is predominant and widely spread throughout the cutaneous ecosystems. This coagulase negative bacterium, previously regarded as a commensal organism of low virulence, has recently been recognized as an emerging etiological agent of numerous clinical conditions. *S.epidermidis* is an opportunistic pathogen that requires a predisposed susceptible host in order to change from a normal inhabitant of human skin to an infectious agent. Most of the infections are hospital acquired and inflict those individuals who have either implanted medical devices or have undergone surgical intervention, as well as immunosuppressed patients (Blum and Rodvold, 1987; Kloos and Bannerman, 1994).

The pathologies caused by *S. epidermidis* range from bacterial keratitis, postoperative wound infections, rejection of indwelling foreign devices (such as prosthetic joints and cardiac valves) to a bacterimia often developed in immunocompromised patients (Baddour *et al*., 1987; Blum and Rodvold, 1987; Tabbara *et al*., 2000).

As many *S. epidermidis* isolates are multi-antibiotic resistant, such infections are very serious and can even be fatal. The pathogenicity of *S. epidermidis* is mainly due to its ability to form biofilms on the surfaces of indwelling synthetic devices and damage heart valves (Bayston and Rodgers., 1990).

The hydrophobic nature of the cell surface is crucial for bacterial adherence during initial colonization of plastic or metal foreign bodies, while in the later stages a copious amount of extracellular polysaccharide material is

synthesized, forming a protective multilayered biofilm preventing the clearance of bacteria by host defense mechanisms and making infection difficult to eradicate (Blum and Rodvold, 1987).

1.2.2.2- Fungal keratitis:-

Mycotic keratitis is an infection caused by fungus that leads to inflammation and ulceration, usually following trauma or treatment for a bacterial infection with steroids or antibiotics (Reddy *et al*., 1972).

Srinivasan, (2004) explained that fungal keratitis is a common cause of blindness worldwide, especially in some developing countries with a warm and wet climate. The ocular surface is constantly exposed to a large number of infectious agents. However, only a few pathogens can cause a corneal infection. Several mechanisms play a major role in the protection of the surface of the eye from infectious agents. Filamentous fungi are frequent causes of fungal corneal ulcers in humans. More than 105 species of fungi, classified in 56 genera, have been identified as the etiological agents of fungal keratitis. Fungal keratitis can cause a deep and severe corneal ulcer. It is caused by *Aspergillus* spp., *Fusarium* spp., Candida spp. and other fungi (Thomas, 2003; Kumara *et al*., 2010).

The typical feature of fungal infection is slow onset and gradual progression, where signs are much more than the symptoms. Small satellite lesions around the ulcer are a common feature of fungal keratitis and hypo yon is usually seen. Keratitis due to filamentous fungi is believed to usually occur following trauma, the key predisposing factor, in healthy young males engaged in agricultural or other outdoor work. The traumatizing agents can be of plant or animal origin (even dust particles), that either directly implant fungal conidia in the corneal stroma or abrade the epithelium, permitting invasion by exogenous fungi. Environmental factors (humidity, rainfall, wind) greatly influence the occurrence of fungal keratitis. Two basic forms have been recognized; first,

filamentous fungi (especially *Fusarium* and *Aspergillus*), and second, yeast (particularly *Candida*). Fungal infections of the cornea must be promptly recognized to facilitate a complete recovery. Symptoms are usually non-specific, although possibly more prolonged in duration (5–10 days) than in bacterial corneal ulcers (Thomas, 1994; Thomas, 2003).

Shukla *et al*., (2008) declared that usually a consequence of injuring the cornea in a farm like setting or in a place where plant material is present, fungal keratitis often develops slowly. The predisposing factors for this condition include immuno-compromised conditions such as excessive use of broad-spectrum antibiotics, in discriminate use of corticosteroids, diabetes, HIV, Hepatitis and AIDS. This infection had been a clinical rarity in the past but at present it is a well-recognized ailment that is also known as mycotic keratitis or hypopyon corneal ulcer. As shown in figure (1-3).

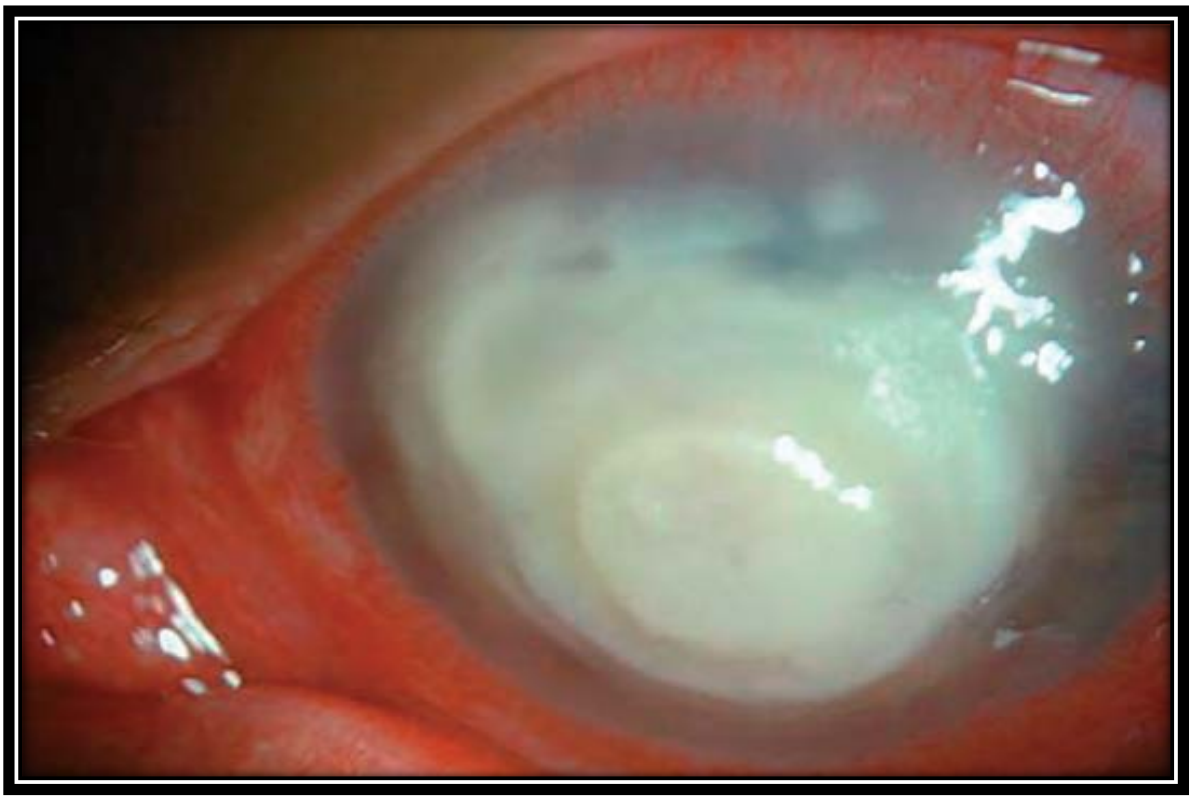


Figure (1-3): Eye infected with fungi and hypopyon appeared (Freda, 2006).

1.2.2.2.1 Fungi causing keratitis:-

1.2.2.2.1.1 *Aspergillus* spp.:

Species of the genus *Aspergillus* are epidemiologically important. These ascomycetes were among the first recognized etiologies of corneal infection and remain common isolates from oculomycoses (Hua *et al*., 2010).

Filamentous fungi have replaced bacteria as the predominant cause of infectious keratitis in some developing countries. Keratomycosis, also known as fungal keratitis, is a superlative, usually ulcerative corneal disease (Thomas, 2003).

Aspergillus keratitis is frequent in agriculture-based geographical regions with hot, humid, tropical, or subtropical climates. Those at the highest risk are young and middle-aged male agricultural workers as they are more exposed to the possibility of corneal trauma with fungus-contaminated material (Kredics *et al*., 2009). Certain *Aspergillus* species, mainly *A.flavus, A. terreus, A. fumigates* and *A. niger*, have long been regarded as important pathogens in eye infections, especially keratitis (Manikandan *et al*., 2008). Other members of the genus less frequently occurring in keratitis include *A. glaucus, A. ochraceus and A. tamarii* (Kredics *et al*., 2007).

Perfect *et al*., (2001) illustrated that *Aspergillus niger* is a filamentous ascomycetes fungus that is ubiquitous in the environment and has been implicated in opportunistic infections of humans (Magnuson *et al*., 2004). It's a member of the microbial communities found in soils by playing a significant role in the global carbon cycle (Baker, 2006).

Identification to the species level of *Aspergillus* causing keratomycosis would be of great importance since the pathogenic potential and antifungal susceptibilities may substantially vary among the species (Manikandan *et al*., 2010). Fungal keratitis can occur among soft contact lenses wearer as a result of inappropriate cleaning or disinfecting procedure, fungal contamination of

clearing or preservation solution for lenses, or due to microtruma of the cornea resulting from continuous use of the lenses. In these cases, the fungus can adhere to the surface of the contact lenses, invade the lenses or the cornea directly, facilitated by fungal enzymes, and cause keratitis (Simmons *et al*., 1986).

Adherence of microorganisms to the surface of contact lenses may be an important step in the pathogenesis of corneal infection associated with contact lenses. Microorganisms may adhere to the contact lens surface and after reaching a critical concentration, adhere to or penetrate the adjacent corneal epithelium. Metabolic and or morphological changes in the corneal epithelium induced by the contact lens may be an important antecedent event before infection of the cornea can be established (Sionov *et al*., 2001).

1.2.2.2.1.2- *Alternaria* spp.*:*

Alternaria spp. has sometimes been involved in human infections. These fungi commonly isolated from plants, soil, food, and indoor air environments. At present, their importance as opportunistic pathogens is clearly increasing with the growing number of patients who are immune-compromised (Ferrer *et al*., 2003).

Ozbek *et al*., (2006) mentioned that *Alternaria* is a filamentous fungus normally isolated from plants or soils. Of the *Alternaria* genus, *A.alternata* is the most common species associated with infections in humans. Fungal keratitis is a corneal infection that is difficult to treat (Flor and Peczon, 2008). *Alternaria* keratitis requires higher *in vivo* concentrations of older generation antifungal agents because of its high in vitro minimum inhibitory concentrations (MICs). *Alternaria* keratitis was first reported in 1975; the patient required penetrating keratoplasty, and the graft eventually failed. Such type of keratitis has been associated with immune-suppression and agricultural trauma (Tuey, 2009). Though a rare infection, *Alternaria* keratitis has recently emerged as an

opportunistic infection after laser-assisted in situ keratomileusis (LASIK) procedures and with soft contact lens use (Neoh *et al*., 2011).

1.2.2.3-Tear film:

Tears are a complex biological mixture, containing electrolytes, proteins, lipids, mucins, some small organic molecules and metabolites which have antimicrobial effects (Harding, 1997).

Berta, (1992) stated that functions of the tear film include lubrication, protection from disease and nutrition of the cornea. It also plays a critical role in the optical properties of the eye. The tear film consists of three layers: an inner mucin layer; a middle aqueous layer which contains electrolytes, proteins and various metabolites; and an outer lipid layer (Gachon *et al*., 1982). Major tear proteins include lysozyme, lactoferrin (lactotransferrin), secretory immunoglobulin A (sIgA), serum albumin, lipocalin previously called tear-specific pre-albumin (Fung, *et al*., 2002).

1.2.2.3.1- Tear film composition:

1.2.2.3.1.1- Lysozyme:

Lysozyme (EC 3.2.17) its belong to hydrolyses group, it's also known as muramidase or N-acetyl muramideglycan hydrolase, is a ubiquitous bacteriolytic enzyme found in most biological secretions, including saliva, mucus, mother milk, egg hen white and tears (Hankiewicz and Swierczek, 1995; Ohashi *et al*., 2006). It's one of the powerful nature antibacterial and antiviral activity. This abundant protein has been explored extensively in many fields of research, including physicochemical (Le Brun *et al*., 2009; Marchal *et al.*, 2002) crystallographic (Strynadka and James, 1996), enzymatic (Barniak *et al*., 2010; Swaminatham *et al.*, 2011) and immunological studies (Mai and Wang, 2010).

Lysozyme was first discovered in 1922 by Alexander Fleming, the discoverer of penicillin and since then has been well known for its antibacterial property (Fleming, 1932). Fleming discovered that his nasal drippings, containing natural lysozyme, triggered the death of bacterial cells in his Petri dish. This phenomenon as a result of the lysozyme-catalyzed hydrolysis of β-1,4-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in a peptidoglycan of the bacterial cell wall (Ghuysen,1994). Consequently, the damaged bacterial cell wall loses its structural integrity and ruptures, which results in subsequent cell death. Lysozyme is part of the innate immune system.

The concentration of lysozyme found in human tears is the highest (1.7 to 1.9 mg/ml in the open eye) of all body fluids (Fullard and Tucker,1994) accounting for 40% of the total protein content of tears (Castillo *et al*.,1986; Keith *et al*.,1997). This enzyme provides antibacterial protection against microbes that may cause ocular infections such as bacterial keratitis (Lal *et al.*, 1991).

Lysozyme can be regarded as an Active Pharmaceutical Ingredient. The main pharmaceutical use of lysozyme is in nasal and throat preparations. Other applications include use as an anti-bacterial (against bacterial or viral inflammatory diseases), in aerosols for the treatment of bronchopulmonary diseases. Also prophylactically for dental caries, and in creams for protection against topical reparation of certain dystrophic and inflammatory lesions of the skin and soft tissues. Apart from the pharmaceutical application of lysozyme, the product is used in the preservation of mainly fermented food products, like cheese and wine (Mai and Wang, 2010).

1.2.2.3.1.2-lactoferrin (lactotransferrin)

Lactoferrin is a glycoprotein, and a member of a transferrin family, thus belonging to those proteins capable of binding and transferring Fe3+ ions (Metz-Boutique *et al.*, 1984). Due to the increase in its concentration during

most inflammatory reactions and some viral infections, several authors classify lactoferrin as an acute-phase protein (Kanyshkova *et al*., 2001). It is considered to be a part of the innate immune system. At the same time, it also takes part in specific immune reactions, but in an indirect way (Legrand *et al*., 2005). Due to its strategic position on the mucosal surface, lactoferrin represents one of the first defense systems against microbial agents invading the organism mostly via mucosal tissues.

