A Dissertation on

CLINICAL AND LAB STUDIES ON SUSPECTED CASES OF MICROBIAL KERATITIS – A HOSPITAL BASED STUDY

Submitted to the

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CERTIFICATE

This is certify that study entitled "**Clinical and Lab Studies on suspected Cases of Microbial Keratitis**" is the result of original work carried out by **Dr.Keerthana.K.E**, under my supervision and guidance at STANLEY MEDICAL COLLEGE, CHENNAI The thesis is submitted by the candidate in partial fulfilment of the requirements for the award of **M.S Degree in ophthalmology**, course from May 2008 to April 2013 at the Stanley Medical College, Chennai.

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DECLARATION

I hereby declare that this dissertation entitled "**CLINICAL AND LAB STUDIES ON SUSPECTED CASES OF MICROBIAL KERATITIS –A HOSPITAL BASED STUDY"** is a bonafide and genuine research work carried out by me under the guidance of **Prof. Dr.K.Basker**, M.S., D.O., HOD, Department of Ophthalmology, Government Stanley Medical College and Hospital, Chennai – 600 001.

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PART-I

INTRODUCTION

Like most socio-economic problems of this subcontinent the problem of blindness in India is of colossal magnitude.

The number of totally blind in India are about 14/1000 population out of which corneal blindness accounts for about 9-12 %.Low standards of health, poverty and ignorance are among the most fundamental causes of this high incidence of blindness in India.

Corneal blindness is a disaster because majority of corneally blind are blind from a young age and therefore cannot be educated and are non-earning members of the family. If the corneally blind are not treated and cured, they remain a liability to the family and community for the rest of their lives.

Corneal infections are sight threatening conditions, which if not treated as an emergency may lead to total blindness.

The organisms most commonly isolated vary from one geographic region to the other depending upon the climate, soil and pattern of patient referrals.

The present study is undertaken to know the aetiology, incidence and pattern of corneal infections amongst patients treated at Stanley medical college hospital Chennai.

HISTORICAL ASPECTS

Basis of modern outlook of fundamental pathology of cornea was laid by fuchs, as indeed may be said of most ocular tissues.

The term **keratitis** was first introduced by **James wardrope (1782-1869)**.

One of his most distinguished followers was **Sri William Lawrence (1783-1867)**. His treatment of corneitis is superb. The onyx, for example, so is now known as a hypopyon, had always been considered to be the "**effused matter**" which insinuated itself between the lamellae of cornea (abscesses intralamellar corneae) was correctly ascribed by him to an effusion of pus into anterior chamber (pures substunica cornea in ocute collectio).

Theoder Axenfield (1867-1930) demonstrated the important role of **pneumococcus** in corneal disease.

In 1895, simultaneously with **Morax**, discovered the **diplobacillus**, which bears their conjoint names.

It is interesting to note that essentially same organisms are found in corneal epithelium as occurs in conjunctival sac. Comparing the growth obtained from smears taken by cotton-wool application, **Blank (1929)**

found an identical flora in 102 eyes, **Pneumococci** 52% **staphylococci** 78%, **cornybacterium xerosis** 66.5%, **diplobacilli** 4% and **Bacilis subtilis** 2.5%.

Given good cultural conditions, the virulence of the organism to cornea is seen in experiments of **Hirotusji (1958)** who found that on intracorneal infection of heat killed staphylococci or **pneumococci** little or no reaction resulted, but when pseudomonads were injected, a violent keratitis with hypopyon resulted.

In this experiments, **Safar** showed the presence of the **organism in normal corneal tissues** far from the site of inoculation 32 hrs. Later, he explained the rapid and malignant course of the ulcer and its failure to respond to local therapy by the vigorous propagation and active migration of the organism in the stroma and its profuse production of endotoxins and exotoxins.

ANATOMY OF THE CORNEA

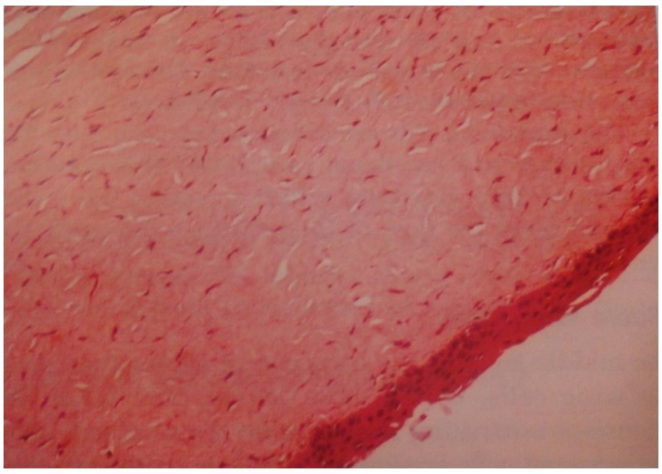
GROSS ANATOMY

The eye is made up of 3 concentric tunics, an outer fibrous shell, middle vascular uveal tract and an inner neurosensory retina. The cornea makes up anterior $1/6^{th}$ of the outer tunic; sclera makes up other $5/6^{th}$. The cornea is clear transparent tissue that joins the opaque sclera at transitional zone limbus.

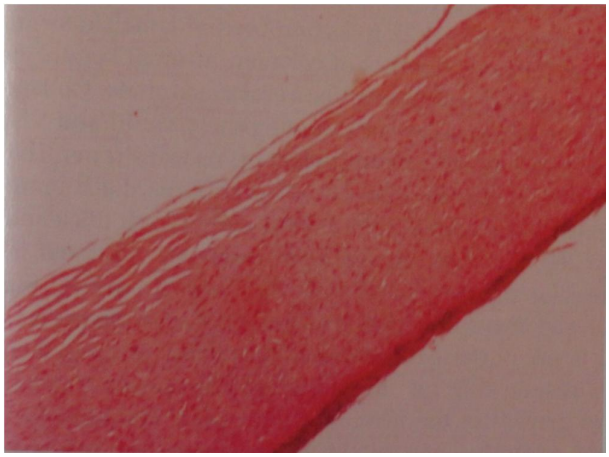
Cornea is circular when viewed from posterior surface, whereas it is oval when viewed from anterior surface. This is because of more prominent limbus superiorly and inferiorly. The diameter of cornea is 12.6 mm horizontally and 11.7 mm vertically.

The anterior surface of cornea is not uniformly curved. The **central $1/3^{rd}$** is called the **optical zone**. It is approximately spherical. The radius of curvature of anterior surface centrally is 7.8 mm and of posterior surface is 6.6 mm [1]. As the major refractive surface of the eye, the anterior surface of cornea provides approximately +48 dioptres of power. The posterior surface of cornea is more spherical than anterior surface. Therefore, central cornea is thinner (0.52mm) peripheral cornea(0.65mm).

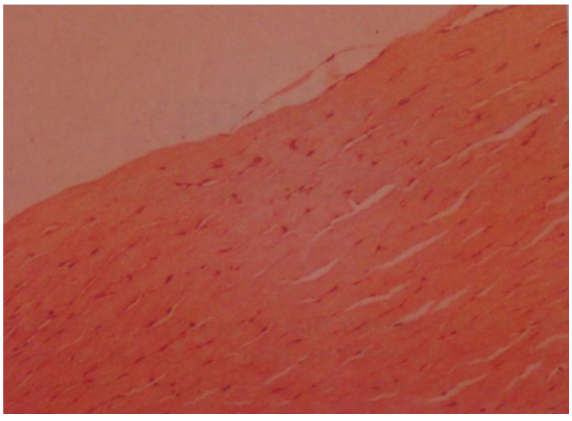
HISTOLOGIC SECTION OF CORNEAL CONSISTING OF EPITHELIUM BASEMENT MEMBRANE, STROMAL LAYERS



HISTOLOGY OF THE CORNEA



HISTOLOGIC SECTION OF THE CORNEA CONSISTING OF THE STROMAL LAYERS, DESCEMET'S MEMBRANE AND ENDOTHELIUM



MICROSCOPIC ANATOMY

The cornea can be divided into 5 layers. The **epithelium, Bowman's membrance, stroma, Descemets membrane and endothelium**.

EPITHELIUM

The epithelium is 50 μ thick and is made up of 5-6 superimposed layers of non-keratinised cells. It consists of a single layer of basal cells, an intermediate zone consisting of 2-3 layer of wing or polyhedral cells and 2 outer layer of flat cells.

BOWMAN'S MEMBRANE

Anterior limiting membrane or anterior elastic lamina does not contain elastic tissue. It is a thin acellular structure and may be regarded as modified portion of stroma. The Bowman's membrane does not regenerate when damaged.