Lactoferrin affects the growth and proliferation of a variety of infectious agents including both Gram-positive and negative bacteria, viruses, protozoa, or fungi (Kirkpatrick *et al*., 1971). *In vitro*, lactoferrin is able to prevent *Pseudomonas aeruginosa* biofilm formation. The lack of iron in the environment forces bacteria to move. Therefore, they cannot adhere to surfaces (Singh *et al*., 2002). Lactoferrin may contribute to defense against the invasion of facultative intracellular bacteria into cells by binding both target cell membrane glycoaminoglycans and bacterial invasions, which prevents pathogen adhesion to target cells. This ability was first reported against *Staphylococcus aureus* (Valenti and Antonini, 2005).

2.2.3.1.3-Secretory immunoglobulin A (sIgA):

Secretory immunoglobulin A (SIgA) is the major immunoglobulin in secretions (Brandtzaeg *et al*., 2001). Tomasi (1994) stated that the predominance of secretary SIgA in secretions suggests that it has a role in protecting mucosal surfaces.

Svanborg-Eden, and Svennerholm (1978) found that SIgA is one of the best-defined effector mechanisms of the mucosal immune system and also it represents an important defense mechanism at the ocular surface. SIgA prevents bacteria from adhering to the mucosa and disposes of bacteria (Williams and Gibbons, 1972). Although SIgA is the predominant immunoglobulin in tears, it

has not been demonstrated to prevent or reduce bacterial colonization of the ocular surface (Knopf et *al*., 1970).

Sen and Sarin (1979) found elevated SIgA levels in patients with acute bacterial conjunctivitis, blepharo-conjunctivitis, and kerato-conjunctivitis. In patients with corneal ulcers, the values were similar to those in control subjects. Specific SIgA antibodies occur naturally against the commensal conjunctival flora (Gregory and Allansmith, 1986; Lan *et al*, 1997) and are induced by the presence of pathologic microbes such as *Acanthamoeba* (Leher *et al*., 1998) and *Pseudomonas* (Masinick *et al*., 1997). SIgA bound microbes are attached via a secretory chain to the mucus layer (Phalipon *et al*., 2002) resulting in their immobilization and discharge with the continuous renewal of the tear film (Argueso and Gipson, 2001).

2.2.3.1.4-Lipocalin:

Tear lipocalins (TL) were discovered in 1995 and first reviewed in the year 2000; they are surface active (Glasgow, 1999; Green-Church, 2011) and may intercalate with lipids at the air-tear interface. Millar (2009) reported that the lipocalin protein family consists mainly of small extracellular proteins that bind hydrophobic ligands and fulfill numerous biological functions including ligand transport, cryptic coloration, sensory transduction, the biosynthesis of prostaglandins, and the regulation of cellular homeostasis and immunity. Lipocalin was thought to be restricted to eukaryotes. The recent crystal structure of a bacterial lipocalin suggests that phospholipids derivatives are likely ligands (Campanacci, 2004).

Bishop (1995) stated that the existence of bacterial lipocalins provides insight into the origins of the lipocalin family and, combined with the powerful tools of bacterial genetics, provides fertile ground for the investigation of

lipocalin structure and function. Tear lipocalin is an unusual lipocalin member, because of its high promiscuity for relative insoluble lipids and binding characteristics that differ from other members. In addition, it shows inhibitory activity on cysteine proteinases similar to cystatins, a feature unique among lipocalins. Although it acts as the principal lipid binding protein in tear fluid, a more general physiological function has to be proposed due to its wide distribution and properties. It would be ideally suited for scavenging of lipophilic, potentially harmful substances and thus might act as a general protection factor of epithelia (Glasgow,1999).

Chapter Two

Materials and Methods

2. Materials and Methods

2.1-Materials:

2.1.1- Apparatus and Equipment:-

Equipment	Company/Origin
Autoclave	Express /Germany
Compound Light Microscope	Olympus /Japan
Digital balance	Ohans /France
ELISA Reader	Bioelisa reader ELx 800/Italy
ELISA Printer	Epson LX-300/Italy
ELISA Washer	Organon Teknlka/Australia
Incubator	Termaks /U.K
Laminar air flow hood	Heraeus /Germany
Micropipette	Witey /Germany
Oven	GallenKamp Sayo/U.K
pH-meter	Radiometer/Denmark
Water bath	Memmert/Germany
Water distiller	GFL/Germany

2.1.2- Kits:

Kit	Company	Origin
Anti-Lysozyme ELISA Kit	DRG	Germany
API 20E	BioMerieux	France

API STAPH.	BioMerieux	France
IgA immunodiffusion plate	LTA	Italy

2.1.3- Chemical and biological materials:

Material	**Company/Origin**
Agar	Biolife /Italy
Glycerol, Ethanol, Sodium hydroxide	Merck /Germany
Peptone , Hydrochloric acid, Methyl red	BDH /U.K
Tetramethyl-*p*-phenylenediamine dihydrochloride, Potassium hydroxide	Sigma /USA
Iodine, Hydrogen peroxide	Fluka /Switzerland
Crystal violet, Methylene blue, Safranine	Himedia /India

2.1.4- Media:

2.1.4.1- Ready-to-use media:

These media were prepared and sterilized according to the instructions fixed on their containers by the manufacturing companies.

Medium	Company\Origin
Brain heart infusion agar	Salucea/Germany
Brain heart infusion broth	Difco /U.S.A
Simmon citrate media	Oxoid / England
Triple sugar iron (TSI) agar	Difco
Nutrient agar	Oxiod
Nutrient broth	Oxiod
Sabrouad agar	Biolife /Italy
Mannitol salt agar, DNase	Oxiod
MacCconky	BDH/England

2.1.4.2- Laboratory-prepared media:-

2.1.4.2.1-Blood Agar (Atlas *et al*., 1995):

It was prepared by dissolving 37g of blood base agar in 950 ml of D.W.; pH was adjusted to 2.0 then sterilized by autoclave. After cooling to 50ºC, 5% of human blood was added and mixed well before distributed into Petri-dishes.

2.1.4.2.2-Methyl Red Vogas-Proskauer (MR-VP) Medium (Atlas *et al*., 1995):

This medium was prepared by dissolving 5g peptone and 5g K2HPO4 in 900 ml of D.W.; then the volume was completed to 950 ml with D.W. and pH was adjusted to 7.6 before sterilized by autoclaving. After cooling to 50˚C, 50 ml of 10% glucose solution (previously sterilized by filtration) was added.

2.1.4.2.3-Urea Agar (Collee *et al*., 1996):

It was prepared by adjusting pH of the urea agar base to 7.0 and autoclaved, after cooling to 50˚C, 50 ml of 40% urea (previously sterilized by filtration) was

mixed with it and distributed into sterilized test tubes (20 ml each), then kept solidified in slant position.

2.1.4.2.4-Semi-solid medium (Atlas *et al*., 1995):

This medium composed of the following components:

Component	Weight(g)
Glucose	20
Yeast extract	2
Peptone	3
Agar	5

All components were dissolved in 900 ml of D.W., pH was adjusted to 7.0, then volume was completed to 1000 ml, distributed in test tubes (10 ml each), sterilized by autoclaving, and left to solidify in vertical position.

2.1.5- Reagents solutions and stains:

2.1.5.1- Catalase reagent (Atlas *et al*., 1995):

This solution was prepared to be consist of 3% hydrogen peroxide.

2.1.5.2- Oxidase reagent (Atlas *et al*., 1995):

A concentration of 1% of tetramethyl phenylene diamine dihydrochloride was freshly prepared in D.W. from the material.

2.1.5.3- Peptone Water (Atlas *et al*.,1995):

It was prepared by dissolving thoroughly 20g peptone and 5g NaCl into a quantity of D.W., then the volume was completed to 1L. pH was adjusted to 7.0 before distributing into test tubes (10 ml each) and autoclaving.

2.1.5.4- Methyl red indicator (Collee *et al*., 1996):

This indicator was prepared by mixing 0.05g of methyl red, 150 ml of 95% ethanol, and 100 ml of D.W.

2.1.5.5- Burritt's reagent (Collee *et al*., 1996):

It is consisted of two solutions:

Solution A: Potassium hydroxide (40%).

Solution B: Prepared by dissolving 5g of α-naphthol into 100 ml of absolute ethanol. Equal volumes from both solutions were immediately mixed before use.

2.1.5.6- Kovac's reagent (Atlas *et al*., 1995):

A portion of 10g of ρ-Dimethyl-aminobenzaldehyde was dissolved in 150 ml of isoamyl alcohol by heating in a water bath at 50C, then hydrochloric acid was added slowly. The reagent was prepared and stored in the refrigerator until use.

2.1.5.7- Gram stains and reagents (Atlas *et al*., 1995):

They were prepared according to Duguid, (1996).

2.1.5.8- Lacto phenol-cotton blue stain (**Atlas *et al*., 1995):**

This stain was prepared from the following components:

Component	Amount
Lactic acid	20 ml
Phenol crystals	20 g
Glycerol	40 ml
Cotton blue or Aniline blue (Poirier's blue)	0.05 g
D.W	20 ml

Phenol crystals were dissolved in lactic acid before added with the glycerol to the D.W. with the help of gentle heating, then aniline blue was added. The mixture was left for 24 hr. at room temperature, before filtration through Whitman filter paper No.1.

2.2- Methods:

2.2.1- Sterilization methods (Baily *et al*., 1990):

2.2.1.1- Glassware's: They were sterilized in the electric oven at 180◦C for 3 hrs.

2.2.1.2- Culture media and physiological solutions: They were sterilized by autoclaving at 121◦C (15 Ib/in^2) for 15 minutes unless otherwise stated.

2.2.1.3- Membrane filtration: It was used to sterilize the thermo-labile component, sugars and amino acid solutions through Millipore filter (0.2 μm).

2.2.2- Collection of specimens:

Specimens were obtained from patients suffering from microbial keratitis referred to Ibn Al-Haitham Eye Teaching Hospital in Baghdad for the period from October (2013) to March (2014). All Specimens were collected by medically qualified personnel, upon completion of the ocular examination and after installation of topical anesthetic. A sterile syringe needle was used to scrape the area of infection, then the scraping was divided into two portions; the first (for bacterial detection) was inoculated onto each of blood agar, chocolate agar, brain heart-infusion agar and MacConky agar and incubated at 37◦C for 2-3 days. The second portion (for fungal detection) was inoculated onto Sabrouad agar and incubated at 28 ◦C for 5-7 days. After incubation**,** positive cultures were further analyzed by subjecting their colonies to some biochemical tests for species identification.

The second part of the work included collection of tear films by the capillary tubes for further analysis.

2.2.3- Identification of bacterial isolates

Colonies of the suspected bacterial isolates were subjected to the following cultural, microscopic and biochemical tests for identification.

2.2.3.1- Cultural examination: (Garrity, 2005):

Colonies grown on the culture media were described according to their shape, size, margin, color and odor.

2.2.3.2 – Microscopical examination: (Atlas *et al*.,1995):

After smears were made from the colonies of bacterial isolates, they were Gram stained to describe cells shape, Gram reaction and grouping.

2.2.3.3- Biochemical tests:

The following tests were performed according to (Collee *et al*., 1996; Macfaddin, 2000; Garrity, 2005).

- **Catalase test:**

With a sterile toothpick, a single colony was placed onto a clean glass microscope slide, then a drop of hydrogen peroxide (3%) was placed onto the colony. Production of gaseous bubbles indicates positive results.

- **Oxidase test:**

This test was done by using moistened filter paper with few drops of a freshly prepared solution of tetra methyl-ρ-phenylenediamine dihydrochloride. Aseptically, a clump of cells was picked up from the slant growth with a sterile wooden stick and

smeared on the moisten paper. The positive result was detected by development of violet or purple color within 10 seconds.

- **Indole test:**

Peptone water was inoculated with fresh culture of the bacterial isolates, separately, before incubated at 37˚C for 24-48 hours. Fifty micro liters of Kovac's reagent (item 2.1.4.5) was added and mixed gently. Positive result was recorded by the appearance of a red ring on the surface of liquid medium.

- **Methyl red test:**

MR-VP broth was inoculated with fresh culture of the bacterial isolates and incubated at 37˚C for 48 hours. Five drops of methyl red reagent was added and mixed. Positive result was detected by changing color to bright red, while the negative is yellow.

- **Vogas-Proskauer test:**

MR-VP broth was inoculated and incubated at 37˚C for 24 hour. Then 1 ml of Burritt's reagent A (item 2.1.4.4) and 3 ml of Burritt's reagent B (item 2.1.4.4) were added to 5 ml of the cultured broth and shacked for 30 seconds. Changing color from pink to red indicates a positive test due to the production of acetoin.

- **Citrate utilization test:**

Simmon citrate agar slants were inoculated with the cultures of the bacterial isolates, then incubated at 37◦C for 24 hr. Changing color to blue represents a positive result.

- **Urease test:**

Urease activity was detected by inoculating the surface of Christensen urea agar slants with the bacterial growth and incubated at 37˚C for 24 hours. Appearance of a red-violet color indicates a positive result.

- **Triple Sugar Iron (TSI) test:**

Isolates were cultured on TSI agar slants by stabbing into the bottom and streaking on surface before incubation for (24-48) hours at 37˚C. The results could be summarized as following:

Slant / Butt	Color	Utilization
Alkaline / acid	Red / Yellow	Glucose fermented; peptones assimilated
Acid / acid	Yellow/Yellow	Glucose, lactose and sucrose fermented
Alkaline/alkaline	Red / Red	Glucose, lactose and sucrose not fermented/peptones assimilated

While the formation of black precipitate is an indication of H_2S production, and pushing the agar to the top indicates CO_2 formation.

- **Motility test:**

Semi-solid agar medium was stabbed with fresh culture of each bacterial isolate and incubated at 30˚C for 24 hrs. Motile bacteria typically give diffuse, hazy growth that spreads throughout the medium rendering it slightly opaque.

- **Coagulase test:**

Several colonies of each bacterial isolate were emulsify in 0.5ml of citrated rabbit plasma and incubated at 35˚C for 1-4 hrs in a water bath. Presence of clots indicates a positive result while negative when absent.

- **DNase test:**

Each bacterial isolate was cultured on DNase agar plates and incubated at 35 ˚C for 18-24 hrs. then the plate was flooded with 1N HCl. Formation of clear zones around the colony indicates a positive result.

2.2.3.4- Identification by APi 20E:

a- Preparation of the strips:-

Five milliliters of tap water were placed into the API inoculation tray, then API test strip was withdrawn from the sealed package and placed into the inoculation tray.

b- Preparation of inoculums:-

Aseptically, one colony of each bacterial isolates grown on MacConky agar was picked up with a sterile loop and transferred to a test tube containing 5 ml of sterile normal saline solution. After shaking well, tube was recapped before comparing its turbidity with the McFarland No.0.5 solution.

c- Inoculation of strips prepare:-

Bacterial suspension was transferred to the API test strip by a sterile pasture pipette, the API strip was tilted and the microtube was filled by placing the pipette tip against the side of the cupules', both the tube and cupules' sections of the **CIT, VP** and **GEL** micro tubes were filled. But the cupules' sections of **ADH, LCD, OCD, H_2S** and **URE** micro tubes were filled completely with sterilize mineral oil to prepare an-aerobic conditions. Then the test strip was incubated at 37˚C for 24 hrs. After incubation the reagents were added to the corresponding micro tubes.

i- One drop of 3.4 % ferric chloride to the **TDA** microtube.

ii- One drop of Kovac's reagent to the **IND** microtube.

iii- One drop of Vogas–Proskauer reagent to **VP** microtube.

The biochemical reactions performed by the APi 20E and their interpretation are listed in Appendix 1.

2.2.3.5- API STAPH.

a- Preparation of the strips:-

Five milliliters of tap water were placed into the API inoculation tray, then API test strip was withdrawn from the sealed package and placed into the inoculation tray.

b- Preparation of inoculums:-

Aseptically, one colony of each bacterial isolates grown on MacConky agar was picked up with a sterile loop and transferred to a test tube containing 5 ml of sterile normal saline solution. After shaking well, tube was recapped before comparing its turbidity with the McFarland No.0.5 solution.

c- Inoculation of strips prepare:-

Bacterial suspension was transferred to the API test strip by a sterile pasture pipette, the API strip was tilted and the microtube was filled by placing the pipette tip against the side of the cupules', both the tube and cupules' sections of the **NIT, PAL** and **VP** micro tubes were filled. But the cupules' sections of **ADH** and **URE** micro tubes were filled completely with sterilize mineral oil to prepare an-aerobic conditions. Then the test strip was incubated at 37˚C for 24 hrs. After incubation the following reagents were added to the corresponding microtubes:

i- One drop of (ZYM A & ZYM B) reagent to PAL microtube.

ii- One drop of (NIT 1 & NIT 2) reagent to NIT microtube.

iii- One drop of Vogas – Proskauer reagent to VP microtube.

The biochemical reaction performed by the APi STAPH and their interpretation are listed in Appendix 2.

2.2.4- Identification of fungal isolates:

2.2.4.1- Microscopical examination:

A loop full from each suspected fungal colonies was transferred onto a glass slide, then lacto phenol–cotton blue stain was added before examined under 40X magnification power of the compound light microscope for hyphae and spore morphological identification.

2.2.5- Maintenance of bacterial isolates:

Bacterial isolates were preserved according to Maniatis *et al*. (1982) as follows:

2.2.5.1- Short time storage:

Bacterial isolates were first cultured on nutrient broth and incubated at 37°C for 24 hr, then maintained for few weeks on nutrient agar slant by keeping them tightly wrapped with parafilm, before storing at 4°C.

2.2.5.2- Long time storage:

Bacterial isolates were first cultured on nutrient broth and incubated at 37°C for 24 hr, then they were maintained as stab cultures for few months with the addition of glycerol (20%) and stored at –20°C.

2.2.6- Maintenance of fungal isolates.

Fungal isolates were maintained for weeks to few months at 4◦C on slants of potato dextrose agar (PDA) with the cultures every 20 days.

2.2.7- Collection of tear samples (Zhou *et al.*, 2004).

Tears samples were collected by using fire-polished 75 µl calibrated glass micro capillary tubes (Drummond Scientific Co, USA), with special care taken not to touch the ocular surface. They were stored at -20◦C until used for further analysis.

2.2.8- Determination of the IgA glycoprotein in human tears:

The radial immunodiffusion plate method (Mancini and Coll., 1965) was used for this purpose**.** The plate was removed from its envelope and left to stand at room temperature for few minutes (to evaporate condensed water in the wells). Each well was filled with 5µl of the sample and\or controls and left until completely adsorbed, then the plate was placed in a moist chamber for 72 hrs. and the formed precipitating ring was measured with an appropriate ruler. IgA concentrations in normal human tear ranges from 9.0 to 45.0 mg/dl according to world health organization (WHO).

2.2.9- Determination of auto antibodies against lysozyme in human tear and other body fluids. (Hankie wiz and Swierczuk**.,** 1995).

2.2.9.1- Principle:

The quantitative immune-enzymatic determination of anti-Lysozyme concentration is based on the ELISA (Enzyme-Linked Immuno Sorbent Assay) technique. Micro-titer strip wells were pre-coated with highly purified anti-lysozyme antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material**,** horseradish peroxidase (HRP) labeled anti-human IgG conjugate was added. This conjugate binds to the captured lysozyme specific antibodies. The immune complex formed by the bound conjugate was visualized by adding tetra methyl benzidine (TMB) substrate which gives a blue reaction product. The intensity of this product was proportional to the amount of lysozyme specific IgG antibodies in the specimen. Sulphuric acid was added to stop the reaction. This products a yellow endpoint color. Absorbance at 450 nm was read using an ELISA micro well plate reader.

2.2.9.2-Reagents supplies:

- Divisible micro plate consisting of 12 modules of 8 wells each, coated with highly purified lysozyme in re-sealable aluminum foil.

- Sample buffer (contains Tris, NaN_3< 0.1 % (w/w)): The contents of each vial of the sample buffer concentrate (5x) were diluted with distilled water to a final volume of 100 ml; pH was adjusted to (7.0 -7.2). yellow color declares ready to use
- Stop solution: 1 vial containing 15 ml sulphuric acid.
- Washing solution (50x conc. Contains PBS, NaN_3 <0.1% (w/w)): 1 vial containing 20 ml of 50-fold concentrated buffer for washing the wells; pH 7.0-7.2.
- Enzyme conjugate solution (PBS, Proclin 300 < 0.5 % (v/v)): 1 vial containing 15 ml of polyclonal anti-human IgG; labeled with horseradish peroxidase; light red color declares ready to use.
- TMB substrate solution: 1 vial containing 15 ml 3, 3',5, 5'-tetramethyl benzidine (TMB) ready to use.
- Anti-lysozyme controls in a serum or tear matrix (PBS, BSA, NaN_3 < 0.1 (w/w)) positive (1) and negative (2) ready to use.
- Anti-Lysozyme IgG calibrators (containing PBS ,BSA, NaN_3< 0.1%(w/w)): 6 vials each containing 1.5 ml; ready to use.

Calibrator A: 0 NTU/ml.

Calibrator B: 6.3 NTU/ml.

Calibrator C : 12.5 NTU/ml.

Calibrator D: 25 NTU/ml.

Calibrator E: 50 NTU/ml.

Calibrator F: 100 NTU/ml.

2.2.9.3- Assay procedure

- Of each calibrators (A, B, C, D, E and F), a portion of 100 µl was dispensed, and samples were diluted into respective wells.
- Wells were covered with the foil supplied in the kit.
- Incubated for 30 minutes at room temperature (20 - 28◦C).

- After incubation, the foil was removed, the content of the wells was aspirated and each washed three times with 300 µl of washing solution.

Overflows from the reaction wells were avoided. The soak between each wash cycle was > 5 sec. At the end, the remaining fluid carefully was removed by tapping strip on tissue paper prior to the next step.

- A volume of 100 µl of enzyme conjugate was dispensed into each well and covered with foil.
- Incubated for 15 minutes at room temperature (20◦C- 28◦C).
- The washing step was repeated.
- A volume of 100 µl of TMB substrate solution was dispensed in each well.
- Incubated for 15min. at room temperature in the dark.
- A volume of 100 µl of stop solution was dispensed into each well of the modules and incubated for 5 min. at room temperature.
- Absorbance of the specimen was read at 450 nm within 30 min. after addition of the stop solution.

2.2.9.4- Interpretation of results:

Normal value ranges for the ELISA with tear samples from healthy donors have been established for the Anti-lysozyme test as follows:

Anti-lysozyme IgG [U/mL]

Normal: <10

Elevated: ≥ 10

2.2.9.4- Statistical Analysis:

The following statistical data analysis approaches were obtained under the application of the statistical package (SSPS) ver. (10.0)(Landau and Everitt, 2004).

- **Descriptive data analysis:**
  a- Tables (Frequencies and percentages).
  b- Descriptive Statistics (Mean, 95% Confidence Interval for mean, Standard deviation, Standard error, two extreme values Min. and Max.).
  c- Person's correlation coefficients.
  d- Graphical presentation by using:
  -pie.

- **Inferential data analysis:**

These were used to accept or reject the statistical hypotheses, which included the following:

a- T-test for testing two independent samples.
b- T-test, for testing the meaningful of the Person's correlation coefficients.

- For the abbreviation of the comparison significant (C.S.), we used the followings:
  -NS: Non significant at $P>0.05$
  -S: Significant at $P<0.05$
  -HS: Highly significant at $P<0.01$

Chapter Three

Results and Discussion

3. Results and Discussion:

3.1- Microbial keratitis patients:

3.1.1- Distribution of microbial keratitis according to gender:

From a total of 73 positive specimens belonged to patients suffering of microbial keratitis , results in table (3-1) show that males had a tendency to get ocular ulcer more than females when 41 (56.16%) of the patients were males and 32 (42.85%) females.

Table (3-1): Distribution of microbial keratitis patients according to age group and gender.

Age group (year)	Gender		Total no. of patients	Percentage (%)
	Male	Female		
≤ 20	9	8	17	23.27
21-40	12	9	21	28.73
41-59	13	9	22	30.20
≥ 60	7	6	13	17.80
Total	41	32	73	100.00

The relatively high percentage of males suffering microbial keratitis as compared with that of females came almost closely to some other similar studies. Chowdhary and Singh (2005) found that men (68%) were more affected by fungal keratitis than women (32%), and Tananuvat *et al*. (2004) reported that out of 214 eyes patients, males comprised about two-third (66.2%) compared to the females who represented only the remaining one-third (33.8%), while age distribution of the patients showed that it was common among the 30-60 years patients. Keshav *et al*. (2008) viewed that from 188 patients infected with microbial keratitis, 121

(64.36%) were males and 67 (35.73%) females, and regarding age group, 24 patients (12.76%) were <30 years of age, 43 (22.87%) aged between 30-60 years and 121 (64.36%) were >60 years. They referred the highest percentage of microbial keratitis among male patients to the greater frequency of subjection to corneal ulcer. Moreover, Sethi *et al*. (2010) found that among 100 patients clinically suspected of microbial keratitis, 67% were males and 33% females. They referred this high percentage by males to the fact that males have more chance of exposure to the accidents or trauma due to their outdoor activities.

Butler *et al.* (2005) found that from a total of 190 patients included in their study, there were 103 (54%) males and 87 (46%) females' patients, with a range of (60–101) recorded in the mean age group of 75.5 years. Gopinathan *et al*. (2002) mentioned in their study that from a total of 156 patients, there were 102 (65.4%) males and 54 (34.6%) females with a mean age of 48 years.

Norina *et al*. (2008) reported that out of 42 patients included in their study, 61.9% were males and 38% females, with a mean age of 44.5 years. Green *et al*. (2008) also found that the microbial keratitis infection was higher in males (60%) than in females with a mean age of 51 years.

3.1.2- Distribution of microbial keratitis patients according to age:

Regarding age groups, results in table (3-1) show that patients of 41-59 years were the highest keratitis infected group with 22 (30.18%) of the total cases, followed by the 21-40 years group which was not too far (28.76%) from the highest one. On the other hand, ≥ 60 years group recorded the lowest cases of microbial keratitis. The highest percentage of infection recorded in the oldest age group (≥ 60) may be due to certain causes such as week immunity and predisposing ocular disease facing this age group (Bourcier *et al*., 2003).

Such results are similar to those of each of Boonpasart *et al* (2002), who found that microbial keratitis patients were mainly of the group (51-60) years, and Al-Yousuf (2009), who reported that the mean age for patients with microbial keratitis was 51 year. While Schufear *et al*., (2001) elucidated that 44.3 years was the mean age of the 85 patients suffering from bacterial corneal infiltrate. Age of those patients was in a range between 7 and 91 years. In a previous study conducted at Ibn Al-Hiatham Teaching Eye Hospital in Baghdad, the mean age of the patients suffering from microbial keratitis was 47.1 years (Al-Shakarchi, 2007). Bharathi *et al*. (2003) reported that the percentage of male patients in microbial keratitis culture was 65.02% compared to 34.98% for female, distributed on the age groups as (14.7% for 21, 20.91% for (21-30), 24.38% for (31-40), 21.55% for (41-50) and 18.45% for 50) years of ages.

3.2.1- Isolations and identifications of bacteria causing keratitis:

The 73 samples of keratitis patients were primary incubated for 24-48 hrs. on blood agar and chocolate agar. After incubation, grown suspected microbial isolates were further identified depending to their cultural, microscopic and biochemical characteristics in accordance with the Bergey's Manual of Determinative Bacteriology (Holt et *al*., 1994). As shown in table (3-2), the microbial cultures considered relevant when growth of the same organism has been observed on more than one position of the inoculation, or if the same organism has been grown from repeated scrap.