SUBSTANTIA PROPRIA OR STROMA

Makes up 90% of corneal thickness. It is a modified connective tissue and has 3 components collagen lamellae, cells and matrix.

(a) Fixed cells

(b) Wandering cells.

Fixed cells are called corneal corpuscles, which resemble corpuscles of bone.

Wandering cells play an important part in inflammation.

THE POSTERIOR LIMITING MEMBRANE (DESCEMETS MEMBRANE)

Is a **strong homogenous** and very **resistant** membrane.

Unlike Bowman's membrane which never regenerates, Descemets membrane can be reformed. **Termination of Descemets** membrane is an important gonioscopic land mark – The **anterior schwalbe's line**.

At the periphery of cornea, the posterior surface of membrane presents wart like elevations, the **Hassal-Henle bodies** which increases with age and in conditions of corneal dystrophies like Fuchs.

THE ENDOTHELIUM

Is the most posterior layer of cornea and is a single layer of flattened epithelial like cells.

Normal average **endothelial cell count is 2800 cells per sq.mm** which varies with age. There is a significant decrease in cell density with age. Techniques for evaluation of endothelium include **specular microscopy**, **pachymetry** and **fluorophotometry**.

VESSELS AND NERVES OF CORNEA

The cornea is avascular. However small loops derived from anterior ciliary vessels invade the periphery for about 1 mm. But, these vessels are not in the cornea. They are in the subconjunctival connective tissue which overlaps it.

Absence of blood vessels contribute to corneal transparency as well as to its priviliged immunologic status – however, this avascularity creates special problems for cornea in meeting its nutritional needs, mobilising defence mechanisms and repairing wounds in various layers.

Nerves

The cornea is supplied by Nasociliary branch of the ophthalmic division of trigeminal nerve via ciliary nerves and those of surrounding conjunctiva. The nerves pass into the cornea as 60-80 myelinated trunks at its junction with the sclera. After 2-4 mm, they usually lose their myelin sheaths and divide into 2 groups. The anterior (40-50) pass through the

substance of the cornea and then form a plexus under Bowman's membrane [15]. Having traversed this, the fibres again inosculate to form a subepithelial plexus and lastly, actually in among epithelial cells an intraepithelial plexus is found.

The posterior (40-50) pass to posterior part of cornea. But, there are no nerves in central posterior part of cornea, nor in Descemets or endothelium.

BIO-CHEMISTRY OF CORNEA

The chemical composition of cornea is the sum of its parts namely the stroma, the epithelium and Descemet's membrane. The number of cells and morphology varies among these areas, and this is reflected in chemical dissimilarities, chemically cornea is a heterogeneous tissue.

Corneal stroma

The corneal stroma forms the bulk of cornea. It is a differentiated connective connective tissue containing 75-80% water on wet weight basis. Of the remaining solids (20-28%) collagen, other proteins and **glycosaminoglycans GAG** or **mucopolysaccharides** constitute the major part. These collagen fibrils form the skeleton of corneal stroma.

Glycosaminoglycans

GAG mucopolysaccharides constitute 4 to 4.5% of dryweight of cornea. The GAG in the interstitial space play an important role in corneal hydration through interactions with electrolytes and water. 3 major fractions of the GAG are found in corneal stroma **keratan sulphate** (50%), **chondroitin sulphate** (25%) and **chondroitin sulphate A** (25%). GAG are implicated in the maintenance of corneal hydration and transparency.

Descemet's membrane

Consists of type IV collagen with glycine, hydroxy glycine and hydroxyl proline. Descemet's membrane is highly elastic and represent a barrier to perforation in corneal ulcers.

Corneal epithelium

The epithelium represents 10% of total weight of cornea. Water in the epithelium represents 70% of wet weight. The solids are

1) Nucleic acids (DNA) and RNA.

2) Lipids – Phospholipids and cholesterol in the cell membranes and proteins.

The epithelium contains high activities of enzymes of glycolysis, kreb's cycle and Na+k+ activated ATP ase.

Corneal Metabolism

In the cornea energy is needed for maintenance of transparency and dehydration.

Energy in the form of ATP is generated by the breakdown of glucose into lactic acid (glycolysis) and CO2 and H20 (Kreb's cycle). The

cornea obtains glucose mainly from aqueous humor[23]. The tears and limbal capillaries contribute minimal amounts of glucose for corneal metabolism.

Corneal dehydration:

The normal cornea maintains a fairly constant thickness during life. It keeps its water content at a steady level of 75-80%.

The following factors affect corneal hydration

1) Anatomic integrity of epithelium and endothelium.
2) Electrolyte and osmotic balance.
3) Metabolism.
4) Evaporation of water through anterior surface.
5) Intraocular pressure.

OCULAR SURFACE DEFENCE MECHANISMS

1. ANATOMIC BARRIERS

Eyelids

The eyelids are an anatomic barrier that protect the eyes against the external environment.

Tear Film

The mechanical flushing action of the tear film is probably its most important function. Constant flushing of the surface of the globe prevents adherence of micro-organisms to the eye, which is believed to be the first step in corneal infection. In addition to their flushing action, the constituents of the teat film help protect the cornea. The tear film contains **immunoglobulins**, **lysozymes**, **β-Lysin**, **lactoferrin** and **complement**.

Corneal Surface

The basement membrane of corneal epithelium constitutes an important anatomic barrier. The basal cells undergo mitosis & they are responsible for replacement and maintenance of epithelial integrity. The **glycocalyx** on the corneal surface forms a scaffold that binds mucus across the **microplicae** and **microvilli** of the anterior plasma membrane of the

epithelial surface. Mucin that adheres to the glycocalyx is essential for tear film stability. The corneal like the conjunctiva, is highly innervated with unmyelinated nerve endings. This is extremely important for corneal well-being and provides the eye with a sensory alert to avert trauma.

Conjunctival Surface

The conjunctival epithelium is crucial in cases of total corneal epithelial damage because it multiplies and slides over to cover the corneal surface and restore its integrity[26]. The cornea is devoid of lymphatic vessels except when vascularized. The conjunctiva on the other hand, is equipped with subepithelial lymphoid tissue (conjunctiva-associated lymphoid tissue (CALT).

2. HUMORAL IMMUNE RESPONSE

Contributed by IgA, Lysozyme, β-Lysins, Lactoferrin.

3. CELLULAR IMMUNE RESPONSE

By Langerhans cells and lymphocytes.

WOUND HEALING

Wound healing in cornea differs from that of other tissues primarily owing to its avascularity. In addition, nature of injury affects the course of wound healing. For instance, infectious ulcers create special problems for wound repair not encountered in clean surgical wounds. Other factors such as location of the wound (central vs. Peripheral) and presence of other corneal, ocular or systemic disease may also affect wound healing. Topical medications may have profound influence on wound healing. This is specially true of steroids.

Epithelial Wound Repair

Normally, the epithelium is continuously renewed from the basal layer with cell division by mitosis every 7 days. A cornea completely denuded of epithelium can recover in 4-7 days. The sequence of events involved is

(1) initial mitotic paralysis.

(2) sliding of epithelial cells bordering the defect to cover the wound and

(3) restoration of normal layer thickness by mitoses.

Stromal Wound Healing

Normal stromal wound healing is most important, for the structural support for the cornea. The effect of corneal avascularity is most readily seen in this layer. Central avascular corneal wounds. Vascularised corneas heal faster than non-vascularised corneas. So, blood vessels play a beneficial role in wound healing although exact mechanism is not known.

After stromal wounding new collagen and new ground substance are synthesized by **fibroblasts**. Soon after injury numerous fibroblasts appear in the area of the wound. Collagen synthesis by **keratocytes** begins within first one and a half hours after injury.