Out of the 105 tested samples, 73 (69.52%) gave positive growth on both medium, while the rest 32 (30.48%) were reported as negative results by showing no growth despite their plates were continued to be incubated up to one week. Such findings were closed to those obtained by Chiquet *et al*., (2007), who reported that the cultural method gave a growth positivity of about 56%. Adversely Anand *et al*. (2001) found that culturing method gave only 25% of positivity, referring that to

various factors such as size of the specimens, fixation of microorganisms on the solid surfaces (intraocular lens, lens fragment), consequent decrease of cells in the vitreous/ aqueous humor, taken antibiotics by patients before collection of clinical material or present of fastidious microorganisms such those causing endophthalmitis (Gupta *et al*., 2008).

Table (3-2) Cultural, microscopical and physiological characteristics of bacteria isolated from patients suffering of eye keratitis.

Test		**Bacterial isolate**		
Colony		***P.aeruginosa*** **(29 isolates)**	***S.aureus*** **(22 isolates)**	***S.epidermidis*** **(17 isolates)**
	Color	**Green**	**Yellow**	**Pale white**
	Shape	**Rod**	**Coccus**	**Coccus**
Gram reaction		–	+	+
Oxidase		+	–	–
Catalase		+	+	+
Urease		+	–	+
DNase		N	+	–
TSI	**H2S**	+	–	–
	CO2	–	–	–
	Acid	**Alk/Alk**	**Acid/Acid**	**Acid/Acid**
MR		–	+	–
VP		–	+	–
Citrate Utilization		+	–	–
Indole		–	–	–

Coagulase	**N**	**+**	**–**
Mannitol	**N**	**+**	**–**

(+): positive result. (-): negative result. N: not tested.
TSI: triple sugar iron agar. MR: methyl red VP: Vogas-Proskauer.

From the (69.52%) of the microbial isolates, (64.76%) were bacterial causatives, in which (39.72%) of them belonged to *Pseudomonas aeruginosa*, (30.13%) to *Staphylococcus aureus* and (23.28%) to *Staphylococcus epidermidis*. In this regard, Ali (2004) reported that out of 111 cases, 72(64.9%) were found to give positive cultures. Gram-positive isolates were prevalent (81.1%) and *Staphylococcus* spp. was the most common, while *Pseudomonas* spp. was the most common among Gram-negative isolates.

Al-Shakarchi *et al*. (2003) found that out of 86 patients referred to Ibn Al-Hiatham Teaching Eye Hospital in Baghdad, positive cultures were obtained in 54 cases; 41 of them were caused by bacteria with *Pseudomonas* spp. (19 cases) as the most common bacterial isolates, followed by *Staphylococcus* spp. (17 cases). Fungal growth, on the other hand was detected in 13 cases.

Tananuvat *et al*. (2004) found that significant growth of microorganisms was obtained in (30.16%) of the specimens they examined. Of these, bacteria were the most occurred when accounted for 61% of the cases, with *P. aeruginosa* as the most common species. While fungi were isolated in 39% of the cases, with *Fusarium* spp as the highest (14.04%) followed by *Aspergillus* (7.02%). Keshav *et al*.(2008) elucidated that *Pseudomonas* spp were seen among over half (53.84%) of the cases of bacterial isolates. while *Staphylococcus* was found in 13 of the cases (20%), *Streptoccocus pneumoniae* was found in 12 cases (18.46 %), other

Streptococcus spp. in 2 (3%) cases and *Klebsiella* spp. was found in 5(7.69%) of patients.

In a study by Leck *et al*. (2002), *Pseudomonas* was found to be the prime isolated among the bacterial isolates in Ghana and South India. While in a study by Laspina *et al*. (2004), coagulase negative staphylococci were the most common isolate, and they related the reason for the variation in the microbial causatives of keratitis to different climatic conditions, socioeconomic standards, culture and occupation that are seen in these geographical areas.

Pseudomonas aeruginosa was the most common dominant organism isolated in the studies of Radford *et al*. (1998) and Jeng and McLeod (2003). They found that *Staphylococcus* and *Streptococcus* isolates were the next most frequent pathogens, and *P.aeruginosa* was associated with the presence of contact lenses. Al-Samarrai and Sunba (1989) were also found that *Staphylococcus* was the most common isolated pathogen. According to Basak *et al*. (2005) in their article about epidemiology of bacterial keratititis, *S. pneumoniae* was the predominant organism, while *Staphylococcus* was the most common organism in the prospective clinical and microbiological study of Schafer *et al*. (2001) in Switzerland.

3.2.2- Isolations and identifications of fungi causing keratitis:

After culturing the 73 specimens of keratitis patients on Sabauroud agar for 7 days at 28◦C, grown fungi were identified according to their morphology and spores as showen in table (3-3). Results indicated that fungal infection was represented by 5 (4.76%) of the total specimens.

Colonies of *Aspergillus niger*, which represented (4.12%), were initially covered with a white, fluffy, aerial mycelium, and as colony matured, their surfaces were covered with black spores, while the reverse of the colony remained as light tan

in color. The microscopic characteristics showed that hyphae were hyaline and distinctly septate, and the conidiophores were long.

Colonies of *Alternaria* sp. (2.73%) were dark greenish surface with gray periphery, black on reverse side. The microscopic featured hyphae distinctly as septet and yellow brown in color, while the conidia were dark brown, multicelled, with both transverse and longitudinal septa, drumstick shaped, arranged in tandem long chains.

Table (3-3): Cultural, microscopical and physiological characteristics of fungal isolates from patients suffering of eye keratitis.

Morphology of fungal isolates and their spores		
	A.niger	***Alternaria spp.***
Colony	White, fluffy, aerial mycelium. Matured colony covered with black spores.	Dark greenish surface with gray periphery.
Hyphae	Hyaline and septet	Yellow brown and septet
Conidiophores	Long	Drumstick shaped

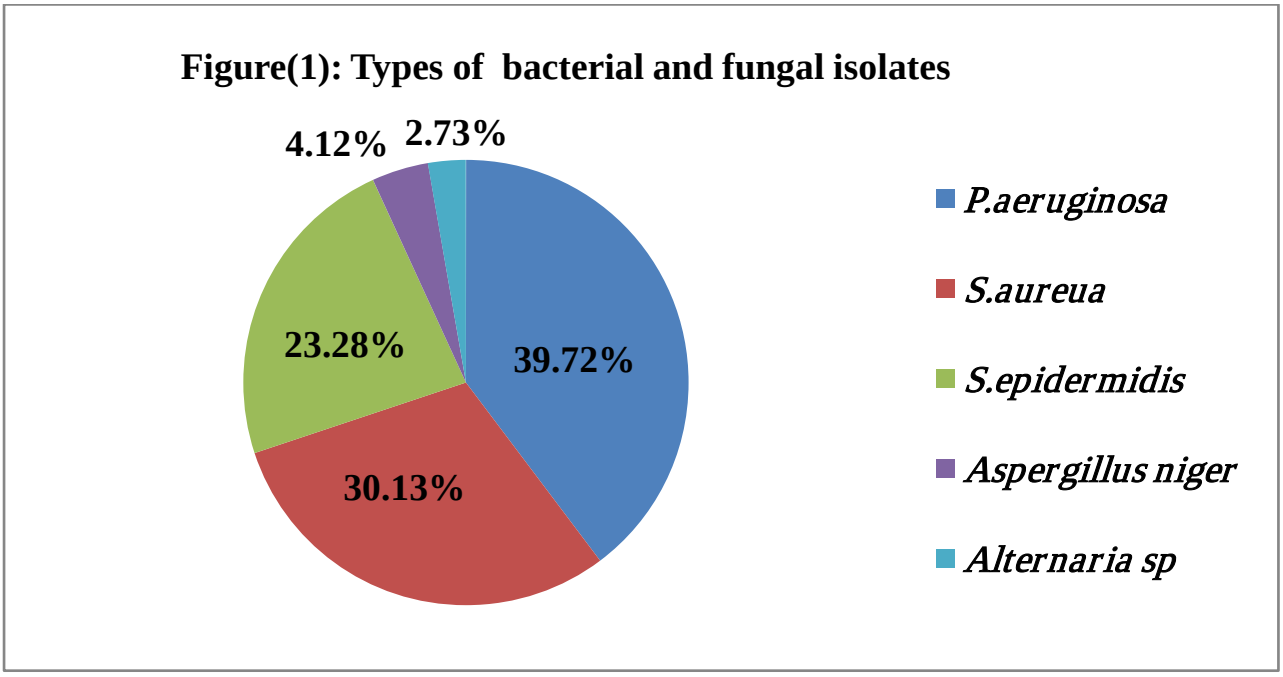
From the results mentioned above, it can be concluded that *A. niger* was the most prevalence fungus isolated from the mycotic keratitis patients. Similar prevalence was achieved by Al-Shakarchi (2007) who found that out of the 396 cases he tested, positive fungal growth was appeared in 74 of the cases (18.7%), and the most common fungi isolated were *Aspergillus* spp. 42 (56.8%) followed by *Fusarium* spp. 20 (27%), *Pencillium* spp. 4 (5.4%), *Scopulariopsis* spp. 2 (2.7%), *Geotrichum* spp. 1 (1.4%), *Alternaria* spp. 1 (1.4%) and *Candida* spp. 4 (5.4%).

When Al-Shakarchi *et al*. (2005) studied the management of fungal keratitis in Iraq with the eye drop amphotercin B 0.15%, they found that from 129 patient specimens of suppurative keratitis, 22 of them had culture proven fungal keratitis (17%). Again here, the most common fungi isolated were *Aspergillus* spp. (10 cases) followed by *Fusarium* spp. (8 cases), *Scopulariopsis* spp. (2 cases), and one case for each of *Penicillium* sp. and *Candida* sp. In another research, Al-Shakarchi *et al*. (2003) investigated the prevalence of fungal keratitis in Iraq and identify types of fungi responsible for corneal ulceration in 100 patients. They found that fungal growth was detected in 16 cases, and the most common fungus isolated was *Aspergillus* spp.(9 cases) followed by *Fusarium* spp. and *Candida albicans* (3 cases for each), while *Scopulariopsis* sp. was isolated from only one case.

Sherwal and Verma, (2008) accounted fungal infection as 32.50%, with *Aspergillus* spp. as the most abundant (56.42%), followed by *Curvurlaria* spp (17.95%), *Cladosporium* spp (7.70%), *Candida* spp (5.13%), *Fusarium* spp (5.13%), *Alternaria* spp (5.13%), *Pencillium* spp (2.57%). They concluded that fungal infection is a sight threatening condition, which needs early diagnosis and treatment to save the patient's eye.

Chowdhary and Singh (2005) found that from a group study of 485 cases, 191(39%) were diagnosed as mycotic (fungal) keratitis. Microscopic examination revealed presence of fungal elements in the corneal scrapings of (62.3%) and (60%) of the subsequently fungal culture-positive cases, respectively. They found also that the *A. niger* was the most common fungus isolated, followed by *Curvurlaria* spp. in the culture-proven cases of fungal keratitis.

Figure (1): Types of bacterial and fungal isolates and their percentages.



3.3- Determination of IgA glycoprotein level in microbial keratitis patients:

IgA was present in measurable quantity among all tear samples (73). The mean, standard deviation, and coefficient of variation (CV) of IgA values obtained in different clinical subgroups and the respective matched control groups are given in table (3-4). It can be observed from the table that the mean IgA levels were found to be significantly higher than those of the matched controls ($t = 2.04$ for 40 df, $P< 0.05$) in the ≤ 20 years age group. In the group age of (21-40) years, the mean IgA levels were found to be highly significant than those of the matched controls ($t = 4.46$ for 54 df, $P<0.001$). In the age groups of (41-59)and (≥ 60) years, also their mean IgA levels ($t = 4.04$ for 42 df, $P<0.001$,and $t = 2.58$ for 59 df, $P<0.001$)were found to be significantly higher than the respective matched controls.

Table (3-4): Age-sex distribution of patients and their tear IgA levels compared to healthy controls.

Age Group (years)	No. of cases		No. of Controls	IgA in (mg/dl)			
	Males	Females		Clinical condition		Matched controls	
				Mean ±SD	CV%	Mean ±SD	CV%
≤ 20	9	8	8	41.8±17.5	41.8	28.0±16.4	58.5
21-40	12	9	11	68.2±34.2	50.1	30.0±15.9	53.0
41-59	13	9	11	54.2±29.5	54.4	26.3±13.6	51.7
≥ 60	7	6	6	47.7±28.5	59.7	27.4±15.2	55.4

Several studies have been reported on immunoglobulin levels in tears of normal persons (Chodirker and Tomasi, 1993; Barnett,1995; Josephson and Weiner, 1997; Little *et al*., 1998; Knopf *et al.,* 1999; Brauninger and Centifanto, 2000; Sen *et al*., 2001), but there is little information on the level of immunoglobulin concentrations in tears in diseased eyes indicating that the IgA levels were found to be increased in bacterial and mycotic ulcerations.

In the few published reports, the numbers of cases studied were small. Upon investigation by Bluestone *et al.* (1995) on a few cases with ocular eye diseases, they found that only the IgA level was rising in the tears. Knopf *et al.* (1999) indicated that whenever there is alteration in the level of immunoglobulin's in tears in the diseased eyes, it is the level of IgA that is predominantly altered. Sen *et al.* (1999) suggested that there is appreciable change in the IgA immunoglobulin concentrations in tears in patients suffering from various ocular diseases. This suggests increased local production of IgA as a result of

stimulation at the site. Barnett (1995) reported that there is no significant differences in the IgA level between men and women and also among different age groups.

3.4- Estimation of tear anti- lysozyme in microbial keratitis patients:

The tear lysozyme level were measured in different age groups of 73 individuals, 48 (65.75%) of them are patients and 25 (34.25%) controls, Low levels of tear lysozyme were observed in patients with infective corneal ulcers, when compared with the controls. Lowest levels were seen in patients with bacterial corneal ulcers. However, the levels of tear lysozyme showed a corresponding decrease with the increase in rate of tear flow in ocular conditions; as a consequence, lysozyme content in the tears tends to be low.

Table (3.5): Tear lysozyme levels of patients and healthy groups (control).

Age (year)	Lysozyme level		P-value
	Patient Mean ± SD	Healthy control Mean ± SD	
≤ 20	0.82 ± 0.31	4.4 ± 0.33	<0.001
21-40	1.66 ± 0.66	8.2± 0.70	<0.001
41-59	1.31 ± 0.37	6.5 ± 0.23	<0.001
≥ 60	1.00 ± 0.40	3.1± 0.19	<0.001

Concentration of lysozyme in the tears rises with age between childhood and maturity; this means that the lysozyme level tends to be low in the age group ≤ 20 years. The highest values were seen in the age group of 21–40 years, and a decrease of lysozyme concentration occurred with an increase in ages from 41-59 and ≥ 60 years, respectively. The increase in the level of lysozyme in tears in

the age groups ≤ 20 years to 21–40 years was statistically significant (P<0.001). Again, the fall in the level of tear lysozyme from the age group 41-59 years to ≥ 60 years was statistically significant (P<0.001). There was no significant differences occurred between the sexes. The mean lysozyme content of tears was 1.768 U/ml in keratitis subjects.

Zhou *et al*. (2009) pointed out that the levels of lysozyme and lactoferrin are decreased in the tear fluid of patients with corneal ulcer compared to healthy patients.

Regan (1999) reported that the lysozyme concentration in tears tended to be low in children under 16, and it raised and attained a plateau in the age group (20-45) years, but decreased in the later life, though still remaining higher than in the children.

Table (3.5): The O.D. titer of tear anti-lysozyme levels among genders of the different age groups.

Age (years)	Males Mean ± SD	Females Mean ± SD	t	df	P-value
≤ 20	0.81±0.31	0.84±0.22	0.41	58	>0.60
21-40	1.68±0.66	1.65±0.70	0.20	85	>0.80
41-59	1.30±0.37	1.19±0.33	1.19	61	>0.20
≥ 60	1.01±0.40	1.00±0.24	0.07	23	>0.90

There are several reports on age-related changes in the level of tear lysozyme which are conflicting (Hirai.,1997).

Bonavida and Sapse (2008) found lower levels of tear lysozyme at the upper and lower extremes of age. Pietsch and Pearlman (2005) declared that the level of lysozyme in tears fell continuously after the age of 10 years.

Mukai (2002) found that in healthy subjects, tear lysozyme reached peak levels in the age group of (11-20) years and tended to decrease thereafter. In another investigation, the lysozyme levels in tear of the diseased people have to be decreased in relative with age especially in the age group of >70 years due to age related changes and decreases in their immunological response. Mackie and Seal (2010) noted a gradual decline of tear lysozyme levels with advancing age. Morsky and Aine (1998) found a tendency to declining concentrations among the (31-45) years age range.

Avisar *et al*. (1998) elucidated that there was a significant decrease when the tear lysozyme level is in the acute stage of keratitis as compared with the level in the healthy subjects ($P<0.001$).

Anderson (1993) reported that the tear lysozyme levels in patients with keratitis have been reported to be low. This low lysozyme level in patients with keratitis probably makes them more vulnerable to secondary bacterial infections, which are frequently associated with keratitis. Ridley (1990) declared that while the normal lysozyme content of tears might be effective against some pathogenic bacteria, the lysozyme level needed to be decreased only very slightly to be totally ineffective against all pathogens.

Chapter Four

Conclusions and Recommendations

4.1. Conclusions:

- *Pseudomonas aeruginosa* was the most dominant bacteria isolated from patient with keratitis, followed by *Staphylococcus aureus*, and S. *epidermidis*, while *Aspergillus niger* was the most common fungus followed by *Alternaria* sp.
- Regarding patients gender, cases of microbial keratitis in this study were more abundant in males than in females.
- The geographical factors may play an important role in the most common microbial causatives of keratitis infections.
- Low levels of tear anti-lysozyme were observed in patients infected with corneal ulcers compared to the controls.
- IgA levels were found to be increased in corneal ulcer patients in comparison to the controls.

4.2. Recommendations:

- Using PCR method in diagnosis of microbial keratitis.
- Using the lysozyme for the dry eye syndrome to decrease the possibility of keratitis infection and as a preservative solution for contact lenses.
- Using Enzyme-Linked Immuno Sorbent Assay (ELISA) to detect the concentration of IgA in the tear.

References

References

(A)

- **Ali, F. I.; Ismail, M. Ch. and Baker, A. W. (2004).** "Demographic and social study of patients with bacterial keratitis in Iraq". Iraqi J preventive med. **1(2)**: 118-124.
- **Alsamarrai, A. R. and Sunba, M. S. (1989).** "Bacterial corneal ulcers among Arabs in Kuwait". J. Ophthalmic Res. **21(3)**:278-284.
- **Al-Shakarchi, F.I. (2007)**. "Initial therapy for supportive microbial keratitis in Iraq". Br J Ophthalmology **91**:1583-1587.
- **Al-Shakarchi, F. I.; Al-Bakri, F.; Amin, M. M. and Al-Sadi (2003).** "Causes of supportive keratitis in Iraq". Iraqi J Med Sci **2 (3)**: 92-95.
- **Al-Shakarchi, F. I.; Hassan, F. K. and Amin, M. M. (2003).** "Fungal keratitis". Iraqi J Comm Med **16 (2)**:142-145.
- **Al-Shakarchi, F. I.; Al-Khashen, M. and Hassan, F. K. (2005).** "Fungal keratitis in Iraq and its management with Amphotercin –B 0.1% eye drops". Journal of Arab Bord of medical specialization. **7(3)**: 244-249.
- **Al-Yousaf, N. (2009).** "Microbial Keratitis in Kingdom of Bahrain: Clinical and Microbiology study". Middle East African Journal of Ophthalmology. **16**: 3-7.
- **Anand, A. R.; Madhavan, H. N.; Neelam,V. and Lily ,T. K. (2001).** "Use of polymerase chain reaction in the diagnosis of fungal endophthalmitis". Ophthalmology. **108(2)**:326–30.
- **Anderson, O.(1993)**. "Lysozyme and the antibacterial properties of tears". Arch Ophthalmology **25**: 491-493.
- **Argueso, P. and Gipson, I.K. (2001).** "Epithelial mucins of the ocular surface: structure, biosynthesis and function". Exp Eye Res **73**:281– 289.

- **Asbell, P. and Stenson, S. (1982).** "Ulcerative keratitis: survey of 30 years' laboratory experience". Arch Ophthalmology **100**: 77–80.
- **Atlas, M.; Parks, C. and Brown, A. (1995).** "Laboratory Manual of Experimental Microbiology". Mosby–Year–Book, Inc., USA.
- **Avisar, R.; Menache, R.; Shaked, P.; Rubinstein, J.; Machtey, I. and Savir,H.(1998).** Lysozyme content of tears in patients with Sjogren's syndrome and rheumatoid arthritis. Am J Ophthalmology **87**:148-151.

(B)

- **Baddour, L. M.; Christensen, G. D. and Bisno, A. L. (1978).** "Bacterial concentration correlations in experimental endocarditis caused by *Staphylococcus epidermidis*". Clin Microbiol **25**: 207-210.
- **Baily, S.; Barono, E. and Finegold, S.(1990).** "Diagnosis Microbiology". 8th ed. The C.V. Mosby Co. London.
- **Baker, S. E. (2006).** "*Aspergillus Niger* genomics: Past, present and into the Future". Medical Mycology, **44**: 517-521.
- **Barnett, E. V. (1995).** "Quantitation of immunoglobulins and L-chains by complement fixation tests". Journal of Immunology, **100**: 1093-1100.
- **Barniak V.L., Burke S.E., Venkatesh S. (2010)**. "Comparative evaluation of multi-purpose solutions in the stabilization of tear lysozyme". Cont. Lens Anterior Eye **33**: 507–511.
- **Basak, S. K.; Basak, S.; Mohanta, A. and Bhowmick, A. (2005).** "Epidemiological and microbiological diagnosis of suppurative keratitis in Gangetic West Bengal, eastern India". Indian Journal of Ophthalmology. **53(1)**:17-22.

- **Bayston, R. and Rodger, J. (1990).** "Production of extracellular slim by *Staphylococcus epidermidis* during stationary phase of growth: its association with adherence to implantable devices". Clin.Pathol.**43**:866- 870.
- **Beare, P. A.; For, R. J.; Martin, L. W. and Lamont, I. L. (2003).** "Siderophores mediated cell signaling in *Pseudomonas aeruginosa*: divergent pathways regulate virulence factor production and siderophores receptor synthesis". Mol. Microbiol **47**: 195–207.
- **Berta A.,(1992).** "Enzymology of the Tears". Boca Raton, Ann Arbor, London, Tokyo: CRC Press,
- **Berthelot, P.; Attree, I.; Plesiat, P.; Chabert, J.; de Bentzmann, S.; Pozzetto, B. and Grattard, F. (2003).** "Genotypic and phenotypic analysis of type III secretion system in a cohort of *Pseudomonas aeruginosa* bacteremia isolates: evidence for a possible association between O serotypes and exo genes". J. Infect. Dis. **188**: 512–518.
- **Bharthi, M. J.; Ramakirhnan, R.; Vasau, S.; Meenakshi, R. and Palaniappan, R. (2003).** "Epidemiological characteristics and laboratory diagnosis of fungal keratitis: a three-years study". Indian J Ophthalmology **51(4)**: 315-21.
- **Bishop R.E., Penfold S.S., Frost L.S. et al**. **(1995)**. "Stationary phase expression of a novel Escherichia coli outer membrane lipoprotein and its relationship with mammalian apolipoprotein D. Implications for the origin of lipocalins". J Biol Chem. **270**:23097–23103.
- **Bluestone, R., Easty, D. L., Goldberg, L. S., Jones, B. R.,** and **Petit, T. H. (1995).** "Lacrimal immunoglobulins and complement

quantified by counter-immunoelectrophoresis". British Journal of Ophthalmology, **59**: 279-281.

- **Blum, R. A. and Rodvold, K. A. (1987).** Recognition and importance of *Staphylococcus epidermidis* infections. Clin. Pharm.**6**:464-475.
- **Bohach, G. A.; Dinges, M. M.; Mitchell, D. T.; Ohlendorf, D. H. and Schlievert, P. M. (1997).** "Exotoxins in crossely KB, Archer GL, eds. The Staphylococci in Human Disease". New York, NY: Churchill Livingstone: 93-111.
- **Bonavida B., Sapse A.T.(2008).** "Human tear lysozyme. II. Quantitative determination with standard Schirmer strips". Am. J. Ophthalmology **66**:70-76.
- **Boonpasart, S.; Kasetuwan, N.; Puangsricharern, V.; Pariyakanok, L. and Jittoonkusol, T. (2002).** "Infectious keratitis at King Chulalongkorn Memorial Hospital: a 12 year retrospective study of 39 cases". J Med Assoc Thai. **85**: 217-230.
- **Brandtzaeg P., Baekkevold E.S., Morton H.C.(2001).** "From B to A the mucosal way". Nat Immunol.**2**:1093–1094.
- **Brauninger, G. E., and Centifanto, Y. M. (2000).** "Immunoglobulin E in human tears". American Journal of Ophthalmology, **72**: 558-561.
- **Butler, T.; Spencer, N.; Chan, C.; Gilhorta, J. and McCellan, K. (2005).** "Infective keratitis in older patients: a 4 year review 1998-2002". Br J. Ophthalmol. **89**: 591-596.

(C)

- **Callaghan, R. J.; Callegan, M. C.; Moreau, J. M.;Green, L. C.; Foster, T. J.; Hartford, O. M.; Engel, L. S. and Hill, J. M. (1997).**

"Specific roles of alpha-toxin and beta-toxin during *Staphylococcus aureus* corneal infection". Infect. Immun. **65(5)**:1571–1578.

- **Callegan, M. C.; Engel, L. S.; Hill, J. M. and O'Callaghan, R. J. (1994).** "Corneal virulence of *Staphylococcus aureus*: roles of alpha-toxin and protein A in pathogenesis". Infect. Immun. **62**:2478–2482.
- **Callegan, M. C.; O'Callaghan, R. J. and Hill, J. M. (1994).** "Pharmacokinetics and the treatment of bacterial keratitis". Clin. Pharmacokinetics **27**:129–149.
- **Campanacci V., Nurizzo D., Spinelli S. et al.,(2004).** "The crystal structure of the Escherichia coli lipocalin Blc suggests a possible role in phospholipid binding". FEBS Lett. **562**:183–188.
- **Castillo E.J., Koenig J.L., Anderson J.M. (1986).** "Characterization of protein adsorption on soft contact lenses. IV. Comparison of in vivo spoilage with the in vitro adsorption of tear proteins". Biomaterials **7**:89–96.
- **Chiquet, C.; Lina, G.; Benito, Y.; Cornut, P. L.; Etienne, J. and Romanet, J. P. (2007).** "Polymerase chain reaction identifications in aqueous humar of pateints with postoperative endophthalmatis". Cataract refract surg. **33(4)**: 635-641.
- **Chodirker, W. B., and Tomasi, T. B. Jr. (1993).** "Immunoglobulins: quantitative relationships in human serum and non-vascular fluids". Science, **142**: 1080-1081.
- **Chowdhary, A. and Singh, K. (2005).** "Spectrum of fungal keratitis in North India". Cornea. **24(1)**:8 –15.
- **Chusid, M. J. and Davis, S.D. (1979)**. "Experimental bacterial keratitis in neutropenic guinea pigs: polymorphonuclear leukocytes in corneal host defense". Infect Immun.**24**:948–952.