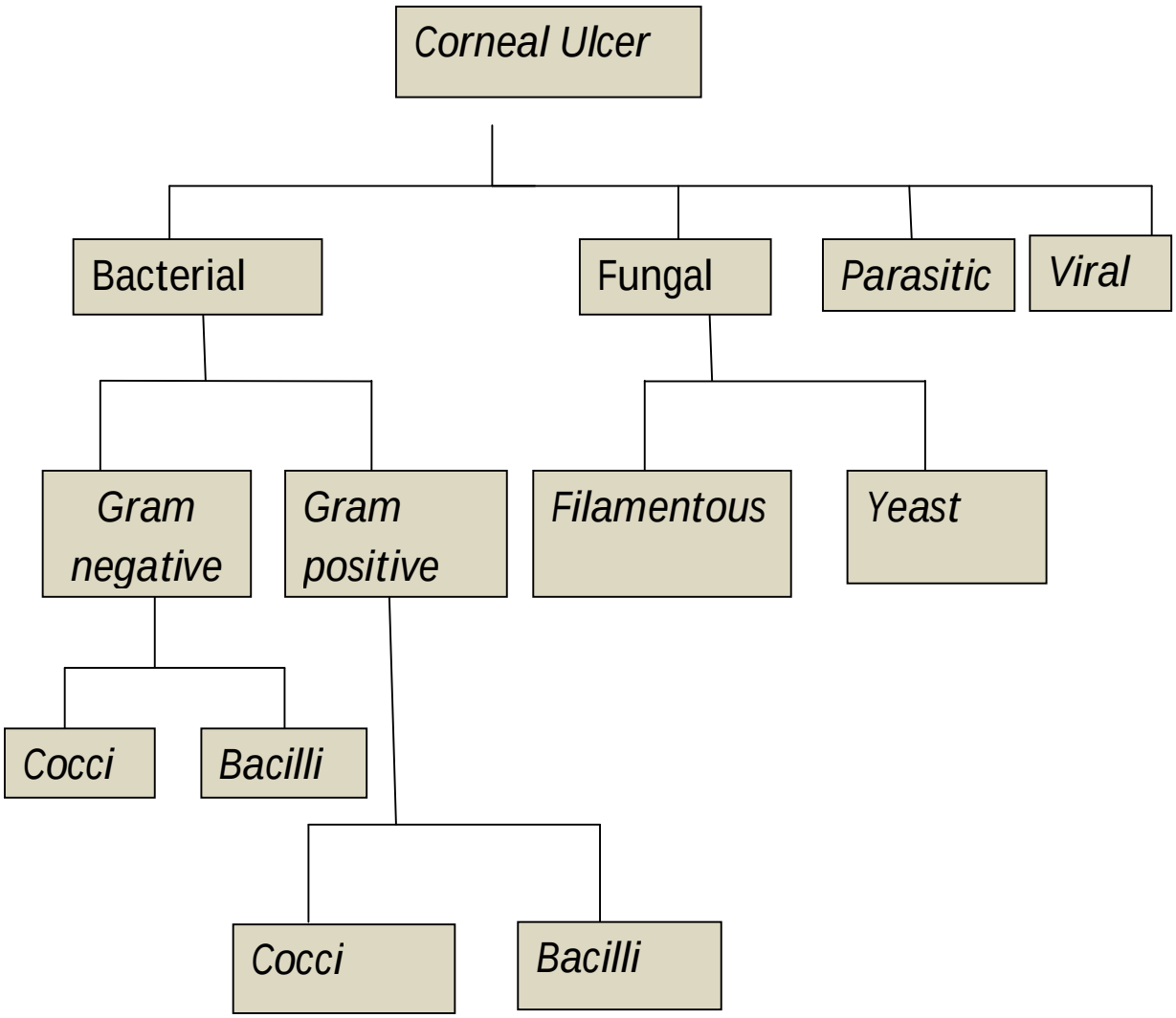
Leucocytes are thought to be important in corneal stromal wound healing. Necrotic debris due to cellular death following injury accumulates in the wound during first few hours after injury[10]. **Polymorphonuclear leucocytes** appear to reach the cornea from limbal vessels via tear film and act either as macrophages engulfing the debris or by enzymatic action of acid hydrolysis in their cytoplasm, lyse the damaged tissue to facilitate resorption.

Endothelial Wound Healing

Present concepts of endothelial wound healing indicate that while mitosis may occur in younger patient (less than 20 years of age) adult human endothelium repairs defects in its layer by a process of enlargement and sliding.

AETIOLOGICAL CLASSIFICATION OF INFECTIVE KERATITIS

1. INFECTIVE KERATITIS



Such infections may be

- Exogenous affecting the cornea primarily.
- Arise by continuity from the conjunctiva, sclera and uveal tract.
- Endogenous in origin

BACTERIAL KERATITIS

Etiology

Common causes of infection are

Gram Positive Cocci

- Staphylococcus aureus
- Coagulase negative streptococci
- Streptococcus pneumonia
- Streptococcus pyogenes
- Streptococcus viridans
- Anaerobic Streptococcus (rare)

Gram negative diplobacilli / cocci

- Moraxella
- Neisseria gonorrhoea
- Neisseria meningitidis

Gram positive rods

- Corynebacterium diphtheria (rare)
- Diphtheroids

Gram negative rods

- Actinobacter species
- Escherichia coli
- Klebsiella pneumonia
- Morganella morgagni
- Proteus species
- Pseudomonas aeruginosa
- Serratia marcescens
- Anaerobes

Acid Fast Bacteria

- Mycobacterium chelonae
- Nocardia asteroids

FUNGAL KERATITIS

Fungi of importance in microbial keratitis

Moniliciae

- Fusarium
- Aspergillus
- Acremonium
- Penicillin

Dematiaceae

- Curvularia
- Alternaria

Yeasts

- Candida (albicans, parapsilosis, glabrata, tropicalis)

PREDISPOSING FACTORS AND PATHOGENESIS

EXOGENOUS FACTORS

- Trauma
- Antibiotics
- Antifungal
- Corticosteroids
- Contact Lens

ENDOGENOUS LOCAL CONDITIONS

LID Disorders

- Lagophthalmos – ulceration is due to exposure
- Exophthalmos
- Entropion: exposure, mechanical irritation and tear film instability contributes to infection.
- Blepharitis.

Lacrimal Disorders

Keratitis sicca – Mechanical flushing and cleaning is lost. There is also lack of immune globulins, lysozymes, β-lysins, lactoferrin and complement which protects the cornea.

Dacryocystitis

Commonly associated with pneumococcal infections.

Conjunctival disorders

- Vernal catarrh
- Trachoma
- Pemphigoid : Keratopathy is secondary to ectropion, aberrant lashes, conjunctival keratinisation and dry eye.
- Steven Johnson syndrome
- Xerophthalmia.

Corneal Disorders

- Trigeminal anaesthesia – Neurotrophic keratitis, because of decreased corneal sensation.

- Bullous keratopathy – endothelial damage and influence of aqueous – subepithelial bullae, necrosis, sloughing, ulceration.

- Herpetic ulcers.

Prior Surgery – Same Eye

Secondary to prolonged use of topical steroids and the presence of loose sutures.

SYSTEMIC CONDITIONS

Vitamin A and Malnutrition

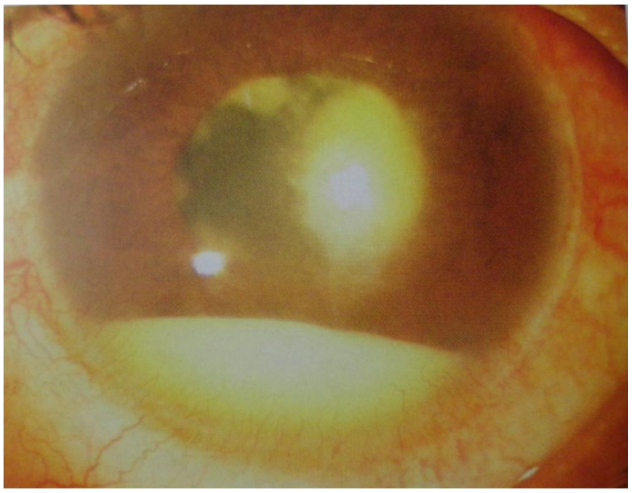
This preventable cause of blindness accounts for around 70% of total causes of blindness.

Severe protein energy malnutrition can interfere with synthesis of proteins in vitamin A metabolism.

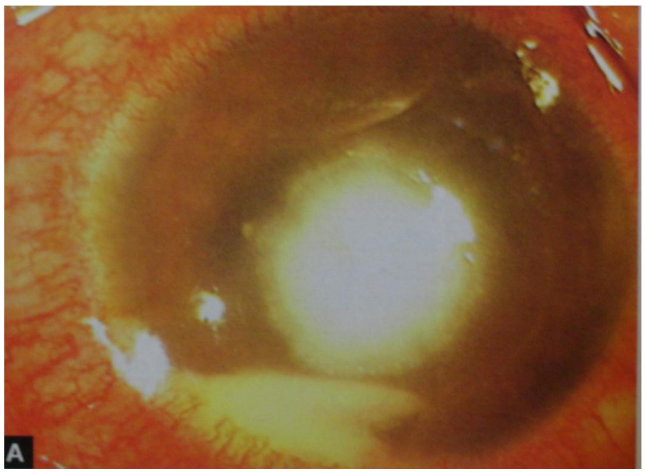
Measles

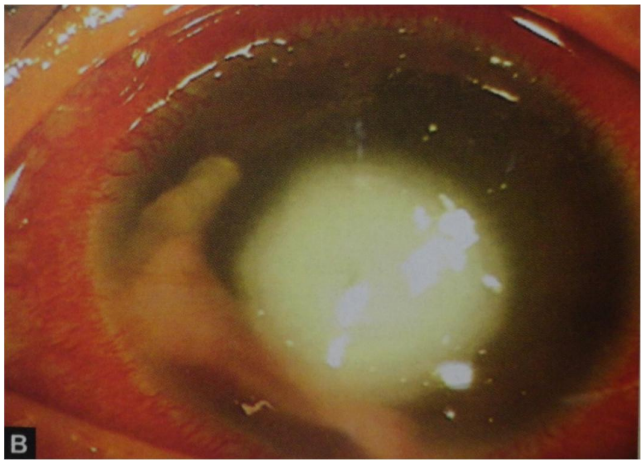
Allergy

HYPOPYON CONREAL ULCER

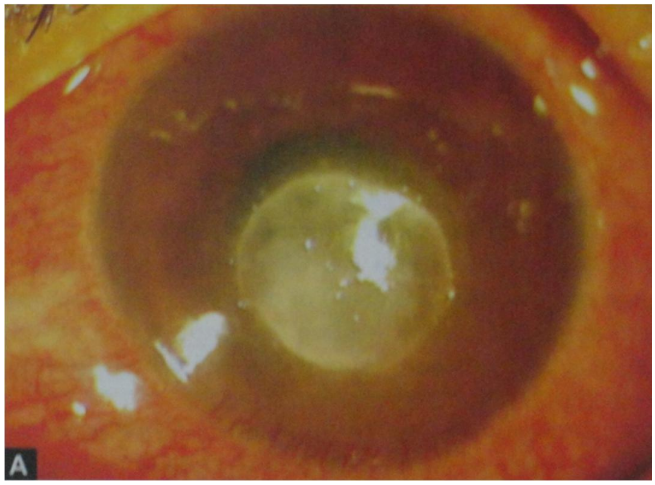


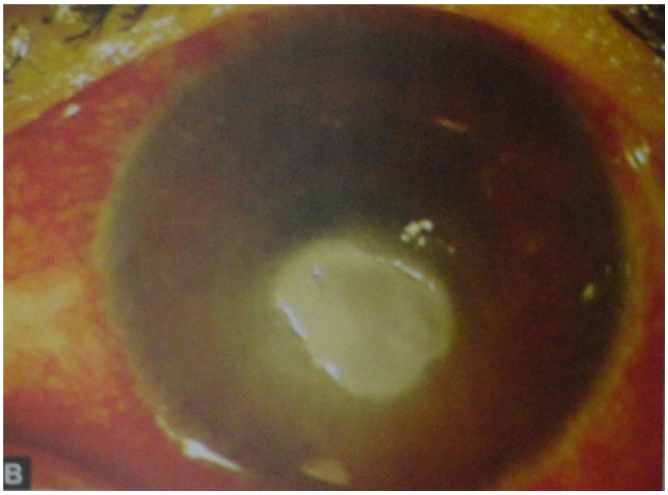
HYPOPYON MOVES AS THE PATIENT MOVES FROM ERECT (A) TO SUPINE POSITION (B)

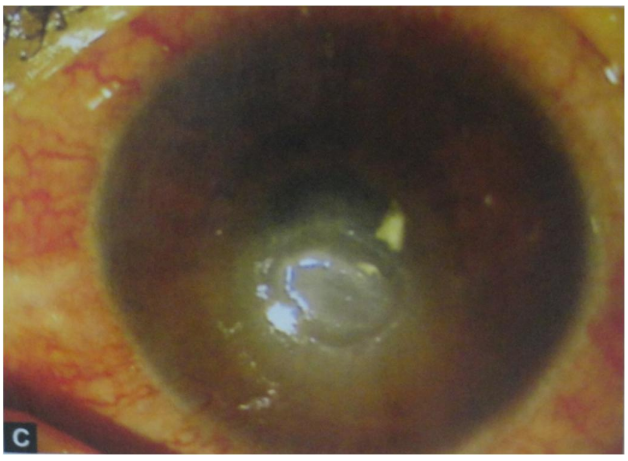




PATHOGENESIS OF CORNEAL ULCER AND STAGES OF HEALING







PATHOGENESIS

N.gonorrhoea, Cornybacterium diphtheria, Listeria and haemophilus aegyptius (Koch-Weeks bacillus) are unique in that they may initiate infection without antecedent epithelial ulceration. Specialised bacterial enzymes aid in the degeneration of corneal substance and in the penetration of the organisms into the deep stroma[8]. Multiple toxins are released, including the alpha , beta, gamma and delta toxins of Staphylococcus, the A,B and C toxins of pseudomonas, and other enzymes, including proteases, coagulase, collagenases, nucleases, fibrinolysins, lipases and hemolysins. Some substances (exotoxins) are released by actively multiplying organisms, and some (endotoxins) only after the death of the organisms and the latter cause ring infiltration. These enzymes or toxins can persist and continue to digest corneal stroma long after the demise of the pathogen. Polymorphonuclear leukocytes are important in host defences but are also implicated in the destruction of the corneal collagen matrix and ground substance by elaborating proteoglycase and collagenase.

HISTOPATHOLOGY

Stage of Progressive Infiltration

There is adherence and entry of organism, followed by invasion into the stroma. The host mounts a polymorphonuclear and lymphocytic infiltrative response into the epithelium and stroma, which gives the clinical appearance of a yellow or white corneal opacity with overlying epithelial edema or loss of epithelium. There is multiplication of the organisms, with production and diffusion of enzymes and toxins.

Stage of active ulceration

The clinical picture varies with the virulence of the organism and toxin production. Symptoms of pain, photophobia, and decreased visual acuity are prominent in this stage. There is necrosis and subsequent sloughing of epithelium and stroma, leaving a demarcated defect with a surrounding infiltration of polymorphonuclear leukocytes, edema of the stroma and overlying epithelium, necrotic ulcer base is surrounded by heaped-up tissue[5].

The anterior chamber reaction may progress to a large hypopyon. The organisms may penetrate deeper, leading to descemetocele formation. Descemet's membrane is resilient against bacterial invasion, but corneal

perforation can occur. Alternatively, the ulcer may progress peripherally toward the sclera[13]. A flat anterior chamber with iris prolapsed, subluxation of the lens, cataract formation, or endophthalmitis is the aftermath of uncontrolled progression.

Stage of regression

Humoral and cellular immune defence mechanisms combine with antimicrobial therapy to neutralize the organism and the cellular debris. A distinct line of demarcation may develop as the ulcer and stromal infiltration round up their edges. Vascularization of the cornea may occur in indolent ulcers.

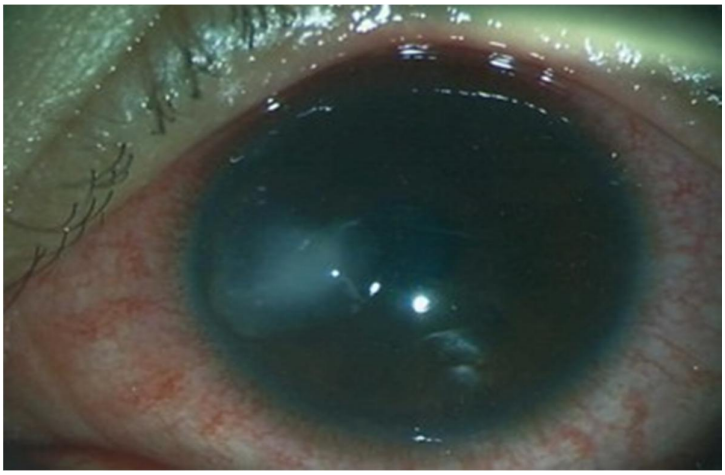
Stage of Healing

There is continuous removal of residual debris and repair of structural alterations, with transformation of histiocytes and keratocytes into fibroblasts. Vascularization occurs in an attempt to fill in the defect. Bowman's layer does not regenerate, but is replaced with fibrous tissue. The epithelium heals slowly over this irregular base[6]. The thinned stroma may again thicken as new lamellae are added, but depressions and facets may remain. Vascularization tends to disappear, leaving ghost vessels