- **Collee, J.; Miles, R. and Watt, B. (1996).** "Tests for the identification of bacteria. In: Practical Medical Microbiology". Collee, J.; Fraser, A.; Marmion, B. and Simmons, A. (Eds.). 4th edition. Churchill Livingstone, U.K.
- **Comolli, J. C.; Hauser, A. R.; Waite, L.; Whitchurch, C. B.; Mattick, J. S. and Engel, J. N. (1999).** "*Pseudomonas aeruginosa* S gene products Pil T and Pil U are required for cytotoxicity in vitro and virulence in a mouse model of acute pneumonia". Infect. Immun. **67**:3625–3630.

(D)

- **Dacheux, D.; Toussaint, B.; Richard, M.; Brochier, G.; Croize, J. and Attree, I. (2000)**. *"Pseudomonas aeruginosa* cystic fibrosis isolates induce rapid, type III secretion dependent, but Exo U-independent, oncosis of macrophage and polymorph nuclear neutrophils". Infect. Immun. **68**:2916-2924.
- **De Chial, M.; Ghysels, B.; Beatson, S. A. ; Geoffroy, V.; Meyer, J. M.; Pattery, T.; Baysse, C.; Chablain, P.; Parsons, Y. N.; Winstanley, C.;Cordwell, S. J. and Cornelis, P. (2003)**. "Identification of type II and type III pyoverdine receptors from *Pseudomonas aeruginosa"*. Microbiol. **149**:821–831.

(F)

- **Feldman, M.; Bryan, R.; Rajan, S.; Scheffler, L.; Brunnert, S.; Tang, H. and Prince, A. (1998).** "Role of flagella in pathogenesis of *Pseudomonas aeruginosa* pulmonary infection". Infect. Immun. **66**: 43–51.
- **Ferrer, C.; Montero, J.; Jorge, L. ;Alio, J. L., Abad ; Jose, M., R.; and Colom, F. (2003)**. "Rapid Molecular Diagnosis of

Posttraumatic Keratitis and Endophthalmitis Caused by *Alternaria infectoria*". J. Clin. Microbiol. **41(7):**3358-3360.

- **Finck-Barbancon, V.; Goranson, J.; Zhu, L.; Sawa, T.; Wiener-Kronish, J. P.; Fleiszig, S. M.; Wu, C.; Mende-Mueller, L. and Frank, D. W. (1997).** "ExoU expression by *Pseudomonas aeruginosa* correlates with acute cytotoxicity and epithelial injury". Mol. Microbiol. **25**: 547–557.
- **Fleiszig, S. M. and Evans, D., J. (2002).** "The pathogenesis of bacterial keratitis: studies with *Pseudomonas aeruginosa*". Clin. Exp. Ophthalmol. **85**: 271– 278.
- **Fleiszig, S. M.; Arora, S. K.; Van, R. and Ramphal, R. (2001)**. "FlhA, a component of the flagellum assembly apparatus of *Pseudomonas aeruginosa*, plays a role in internalization by corneal epithelial cells". Infect. Immun. **69**: 4931–4937.
- **Fleiszig, S. M.; Wiener-Kronish, J. P.; Miyazaki, H.; Vallas, V.; Mostov, K. E.; Kanada, D.; Sawa, T.; Yen, T. S. and Frank, D.W. (1997).** *"Pseudomonas aeruginosa*-mediated cytotoxicity and invasion correlate with distinct genotypes at the loci encoding exo enzyme S". Infect**.** Immun. **65**: 579–586.
- **Fleming A. (1932)**. "Lysozyme: president's address". Proc. R. Soc. Med. **26**:71–84.
- **Flor, C., N. and Peczon, I. V. (2008)**. "Interventions for fungal keratitis". Cochrane Database of Systematic Reviews J. Medical.
- **Foster, T. J. and McDevitt, M. (1994)**. "Surface-associated proteins of *Staphylococcus aureus*: their possible roles in virulence". FEMS Microbiol. Lett.**118**:199–206.

- **Freda, R. (2006).** "Use of oral voriconazole as adjunctive treatment of severe cornea fungal infection: case report". Arq. Bras. Ophthalmol. **69(3)**:431-444.
- **Fullard R.J. and Tucker D. (1994).** "Tear protein composition and the effects of stimulus". Adv. Exp. Med. Biol. **350**:309–314.
- **Fung K., Morris C., Duncan M. (2002).** "Mass spectrometric techniques applied to the analysis of human tears: a focus on the peptide and protein constituents". Adv. Exp. Med. Biol. **506**: 601-605.

(G)

- **Gachon A.M., Richard J., Dastugue B. (1982).** "Human tears: normal protein pattern and individual protein determinations in adults". Curr. Eye Res. **83(2):**301-308.
- **Gao, A. (2009).** "Population biology of *Propionibacterium acnes* and *Pseudomonas aeruginosa* in ophthalmic infections and the development of novel diagnostic tools". Ph.D. Dissertation. Warwick University.
- **Garrity, G**. **(2005)**. "Bergey's Manual of Systemic Bacteriology". (2^{nd} ed.). Vol 2. Williams and Wilkins, Bailtimol. London.
- **Ghuysen J.M. H.R., ed. (1994).** "Bacterial cell wall". Amsterdam: Elsevier B.V.
- **Glasgow B.J., Marshall G, Gasymov O.K, Abduragimov A.R, Yusifov T.N., Knobler C.M. (1999).** "Tear lipocalins: potential lipid scavengers for the corneal surface". Invest. Ophthalmol Vis. Sci. **40**:3100–3107.
- **Gopinathan, U. V. and Sangwan, T. E. (2002).** "Polymerase chain reaction based detection of fungi in infected corneas". Br J Ophthalmol. **86**:755–760.

- **Green, M.; Andrew, A. and Fiona, S. (2008).** "Risk Factors and Causative Organisms in Microbial Keratitis". J. Cornea . **27 (1):** 22-27.
- **Green-Church K.B., Butovich I., Willcox M., et al., (2011)**. "The international workshop on meibomian gland dysfunction: report of the subcommittee on tear film lipids and lipid protein interactions in health and disease". Invest. Ophthalmol. Vis. Sci. **52**:1979–1993.
- **Gregory R.L., Allansmith M.R. (1986)**. "Naturally occurring IgA antibodies to ocular and oral microorganisms in tears saliva and colostrums: evidence for a common mucosal immune system and local immune response". Exp Eye Res. **43**:739–749.
- **Gupta, C. P.; Dubey, R. C.; Kang, S. C. and Maheshwari, D. K. (2001).** "Antibiosis-mediated necrotrophic effect of Pseudomonas GRC2 against two plant pathogens". Curr Sci. **81(1)**: 91-94.

(H)

- **Hahn, H. P. (1997)**. "The type-4 piles is the major virulence-associated adhesion of *Pseudomonas aeruginosa* -a review". Gene **192**: 99–108.
- **Hall, L. R.; Lass, J.H.; Diaconu, E.; Strine, E.R. and Pearlman, E. (1999)**. "An essential role for antibody in neutrophil and eosinophile recruitment to the cornea: B-cell deficient (l MT) mice fail to develop TH-2dependent, helminthes mediated keratitis". J. Immuno. **163**: 4970–5000.
- **Hankiewiz, J**. **and Swierczuk, E.(1995).** "Lysozyme in human body fluids". Clinic. Chemical. Acta.,**57**: 205-209.
- **Harding J.J. (1997).** "Biochemistry of the Eye". London: Chapman and Hall.

- **Harry, J. and Misson, G. (2001).** "Clinical ophthalmic. Pathology".**91**.UK.
- **Hazlett, L. D. (2002)**. "Pathogenic mechanisms of *P. aeruginosa* keratitis: a review of the role of T cells, Langerhans cells, PMN, and cytokines". DNA Cell Biol. **21(5–6):** 383–390.
- **Hirai, T.(1997).** "Studies of Lysozyme in tears". J. Tokyo Womens Med. Coll. **42**:167-177.
- **Hua, X.,; Yuan, X., and Wilhelmus, K. R. (2010).** "A fungal PH-responsive signaling pathway regulating *Aspergillus* adaptation and invasion into the cornea". IOVS. **51(3)**.

(I)

- **Ikonne, E. U. and Odozor, O. (2009).** "Comparative efficacy of tropical ciprofloxacin on *Staphylococcus aureus* and *Pseudomonas aeruginosa".* In vitro. NOA: 8-11.
- **Ismail. M. CH.; Ali, F. I. and Mohammed, S. W. (2011).** "Production of slim layer by *Staphylococcus epidermidis* isolated from corneal infection". Baghdad Science. **8(3):**741-744.
- **Itahashi, M.; Higaki, S.; Fukuda, M. and Shimomura, Y. (2010).** "Detection and Quantification of Pathogenic Bacteria and Fungi Using Real- Time Polymerase Chain Reaction by Cycling Probe in Patients With Corneal Ulcer". Arch. Ophthalmol.**128(5):**535-540.

(J)

- **Josephson, A. S., and Weiner, R. S. (1997).** "Studies of the proteins of lacrimal secretions". Journal of Immunology, **100**:1080-1092.
- **Jeng, B. H. and McLeod, S. D. (2003).** "Microbial keratitis". British Journal of Ophthalmology. **87**:805-808.

(K)

- **Kanyshkova T.G., Buneva V.N., Nevinsky G.A. (2001)**. "Lactoferrin and its biological functions". Biochemistry (Moscow), **66**: 1–7.
- **Keay, L.; Edwards, K.; Naduvilath, T.; Forde, K. and Stapleton, F.(2006).** "Microbial keratitis: predisposing factors and morbidity". Ophthalmol. **113**:109–116.
- **Keith D., Hong B., Christensen M. (1997).** "A novel procedure for the extraction of protein deposits from soft hydrophilic contact lenses for analysis". Curr. Eye Res. **16**:503–510.
- **Kernacki, K. A.; Goebel, D. J.; Poosch, M. S. and Hazlett, L. D. (1998).** "Early cytokine and chemokine gene expression during *Pseudomonas aeruginosa* corneal infection in mice". Infect. Immun. **66**: 376–379.
- **Keshav, B.; Zacheria, G.; Ideculla, T.; Bhat, V. and Joseph, M. (2008).** "Epidemiological characteristics of corneal ulcers in South Sharqiya Region". J of Oman Medical. **23**: 1-6.
- **KirkpatrickC.H., Green I., Rich R.R., Schade A.L. (1971)**. "Inhibition of growth of *Candida albicans* by iron-unsaturated lactoferrin: relation to host-defense mechanisms in chronic mucocutaneous candidiasis". The Journal of Infectious Diseases, **124**: 539–544.
- **Kloos, W. E. and Bannerman, T. L. (1994)**. "Update on clinical significance of coagulase- negative Staphylococci". Clin. microbiol. **7**: 117-140.
- **Knopf, H. L. S., Bertran, D. M., and Kapikian, A. Z. (1999).** "Demonstration and characterization of antibody in tears following

intranasal vaccination with inactivated type 13 rhinovirus. A preliminary report". Investigative Ophthalmology, **9**: 727-734.

- **Knopf, H. L. S., D. M. Bertran, and A. Z. Kapikian (1970).** "Demonstration and characterization of antibody in tears following intranasal vaccination with inactivated type 13 rhinovirus: a preliminary report". Invest. Ophthalmology **9**:727-734.
- **Kordic, R.; kica, S. and Vladusic, I. (2008).** "Bacterial keratitis-empirical treatment or therapy according to antibiotic report". Acta. Clinic. Croat. **47(1):**27-29.
- **Kornblum, J.; Kreiswirth, B. N.; Projan, S. J.; Ross, H.; and Novick, R. P. (1990).** "Agr: a polycistronic locus regulating exo protein synthesis in *Staphylococcus aureus*". In R. P. Novick (ed.), Molecular biology of the staphylococci. VCH Publishers, New York, N.Y.: 373–402.
- **Kredics, L. ; Varga, J.; Kocsube, S.; Doczi, I.; Samson, R.A.; Rajaraman, R.; Narendran, V.; Bhaskar, M.; Vagvolgyi, C. and Manikandan, P.(2007).** "Case of keratitis caused by *Aspergillus tamarii*". J. Clin. Microbiol. **45**:3464-3467.
- **Kredics, L.; Varga, J.; Kocsube, S.; Rajaraman, R.; Raghavan, A.; Doczi, I.; Bhaskar, M.; Nemeth, T.; Antal, Z.; Venkatapathy, N.; Csaba, V.; Samson, R.; Chockaiya, M. and Palanisamy, M. (2009).** "Infectious Keratitis Caused by *Aspergillus tubingensis"*. Cornea.
- **Kumara, A.; Pandy, S.; Madan, M.; Kavathia, G. (2010).** "Keratomycosis with Superadded Bacterial Infection Due To Corticosteroid Abuse-A Case Report". Journal of Clinical and Diagnostic Research. **(4):**2918-2921.

(L)

- **Ladage, P. M. (2004).** "Eye Constant Lens". 30:194-197.
- **Lal H., Ahluwalia B.K., Khurana A.K., Sharma S.K., Gupta S. (1991).** "Tear lysozyme levels in bacterial, fungal and viral corneal ulcers". Acta. Ophthalmol. (Copenh) **69**:530–532.
- **Lan J., Willcox M.D., Jackson G.D. (1997).** "Detection and specificity of anti- *Staphylococcus* intermedius secretary IgA in human tears". Aust. NZJ. Ophthalmology **25 (suppl 1):** 517-519.
- **Landau, S. and Everitt, B.(2004).** A Hand Book of Statistical Analysis using SSPS. USA, New York. Chapman and Hall/ CRC Press LLC.
- **Lanotte, P.; Watt, S.; Mereghetti, L.; Dartiguelongue, N.; Rastegar-Lari, A.; Goudeau, A. and Quentin, R. (2004).** "Genetic features of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients compared with those of isolates from other origins". J. Med. Microbiol. **53**: 73–81.
- **Laspina, F.; Samudio, M.; Cibils, D. Ta, C.; Farina, N.; Sanabria, R.; Klauss, V. and Mino de Kaspar, H. (2004).** "Epidemiological characteristics of microbiological results on patients with infectious corneal ulcers: a 13-year survey in Paraguay". Clin Exp Ophthalmol. **242**:204-209.
- **Le Brun V., Friess W., Schultz-Fademrecht T., Muehlau S., Garidel P.(2009).** "Lysozyme-lysozyme self-interactions as assessed by the osmotic second viral coefficient impact for physical protein stabilization". Biotechnol. J. **4**:1305–1319.
- **Leck, A. K.; Thomas, P. A.; Hagan, M.; Kaliamurthy, J.; Ackuaku, E.; John, M.; J Newman, M.; Codjoe, F. S.; Opintan, J. A.; Kalavathy, C. M.; Essuman, V.; Jesudasan, C.**

and Johnson, G. J.(2002). "Aetiology of supportive corneal ulcers in Ghana and South India and epidemiology of fungal keratitis". Br J Ophthalmol. **86**: 1211-1215.