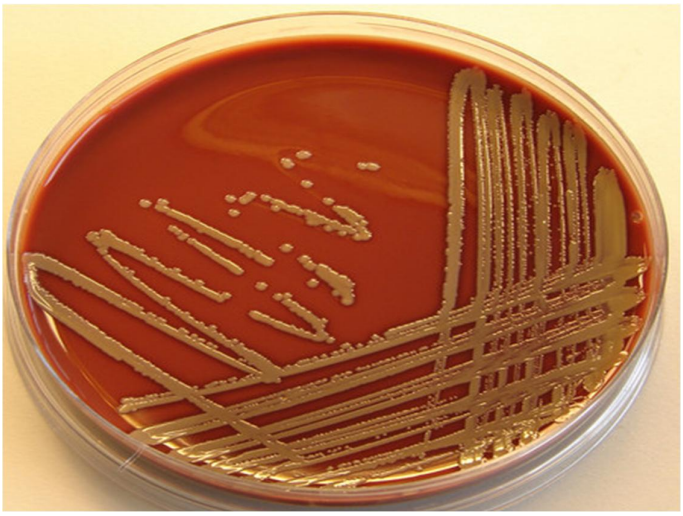
GRADING OF CORNEAL ULCERS[8]

Characteristic	**Mild**	**Moderate**	**Severe**
Size of the ulcer (mm)	<2	2-5	>5
Depth of the ulcer (%)	<20	20-50	>50
Infiltrate	Infiltrate may be dense but superficial and limited to ulcer base	Infiltrate dense and extending to mid stroma	Infiltrate dense extending deeper to the midstroma or into sclera
Sclera	Not involved	Not involved	May be involved

STAPHYLOCOCCUS AUREUS



STAPHYLOCOCCUS AUREUS IN BLOOD AGAR & NUTRIENT AGAR



SPECIFIC CHARACTERISTICS OF EACH ORGANISM

Gram positive cocci: tend to cause localised, round or oval, greyish white ulcers that have distinct borders and minimal surrounding epithelial oedema and stromal infiltrate.

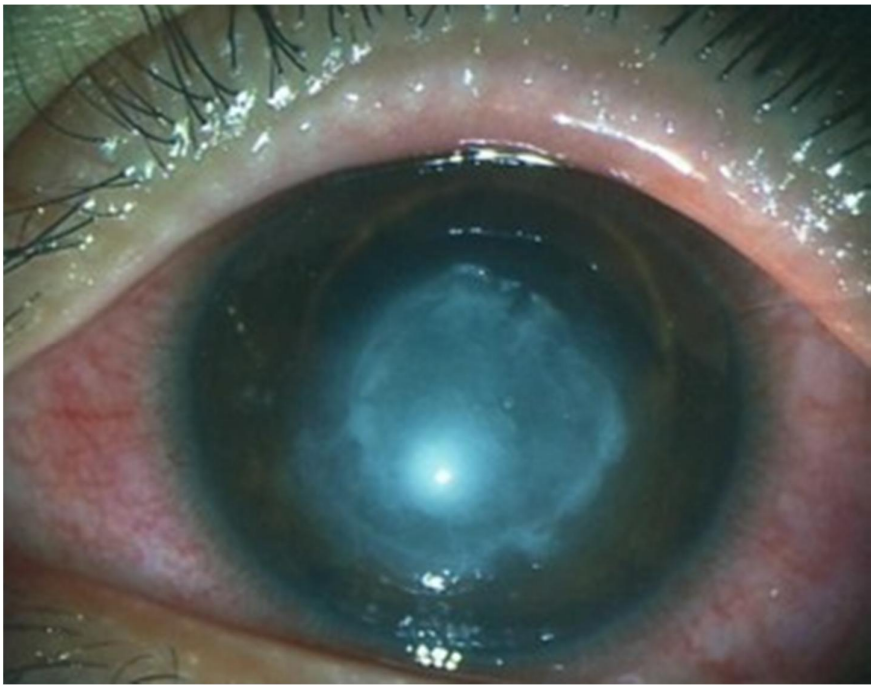
Staphylococcus aureus: central ulcerations are frequently superinfections in the compromised corneas, especially in association with herpes zoster, atopic dermatitis.

Staphylococcus epidermidis: presents with a more indolent ulceration and infiltration.

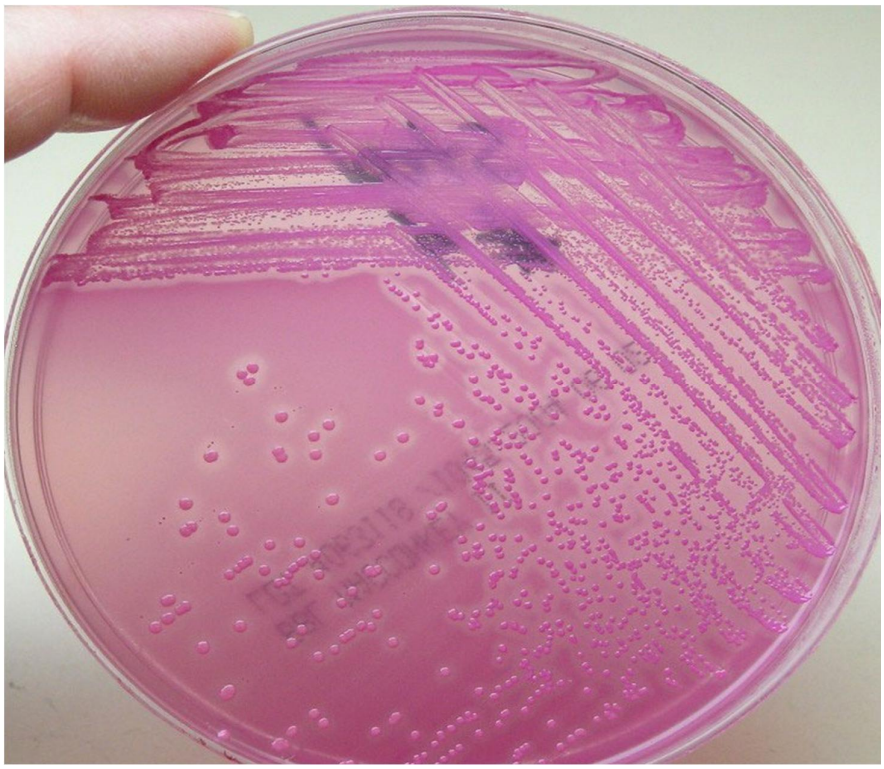
Streptococcus pneumonia

- There is a serpiginous edge of activity to the ulceration, which advances on one edge while healing on the opposite edge.
- Increasing dense stromal abscess formation with radiating folds in the Descemets membrane and moderate stromal edema.
- Hypopyon is a common feature, perforation is possible.

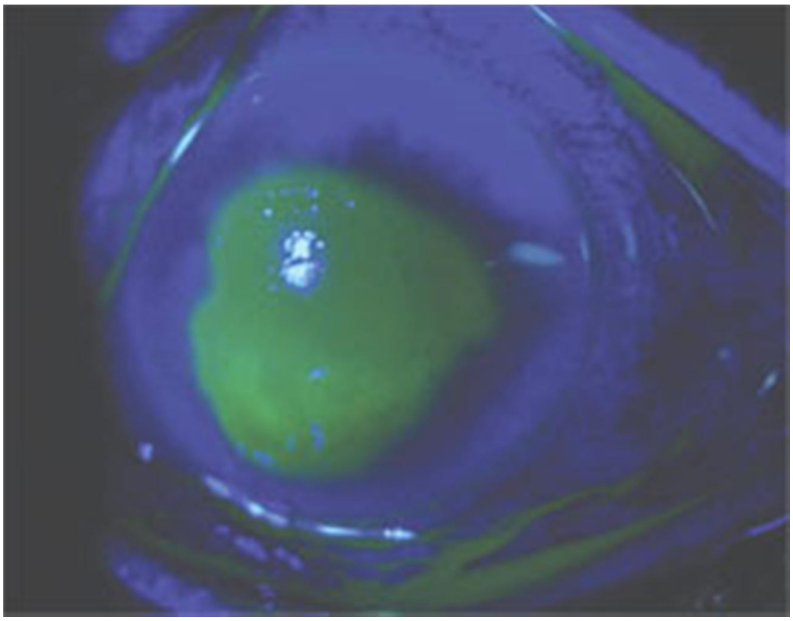
KLEBSIELLA SPECIES



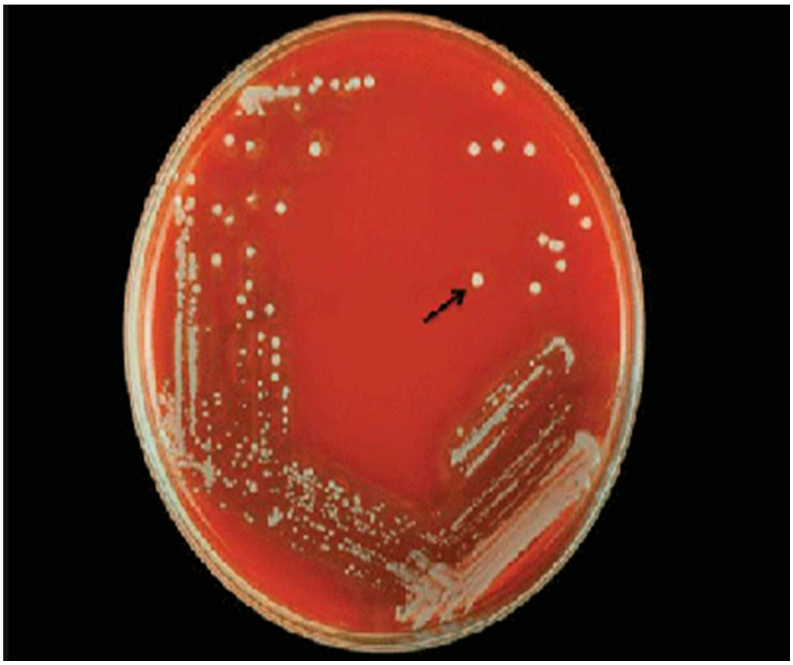
KLEBSIELLA SPECIES IN MAC CONKEY AGAR



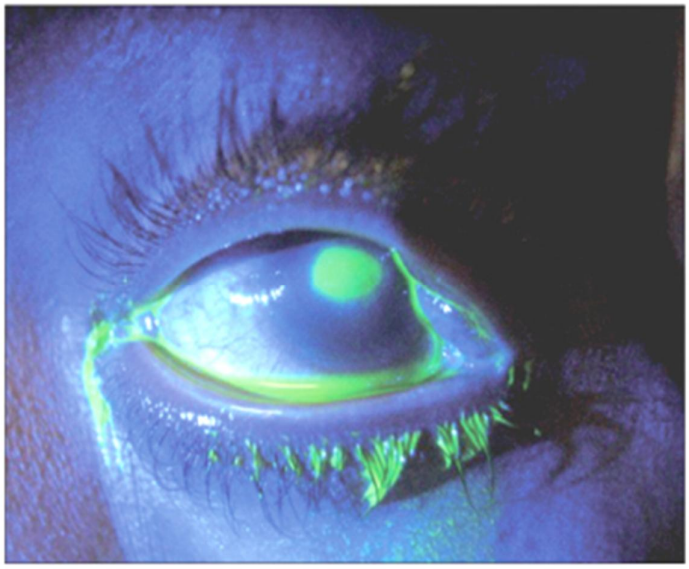
STAPHYLOCOCCUS ALBUS



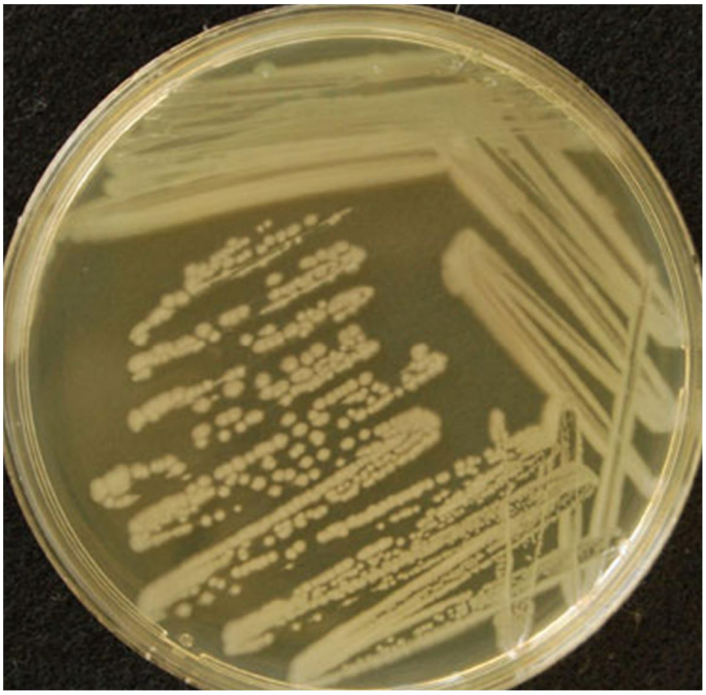
STAPHYLOCOCCUS ALBUS IN BLOOD AGAR



PSEUDOMONAS AERUGINOSA



PSEUDOMONAS AERUGINOSA IN NUTRIENT AGAR



Gram negative Bacilli

Pseudomonas aueroginosa : Rapid progression of central or paracentral broad, shallow ulceration with copious mucopurulent, yellowish green exudates. Rest of cornea has a ground glass appearance with loss of transparency or a diffuse graying of epithelium away from the ulcer site. Ulcer may progress rapidly with stromal abscess that may spread concentrically and symmetrically to form a ring ulcer with large hypopyon. Perforation is distinct threat.

The remaining Gram negative bacteria lack such distinct features. **Moraxella lacunata - pericentral or perilimbal area** and produce a shallow, irregular, indolent, grey white ulcer in the inferior zone. It is usually associated with **alcoholism, debilitation or diabeties**[2].

Neisseria - papillary conjunctival injection, chemosis, copious purulent discharge.

Bacillus cereus – a distinctive feature is the presence of ring infiltrate of the cornea, remote from the site of injury with rapid progression. Nocardia, Actinomyces, Streptomyces – indolent and may simulate fungal keratitis.

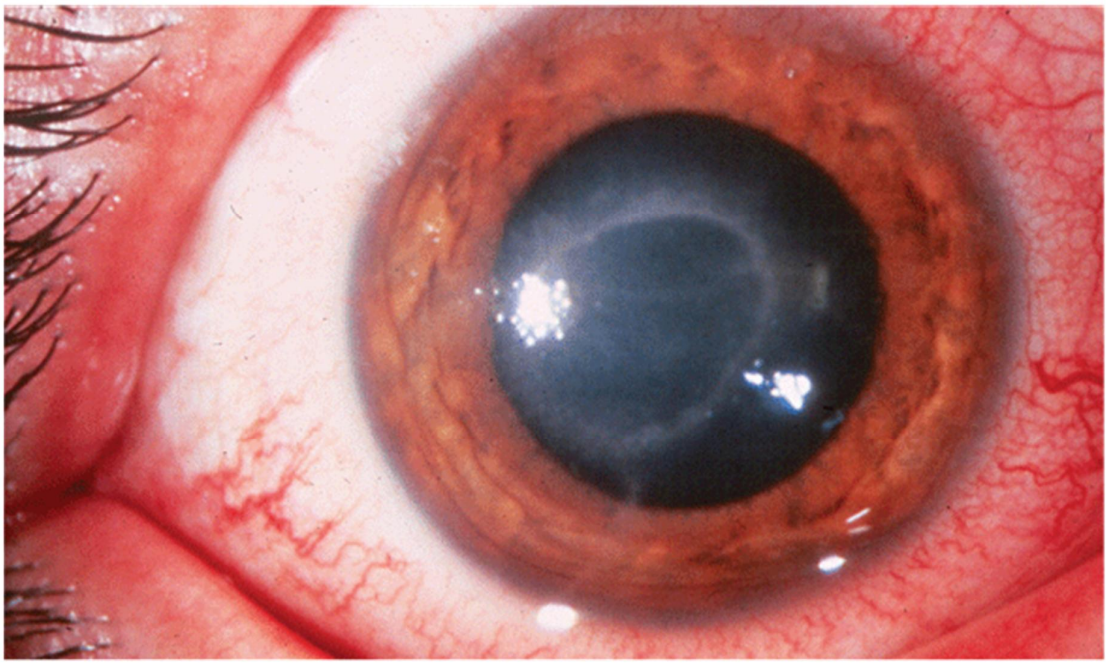
Mycobacterium fortituitum and mycobacterium chelonei – indolent slowly progressive lesions with a cracked **wind shield appearance**.

Ring abscess of the cornea have distinctive appearance and are associated with Proteus, Pseudomonas aueroginosa, Bacillus cereus, Streptococcus. They frequently occur following penetrating injury at the limbus.