- **Lee, E. J.; Cowell, B. A.; Evans, D. J. and Fleiszig, S. M. (2003).** "Contribution of ExoS A-regulated factors to corneal infection by cytotoxicity and invasive *Pseudomonas aeruginosa* in a murine scarification model". Invest. Ophthalmol. Vis. Sci. **44**: 3892–3898.
- **Legrand D., Elass E., Carpentier M., Mazurier J. (2005)**. "Lactoferrin: a modulator of immune and inflammatory responses". Cellular and Molecular Life Sciences, **62**: 2549–2559.
- **Leher H.F., Alizadeh H., Taylor W.M., et al.,(1998).** "Role of mucosal IgA in the resistance to *Acanthamoeba* keratitis. Invest Ophthalmol Vis Sci. **39**:2666–2673.
- **Liesegang, T. J.(1998).** "Bacterial and fungal keratitis". In: Kaufman HE, Baron BA, McDonald MB, eds. The Cornea. Boston, MA: Butterworth- Heinemann. 159–219.
- **Little, J. M., Centifanto, Y. M., and Kaufman, H. E. (1998).** "Immunoglobulins in human tears". American Journal of Ophthalmology, **68**: 898-905.
- **Lomholt, J. A.; Poulsen, K. and Kilian, M. (2001).** "Epidemic population structure of *Pseudomonas aeruginosa*: evidence for a clone that is pathogenic to the eye and that has a distinct combination of virulence factors". Infect. Immun. **69**:6284–6295.

(M)

- **Macfaddin**, **J.(2000).** "Biochemical Tests of Medical Bacteria". 3th ed. Lippincott. Williams and Wilkins, USA.

- **Mackie I.A., Seal D.V.(2010).** "Quantitative tear lysozyme assay in units of activity per microlitre". Br. J. Ophthalmol. **60**: 70-74.
- **Magnuson, J. and Lasure, L. (2004).** "Organic acid production by filamentous fungi". In: Tkacz J., Lange L. (eds). Advances in Fungal Biotechnology for Industry, Agriculture, and Medicine. New York: Kluwer Academic and Plenum Publishers. 307-340.
- **Mai W.J and Wang W.N. (2010).** "Protection of blue shrimp (Litopenaeusstylirostris) against the White Spot Syndrome Virus (WSSV) when injected with shrimp lysozyme". Fish Shellfish Immuno. **28**:727–733.
- **Mancini and Coll (1965).** "Determination of the IgA protein, by radial immunodiffusion plate". Immuno. Chemistry. **2**: 235.
- **Maniatis, T.; Fritch, E. and Sambrock, J. (1982).** Molecular Cloning: A laboratory Manual. Gold Spring Harbor Laboratory. N.Y.,USA.
- **Manikandan, P.; Dóczi, I.; Kocsube, S.; Varga, J.; Nemeth, T.M.; Antal, Z.; Vagvolgyi, C. ; Bhaskar, M.and Kredics,L.(2008).** "Aspergillus species in human keratomycosis". Aspergillus in the genomic era Wageningen: Wageningen Academic Publishers Varga., J.; Samson, R. :293-328.
- **Manikandan, P.; Varga, J. ; Kocsube, S. ; Revathi, R.; Anita, R.; Nemeth, M. T.; Narendran, V.; Vagvolgyi, C.; Bhaskar, M.; Manoharan, C.; Samson, R. and Kredics, L. (2010).** "Keratitis caused by the recently described new species *Aspergillus brasiliensis*: two case reports". Journal of Medical Case Reports, **4**:68-70.

- **Marchal R., Chaboche D., Douillard R., Jeandet P.(2002).** "Influence of lysozyme treatments on champagne base wine foaming properties". J. Agric. Food. Chem. **50**:1420–1428.
- **Masinick S.A., Montgomery C.P., Montgomery P.C., Hazlett L.D. (1997).** "Secretory IgA inhibits *Pseudomonas aeruginosa* binding to cornea and protects against keratitis". Invest Ophthalmol Vis Sci. **38**: 910–918.
- **Metz-Boutique M.H., Jolles J., Mazurier J., Schoentgen F., Legrand D., Spik G., Montreuil J., Jolles P. (1984)**. "Human lactotransferrin: amino acid sequence and structural comparisons with other transferrins". European Journal of Biochemistry, **145**: 659–676.
- **Millar T.J., Mudgil P., Butovich I.A., Palaniappan C.K. (2009).** "Adsorption of human tear lipocalin to human meibomian lipid films". Invest. Ophthalmol. Vis. Sci. **50**:140–151.
- **Moreau, J. M.; Sloop, G. D.; Engel, L. S.; Hill, J. M. and O'Callaghan, R. J. (1997)**. "Histopathological studies of Staphylococcal alpha-toxin: effects on rabbit corneas". Curr. Eye Res. **16**:1221-1228.
- **Moreira, B. M. and Daum, R. S. (1995).** "Antimicrobial resistance in Staphylococci". Editor. Clin. North Am. **42**:619–648.
- **Morsky P. and Aine E.(1998).** "Determination of lysozyme in tears by immunoturbidimetric and optimised kinetic bacteriolytic method". Clin. Chim. Acta. **129**: 201-209.
- **Mukai M. (2002).** "Tear protein. Low molecular protein and lysozyme". Nippon Ganka Gakkai Zasshi **79**: 607- 613.

(N)

- **Nayak, N. and Satpathy, G. (2000)**. "Slime production as a virulence factor in *Staphylococcus epidermidis* isolated from bacterial keratitis". Indian J. Med. Res. **111**:6-10.
- **Nayak, N.; Nag, T.C.; Satpathy, G. and Ray, S.B. (2007).** "Ultra-structural analysis of slime positive and slime negative *Staphylococcus epidermidis* isolates in infectious keratitis". Indian J. Med. Res. **125**: 767-771.
- **Nayak, N.; Satpathy, G.; Vajpayee, R. B.; Sethi, A.; Ray, M. and Sinha, R. (2002**). "Clinical significance of slime productionby *Staphylococcus epidermidis* in bacterial keratitis". Ann Ophthalmol. **34**:204-210.
- **Neoh, C. F.; Leung, L.; Vajpayee, R. B.; Stewart, K. and Kong, D. C.M. (2011).** "Treatment of Alternaria Keratitis with Intrastromal and Topical Caspofungin in Combination with Intrastromal, Topical, and Oral Voriconazole" .The Annals of Pharmacotherapy. **45**.
- **Nilsson, L.; Flock, P. and Lindberg, G. (1998).** "A Fibrinogen-Binding Protein of *Staphylococcus epidermidis*". Infection and Immunity. **66(6):** 2666-2673.
- **Norina, T. J.; Raihan, S.; Bakiah, S.; Ezaqnee, M.; Liza, S. and Wan, H. (2008).** "Microbial Keratitis: aetiological diagnosis and clinical features in patients admitted to Hospital Universiti Sains Malaysia". Singapore Med J. **49**: 67-71.

(O)

- **Ohashi Y., Dogru M., TsubotaK. (2006).** "Laboratory findings in tear fluid analysis". Clin. Chim. Acta. **369**:17–28.

- **Ozbek, Z.; Kang, S.; Sivalingam, J.; Rapuano, C.J.; Cohen, E. J. and Hammersmith, K. M. (2006).** "Voriconazole in the management of Alternaria keratitis". Cornea **25**:242-244.

(P)

- **Parsons, Y. N.; Panagea, S.; Smart, C. H.; Walshaw, M. J.; Hart, C. A. and Winstanley, C. (2002).** "Use of subtractive hybridization to identify a diagnostic probe for a cystic fibrosis epidemic strain of *Pseudomonas aeruginosa"*. J. Clin. Microbiol. **40**: 4607–4611.
- **Pearlman, E.; Lass, J. H.; Bardenstein, D.S. Diaconu, E. Hazlett, F.E. Albright, J. Higgins, A.W. and Kazura J.W. (1997).** "IL-12 exacerbates helminthes-mediated corneal pathology by augmenting cell recruitment and chemokine expression". J. Immuno. **158**: 827–833.
- **Perfect, J.R.; Cox, G.M.; Lee, J.Y.; Kauffman, C. A.; de Repentigny, L.; Chapman, S. W.; Morrison, V. A.; Pappas, P.; Hiemenz, J. W. and Stevens, D. A. (2001).** "The impact of culture isolation of Aspergillus species: a hospital-based survey of a spergillosis". Clin. Infect. Dis. **33**:1824-1833.
- **Phalipon A., Cardona A., Kraehenbuhl J.P., Edelman L., Sansonetti P.J., Corthesy B.(2002).** "Secretory component: a new role in secretory IgA mediated immune exclusion in vivo". Immunity. **17**:107–115.
- **Pietsch R.L., Pearlman M.E.(2005).** "Human tear lysozyme variables". Arch. Ophthalmol. **90**: 94-96.
- **Pillar, C. M. and Hobden, J. A. (2002).** *"Pseudomonas aeruginosa* exotoxin A and keratitis in mice". Invest. Ophthalmol. Vis. Sci. **43:** 1437– 1444.

- **Prescott R.; Narendran, V.; Bhaskar, M.; Vagvolgyi, C. and Manikandan, P. (2005).** "Case of keratitis caused by *staphylococcus aureus*" .J. Clin. Microbiol. , **45**:3464-3467.

(R)

- **Radford, C. F.; Minassian, D. C. and Dart, J. K. G. (1998).** "Disposable contact lens use as a risk factor for microbial keratitis". Br J. Ophthalmol. **82**:1272–1275.

- **Reddy, P. S.; Satyendran, O. M.; Satapathy, M.; Kumar, H. V.; Reddy, P. R. (1972).** "Mycotic keratitis". Invest. Ophthalmol. **20**: 101-108.
- **Regan, E.(1999).** "The Lysozyme content of tears". Am. J. Ophthalmol. **33**:600-605.
- **Ridley F.(1990).** "The tears". In: Ridley F. Sorsby A. eds Modern Trendsmin Ophthalmology. New York: Hoeber**15**: 382-387.
- **Roy-Burman, A.; Savel, R. H.; Racine, S.; Swanson, B. L.; Revadigar, N. S.; Fujimoto, J.; Sawa, T.; Frank, D. W. and Wiener-Kronish, J. P. (2001).** "Type III protein secretion is associated with death in lower respiratory and systemic *Pseudomonas aeruginosa* infections". J. Infect. Dis.**183**: 1767–1774.
- **Rudner, X. L.; Kernacki, K. A.; Barrett, R. P. and Hazlett, L. D.(2000)**. "Prolonged elevation of IL-1 in *Pseudomonas aeruginosa* ocular infection regulates macrophage-inflammatory protein-2 production, polymorphonuclear neutrophil persistence, and corneal perforation". J. Immunol. **164**: 6576–6582.
- **Ryan K.J., Ray C.G. (editors) (2004).** Sherris Medical Microbiology (4th ed.). McGraw Hill. ISBN: 8385-8529.

(S)

- **Sato, H.; Frank, D.W.; Hillard, C. J.; Feix, J. B.; Pankhaniya, R. ; Moriyama, K.; Finck-Barbançon, V.; Buchaklian, A. ; Lei, M.; Long, R. M.; Wiener-Kronish, J. and Sawa, T. (2003).** The mechanism of action of the *Pseudomonas aeruginosa*-encoded type III cytotoxin, ExoU. J. of EMBO. **22**: 2959–2969.
- **Schafer, F. ; Bruttin, O. ; Zografos, L. and Guex-Crosier, Y. (2001).** "Bacterial keratitis: a prospective clinical trial and microbiological study". British Journal of Ophthalmology. **85**:842-847.
- **Schulert, G. S.; Feltman, H.; Rabin, S. D.; Martin, C. G.; Battle, S. E.; Rello, J. and Hauser, A. R. (2003).** "Secretion of the toxin ExoU is a marker for highly virulent *Pseudomonas aeruginosa* isolates obtained from patients with hospital-acquired pneumonia". J. Infect. Dis. **188**: 1695–1706.
- **Sen D.K and Sarin G.S. (1979).** "Immunoglobulin concentrations in human tears in ocular disease". Br. J. Ophthalmol **63**:297-300.
- **Sen, D. K., Sarin, G. S., Mani, K., and Saha, K. (2001).** "Immunoglobulins in tears of normal Indian people". British Journal of Ophthalmology, **60**: 302-304.
- **Sethi, S.; Sethi, M., and Iqbal, R. (2010).** "Causes of microbial keratitis in patients attending an eye clinic at Peshawar". Gomal Journal of Medical Sciences. **8 (1)**: 20-22.
- **Sherwal, B. L. and Verma, A. K. (2008).** "Epidemiology of Ocular Infection Due to Bacteria and Fungus – A Prospective Study". J. B science. **10 (3):** 1-4.