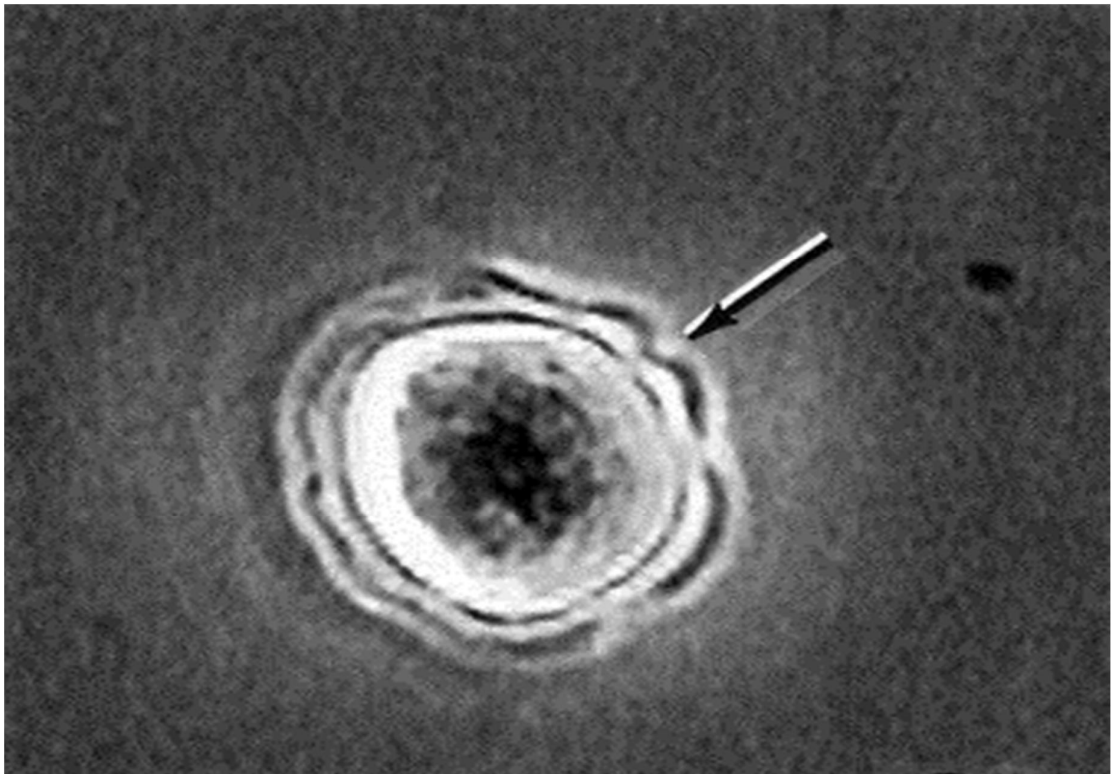
Fungal Keratitis

- Fine or coarse granular infiltrates
- The epithelial surface has a dry, rough texture, dirty grey – white colour.
- The lack of marked stromal inflammation may permit direct visualisation of pigment and delicate feathery and branching hyphae with surrounding stromal infiltration.
- Multifocal suppurative micro abscesses or satellite lesions, immune ring, endothelial plaques and hypopyon.
- Advanced disease, the entire cornea becomes homogeneously yellow white, stromal ulceration and necrosis may lead to perforation and endophthalmitis.

ACANTHAMOEBA KERATITIS WITH RING LIKE STROMAL INFILTRATE



CYST OF ACANTHAMOEBA



- Yeast keratitis – trauma is rarely the initiating factor. It occurs in immunosuppressed individuals. Causes small oval ulceration with an expanding discrete sharply demarcated dense yellow white stromal suppuration.

ACANTHAMOEBA KERATITIS

Acanthamoeba species have been found in soil, fresh water, sewage, sludge, brackish water, sea water, swimming pools, soft contact lens. Exists in trophozoite and cystic form.

- Young healthy immunocompetent individual
- History of direct contact to cornea with contaminated fluids with contact lens, foreign body, trauma.
- Severe eye pain
- Recurrent epithelial erosions, pseudo dendrites
- Ring infiltrates, nummular infiltrates, satellite lesions.
- Scleral nodules, scleritis.
- Usually misdiagnosed as HSV keratitis.
- A chronic progressive course despite treatment.
- Repeated negative cultures for bacteria, virus and fungus.

KERATOMYCOSIS

INTRODUCTION

Over one hundred fungal species, representing a wide spectrum of filamentous fungi, yeasts and dimorphic organisms have been reported as corneal pathogens. Although fungi are uncommon corneal pathogens, keratomycosis is becoming more common during the past two decades.

The pathogenicity of fungi to produce disease in humans was recognised very long back.

Schonlein, in 1837 had shown that fungi are capable of producing disease[3].

Remak, in 1945, Successfully transferred fungi from an apple to his own skin.

Gruby had shown in 1841 that favus mold was the culprit for alopecia which was confirmed by sabourard in 1892.

In 1856 Virchow – coined the term mycosis.

In 1897 Leber reported ‘Aspergillus’ as the cause of corneal ulcer with hypopyon.Several thousand species of fungi have been identified.

About 50 species are capable of producing disease in humans and animals. Only few can spread from animal to man or man to man.

After introduction of antibiotics, it became evident that there existed, in the body, really a rather delicate homoeostasis involving the endogenous fungi and bacteria, and that when the equilibrium was disturbed by suppression of normal bacterial flora, the hitherto normal harmonious fungi through either qualitative or quantitative changes became pathogenic.

As the steroids became freely available the picture became more complicated. It enhances the fungal growth, simultaneously producing deleterious effects on the structures of the cornea, thus enabling the fungi to invade.

CLASSIFICATION

Fungi are primitive, non-motile, plant like structures that may grow as unicellular organisms, called yeasts and multicellular filamentous structures called molds.

True fungi can be classified into 4 major classes[35].

A. **Zygomycetes**: They are non-septate filamentous fungi which are rare human corneal pathogens. Genera of this class are mostly associated with the orbital phycomycosis.

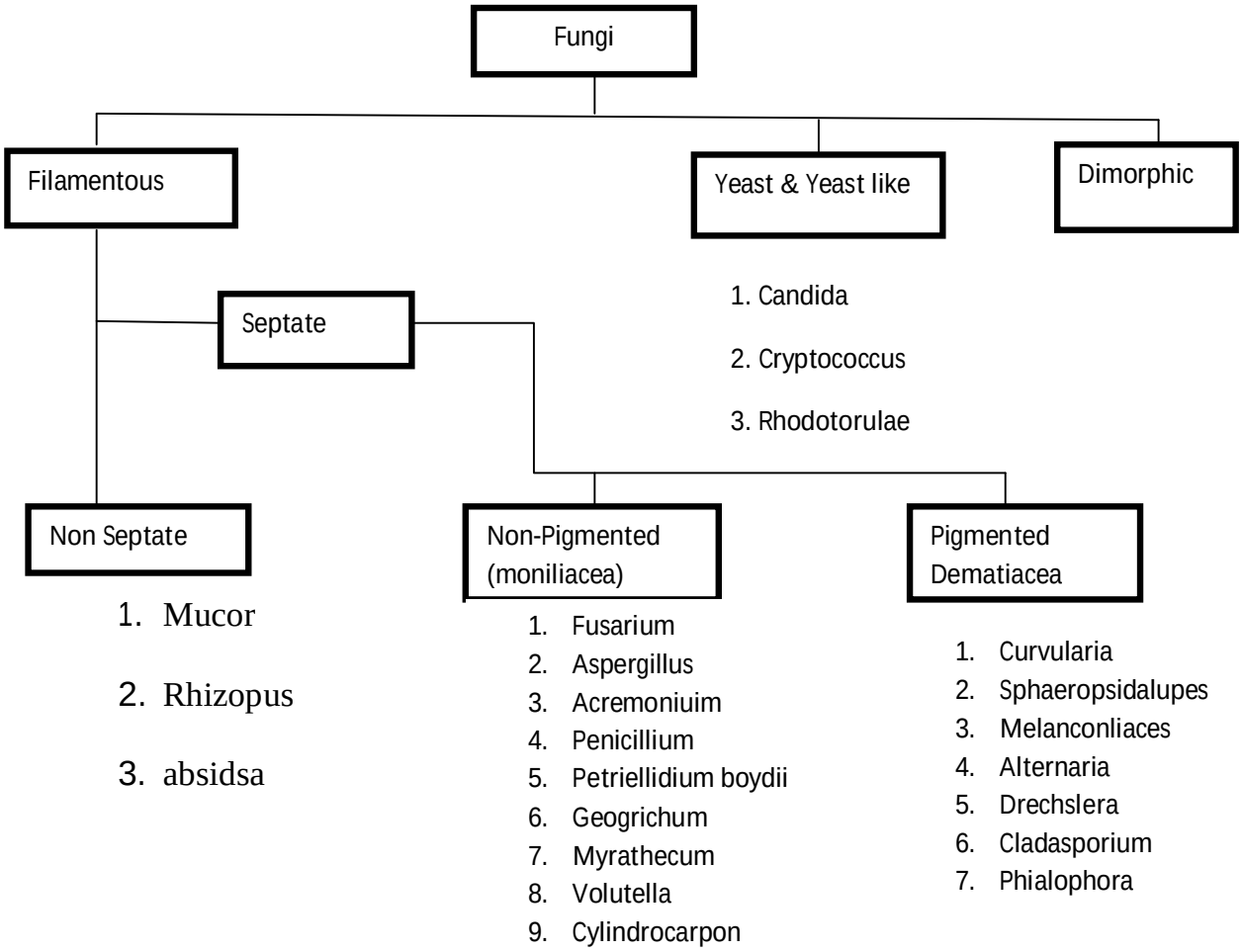
B. **Ascomycetes**: They are fungi with septate hyphae and containing spores in the sacs or asci. Corneal pathogens, representing this class include the Genera, Asperigillus and Penicillium.