- **Shukla, P. K.; Kumar, M. and Keshava, G. B. S. (2008).** Mycotic keratitis: an overview of diagnosis and therapy. Mycoses. **51**: 183–199.
- **Simmons, R. B.; Buffington, J. R.; Ward, M.; Wilson, L. A. and Ahearn, D.G. (1986).** Morphology and Ultra structural of Fungi In Extended Wear Soft Contact Lens. J. Clinic. Microbiol. **24**:21-25.
- **Singh P.K., Parsek M.R., Greenberg E.P., Welsh M.J. (2002).** A component of innate immunity prevents bacterial biofilm development. Nature, **417**: 552–555.
- **Sionov, E.; Sandovsky-Losica, H.; Gov, Y. and Esther, S. (2001)**. Adherence of Aspergillus species to soft contact lenses and attempts to inhibit the adherence. Mycosis **44**:464-471.
- **Snell, R. S. and Lemp, M. A. (1998)**. Clinical anatomy of the eye. 2nd edition. UK. 2.
- **Snyder, M. E. and Katz, H. R.(1992)**. Ciprofloxacin-resistant bacterial keratitis. Am J.Ophthalmol.**114**: 336-338.
- **Srinivasan, M. (2004).** Fungal keratitis. Curr. Opin. Ophthalmol. **15**:321–327.
- **Strynadka N.C. and James M.N. (1996)**. Lysozyme: a model enzyme in protein crystallography. EXS **75**:185–222.
- **Svanborg-Eden, C., and A. M. Svennerholm. (1978).** Secretory immunoglobulin A and G antibodies prevent adhesion of Escherichia coli to human urinary tract epithelial cells. Infect. Immun. **22**:790-791.
- **Swaminathan R., Ravi V.K., Kumar S., Kumar M.V., Chandra N. (2011)**. Lysozyme: a model protein for amyloid research. Adv. Protein Chem. Struct. Biol. **84**: 63–111.

(T)

- **Tabbra, K. F.; El-Sheikh, H. F. and Aabed, B. (2000).** Extended wear contact lens related bacterial keratitis. Br. J. Ophthalmology **84**:327-328.
- **Tananuvat, N.; Sienglew, S. and Ausayakhun, S. (2004).** Microbial Keratitis leading admission at Maharaj Nakorn Chiang Mai Hospital. Chiang Mai Med Bull. **43:** 93-103.
- **Tang, A.; Marquart, M. E.; Fratkin, J. D.; McCormick, C.C. ;Caballero, A. R. ; Gatlin, H.P. and O'Callaghan, R. J. (2009).** "Properties of PASP: a Pseudomonas protease capable of mediating corneal erosions". Invest. Ophthalmology Vis. Sci. **50 (8):**3794–3801.
- **Thomas, P. A. and Geraldine, P. (2007)**. Infectious keratitis. Curr. Opin. Infect. Dis. **20(2):**129-141.
- **Thomas, P.A. (1994)**. Mycotic keratitis: an underestimated mycosis. J. Med. Vet. Mycol. **32**:235–254.
- **Thomas, P.A. (2003).** Current perspectives on ophthalmic mycoses. Clin. Microbiol. Rev. **16**:730–797.
- **Thomas, P.A. (2003).** Fungal infection of the cornea. Eye **17**: 852-862.
- **Todar, K. (2004)**. Bacteria Resistance to Antibiotics. Todar's Online Textbook of Bacteriology. Sci. **304:**1421-1425.
- **Tomasi T.B.J. (1994).** Introduction: an overview of the mucosal immune system. In: Ogra PL, Mestecky J, Lamm ME, Strober W, McGhee JR, Bienenstock J, eds. Handbook of Mucosal Immunology. San Diego, CA: Academic Press: 3–8.

- **Tu, E.Y. (2009)**. Alternaria keratitis: clinical presentation and resolution with topical fluconazole or Intrastromalvoriconazole and topical Caspofungin. Cornea. **28**:116-119.

(V)

- **Valenti P., Antonini G. (2005).** Lactoferrin: an important host defense against microbial and viral attack. Cellular and Molecular Life Sciences, **62**: 2576–2587.
- **Vermon, M. O.; Hayden, M. K.; Truck, W. E.; Hayes, R. A.; and Blom, D. W.(2006).** Antimicrobial Resistance Project Arch. Int. Med. **166**:306-312.

(W)

- **Williams, R. C., and R. J. Gibbons.(1972**). Inhibition of bacterial adherence by secretary immunoglobulin A: a mechanism of antigen disposal. Science **177**:697-699.

(Z)

- **Zhang, J.; Wu, X.Y. and Yu, F.S.** (2005). Inflammatory response of corneal epithelial cells to *Pseudomonas aeruginosa* infection Curr Eye Res. **30**:527-534.
- **Zhang, J.; Xu, K.; Ambati, B. and Yu, F. S.** (2003). Toll-like receptor 5- mediated corneal epithelial inflammatory responses to *Pseudomonas aeruginosa* flagellin. Invest Ophthalmol Vis Sci. **44:** 4247–4254.
- **Zhou L., Huang L.Q., Beuerman R.W., Grigg M.E., Li S.F., Chew F.T., et al (2004)** . Proteomic analysis of human tears: defensin expression after ocular surface surgery. J. Proteome Res. **3**:410-416.

- **Zhou, L.; Beuerman, R.W.; Chan, C.M. et al (2009).** Identification of tear fluid biomarkers in dry eye syndrome using iTRAQ quantitative. J. proteome Res.**8**: 4889-4905.
- **Zolfaghar, I.; Evans, D. J. and Fleiszig, S. M.(2003).** Twitching mobility contribute to the role of pili in corneal infection caused by *Pseudomonas aeruginosa*. infect. immun. **71**: 5389-5393.

Appendices

Appendix (1): Biochemical reactions and results of identification of bacterial isolates by API 20E system.

TEST	ACTIVE INGREDIENT	REACTION / ENZYME	RESULT NEGATIVE	POSITIVE
ONPG	2-nitrophenyl-ß D-Galactopyranosidase	ß-galactosidase (Ortho NitroPhenyl-ß D-Galactopyranosidase)	Colorless	Yellow (1)
ADH	L-Arginine	Arginine DiHydrolase	Yellow	Red/orange (2)
LDC	L-lysine	Lysine DeCarboxylase	Yellow	Red/orange (2)
ODC	L-Ornithine	Ornithine DeCarboxylase	Yellow	Red/orange (2)
CIT	Trisodium citrate	CITrate utilization	Pale green/ yellow	Blue-green / blue (3)
H2S	Sodium thiosulfate	H2S production	Colorless/ grayish	Black deposit/thin line
URE	urea	UREase	Yellow	Red / orange (2)
TDA	L-Tryptophan	Tryptophan DeAminase	TDA / immediate Yellow	Reddish brown

IND	L-Tryptophan	Indole production	JAMES / immediate Colorless / Pale green / Yellow / Pink	
VP	sodium pyruvate	acetoin production (Vogas-Proskauer)	VP 1 + VP 2 / 10 min Colorless / Pink / red (5)	
GEL	Gelatin (bovine origin)	GELatinase	No diffusion	Diffusion of black pigment
GLU	D-Glucose	fermentation /oxidation (GLUcose) (4)	Blue / blue-green	Yellow
MAN	D-Mannitol	fermentation / oxidation (MANnitol) (4)	Blue/ blue-green	Yellow
INO	inositol	fermentation / oxidation (INOsitol) (4)	Blue / blue-green	Yellow

The following reagents were added to the corresponding micro-tubes:-

i- One drop of 3.4 % ferric chloride to the TDA microtube.

ii- One drop of Kovac's reagent to the IND microtube.

iii- One drop of Vogas – Proskauer reagent to VP microtube.

Appendix (2): Biochemical reactions and results of identification of bacterial isolates by API STAPH system.

TESTS	ACTIVE INGREDIENTS	QTY (mg/ cup)	REACTIONS / ENZYMES	RESULT	
				Positive	**Negative**
GLU	D-glucose	1.56	(Positive control) (D-GLUcose)	Yellow	Red*
FRU	D-fructose	1.4	Acidification (D-Fructose)		
MNE	D-mannose	1.4	Acidification(D-Mannose)		
MAL	D-maltose	1.4	Acidification (Maltose)		
LAC	D-lactose	1.4	Acidification (Lactose)		
TRE	D-TREhalose	1.32	Acidification (D-TREhalose)		
MAN	D-MANnitol	1.36	Acidification (D-MANnitol)		
XLT	XyLiTol	1.4	Acidification (XyLiTol)		
MEL	D-MELibiose	1.32	Acidification (D-MELibiose)		
NIT	Potassium nitrate	0.08	Reduction of Nitrates to nitrites	NIT 1 + NIT 2 / 10 min Light Pink Red	Colorless

PAL	ß-naphthyl phosphate	0.0244	Alkaline Phosphates	ZYM A + ZYM B / 10 min Violet	Yellow
VP	sodium pyruvate	1.904	Acetyl-methyl-carbinol production (Vogas- Proskauer)	VP 1 + VP 2 / 10 min Violet-pink	Colorless
RAF	D-raffinose	1.56	Acidification (Raffinose)	Yellow	Red
XYL	D-XYLose	1.4	Acidification (XYLose)		
SAC	D-saccharose (sucrose)	1.32	Acidification (Saccharose)		
MDG	methyl-α D-glucopyranoside	1.28	Acidification (Methyl-α D-glucopyranoside)		
NAG	N-acetyl-glucosamine	1.28	Acidification (N-Acetyl-Glucosamine)		
ADH	L-Arginine	1.904	Arginine DiHydrolase	Orange-red	Yellow
URE	urea	0.76	UREase	Red-violet	Yellow

The following reagents were added to the corresponding micro tubes:-

i- One drop of (ZYM A & ZYM B) reagent to PAL microtube.

ii- One drop of (NIT 1 & NIT 2) reagent to NIT microtube.

iii- One drop of Vogas – Proskauer reagent to VP microtube.

الخلاصة

هدفت الدراسة إلى التحري عن تأثيرضد اللايسوزايم والاجسام المناعية IgA في الدموع على تواجد ونموالأحياء المجهرية(بكتريا و/اوفطريات)المسببة لألتهابات قرنية العين.

لهذا الغرض فقد تم أخذ(105) عينة كشطات من مرضى التهاب قرنية العين المراجعين لمستشفىابن‌الهيثم‌للعيون ف ي بغداد. أظهرت النتائج بعد زرع العينات على الاوساط الزرعية الاختيارية ، أن 37 من هذا ملعينات كانت إيجابية لتواجد الأحياء المجهرية: منها (67.46%)86 عينة حاوية على البكتريا و(4.76%) 5 على الفطريات.

بعد ان تم تشخيص العزلات البكتيرية بالاعتماد على الاختبارات الزرعية والمجهرية والفسيولوجية امكن الحصول على الأنواع والنسب المئوية التالية:

Pseudomonas aeruginosa 29 (39.72%), *Staphylococcus* aureus 22 (30.13%) and *Staphylococcus epidermidis* 17 (23.28%).

اما بالنسبة للفطريات فقد تم تشخيصها اعتمادا على صفاتها الزرعية والمجهرية اضافة الى نوع وأشكال الأبواغ التي تكونها، وكانت ممثلة بالنوعين الأتيين ونسبتيهما المئويتين:

Aspergillus niger 3 (4.12 %) و*Alternaria* spp. 2 (2.73 %).

اعتمادا على ماتم الحصول عليه من نتائج اعلاه عدت *P.aeruginosa* الاكثر تواجدا في عينات التهابات قرنية العين البكتيرية، بينما كان عفن *Aspergillus niger* هو السائد في التهابات القرنية التي سببتها الفطريات.

فيما يتعلق بجنس المرضى، تبين أن حالات التهاب القرنية الجرثومية في الذكور والتي بلغت (41) حالة (56.16%) هي أكثر منها في الاناث التي تمثلت ب (32) حالة (58.24%). وفيام يتعلق باتائفل العمرية التي شملتها الدراسة ، فقد كان مرضى الفئة العمرية)59-41 (عاما الأكثر تعرض ا

لإصابة قرنية العين الجرثومي بنسبة مئوية بلغت (30.20 %)، فيما كانت الفئة العمرية (60 ≥) عاما الأقل عرضة وبنسبة (17.80%).

حصلت زيادة احصائية معنوية في مستويات (IgA) لمرضى التهابات قرنية العين الجرثومي . وذلك عندما كان متوسط مستويات (IgA) اعلى كثيرا مقارنة بالسيطرة (t = 2.04 for 40 df, P< 0.05) في الفئة العمرية 20≤ عاما . وفي الفئة العمرية (21-40) امكن الحصول على متوسط مستويات (IgA) تفوق احصائيا تلك العائدة الى عينات السيطرة يقابل (t=4.46 for 54 df, P<0.001). وهذا انطبق أيضا على الفئتين العمري تين (41-59) و (60≥) عاماعندما فاق مستوى (IgA) ذلك المسجل للسيطرة (t=4.04 for 42 df, P<0.001) و t=2.58 for 59 df, (P<0.001).

اكدت النتائج حصول انخفاض في مستويات ضد اللايسوزايم في المرضى الذين يعانون من التهابات قرحة قرنية العين بالمقارنة م ع السيطرة وذلك عندما كانت الزيادة في مستوى ضد اللايسوزايم في الدموع في الفئ تين العمري يتين 20≤ و(21-40)عاما ذات فرق معنوي (P<0.001). كذلك كان انخفاض مستوى ضد اللايسوزايم في ا لدموع للفئتين العمري يتين (41-59) و60≥ عاما ذوفرق معنوي (P<0.001). كما ودلت النتائج على عدم وجود فروقات معنوية بين الجنسين المشمولين بالدراسة.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم
قسم التقانة الاحيائية

تأثير ضد اللايسوزايم والاجسام المناعية IgA في الدمع على الاحياء المجهرية المسببة لالتهاب قرنية العين

رسالة ماجستير
مقدمة الى مجلس كلية العلوم/جامعة النهرين كجزء من متطلبات نيل درجة الماجستير علوم/تقانة احيائية

من قبل
مروة عادل حسين العكيدي
بكالوريوس علوم/تقانة احيائية/كلية العلوم/جامعة النهرين
(2012)

إشراف

د.عبدالواحد باقر الشيباني (استاذ)

د.فائز اسماعيل الشكرجي (استاذ مساعد)

ذي القعدة 1435

ايلول 2014