C. **Basidomycetes**: They are fungi with septate hyphae and sexual spores contained within club-shaped structures called Basidia. Mushrooms and plant rusts belong to this class.

D. **Deutromycetes**: Fungi Imperfecti: Most of the human corneal pathogens belong to this class. The term imperfecti means lack of sexual spores.

More practical way of classification of the Pathogenic fungi would be the following [23]:

a) Filamentous fungi
    a. Septate
    b. Non septate
b) Yeast and yeast like fungi
c) Dimorphic fungi



Biology of the fungi

Fungi are plant like organism but lacking chlorophyll and that is why they are saprophytic. Understanding the biology of the fungi enables us to manage the keratomycosis effectively[31]. The important structure in the fungus is the cell wall.

It is composed mostly of

a) Polysaccharide:80% -90%

1) Cellulose

Or

Chitin

In each type of fungi only one of the above two is present and defines the characters of the fungi.

b) Lipids

Ergosterol is the unique component of the fungal cell wall whereas cholesterol is the counterpart in the animal cell wall. This difference plays an important role in designing the antifungal agents.

The fungal cell wall controls the influx and out-flow of ions into the fungi protoplasm.

So by altering this mechanism the antifungal agents could be designed. (Smollin – Liesigang) [26].

COMMON TRAUMATISING AGENTS

a) Plant Origin ---- a) Leaves

b) Tree branches

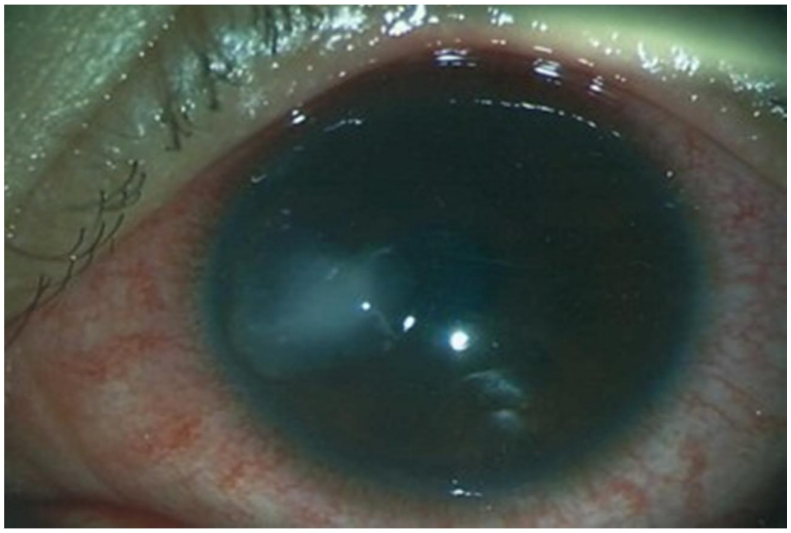
c) Paddy grain

d) Wooden chips (carpenter)

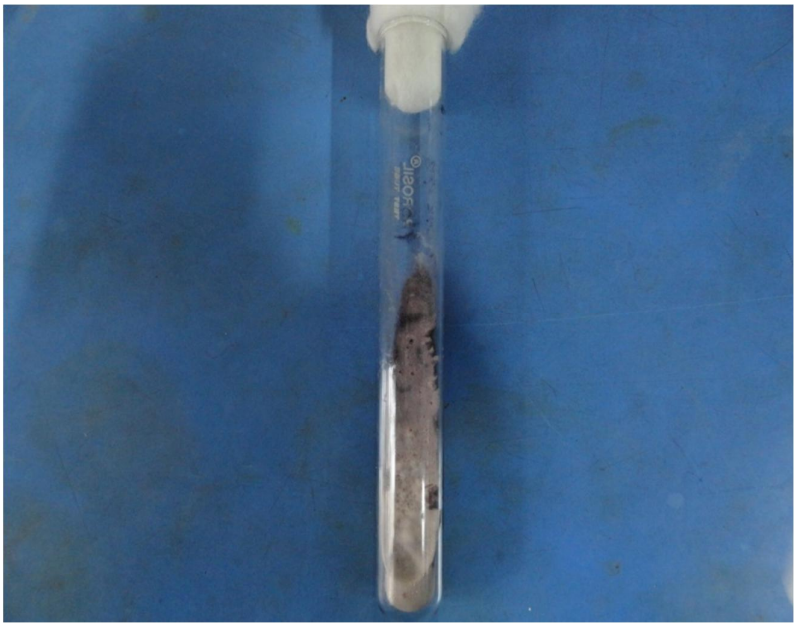
e) Quarry Infection

b) Animal Origin ------ Cow Tail etc.

FUSARIUM



FUSARIUM IN SDA



MORPHOLOGY AND CULTURAL CHARACTERISTICS OF COMMON CORNEAL PATHOGENIC FUNGI

Filamentous fungi can be easily identified if large segments of hypae are seen intact. But the identification of genera and species is usually not possible from morphologic examination of a tissue specimen, since spores and others supporting structures which are the principle identifying features in taxonomy are absent.

FUSARIUM

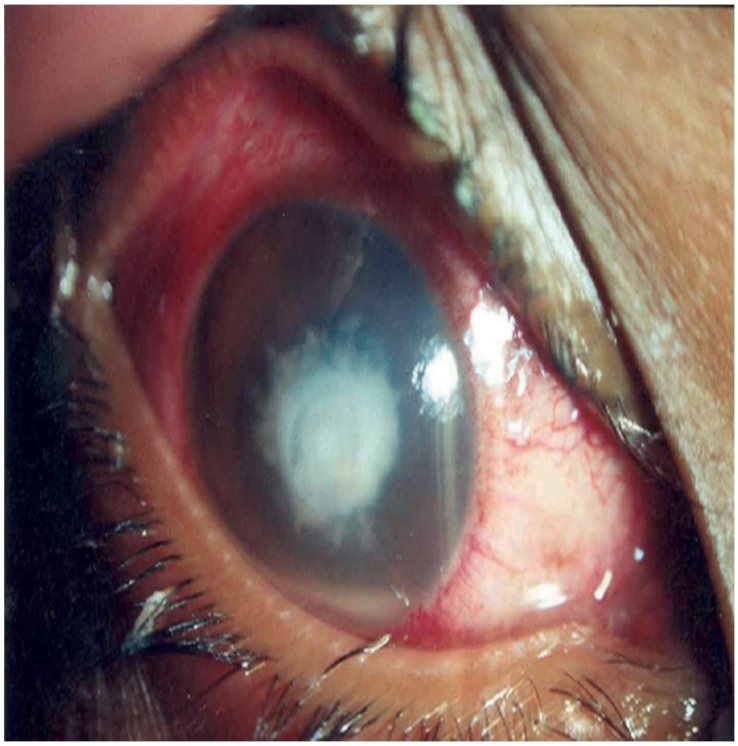
Fusarium ap. are characterised by the distinct spores referred to as macroconidia and in some by small coccoid spores known as microconidia[6].

The aggregates of macroconidia bear a striking resemblance to a '**bunch of bananas**' . The conidia often have transverse septation.

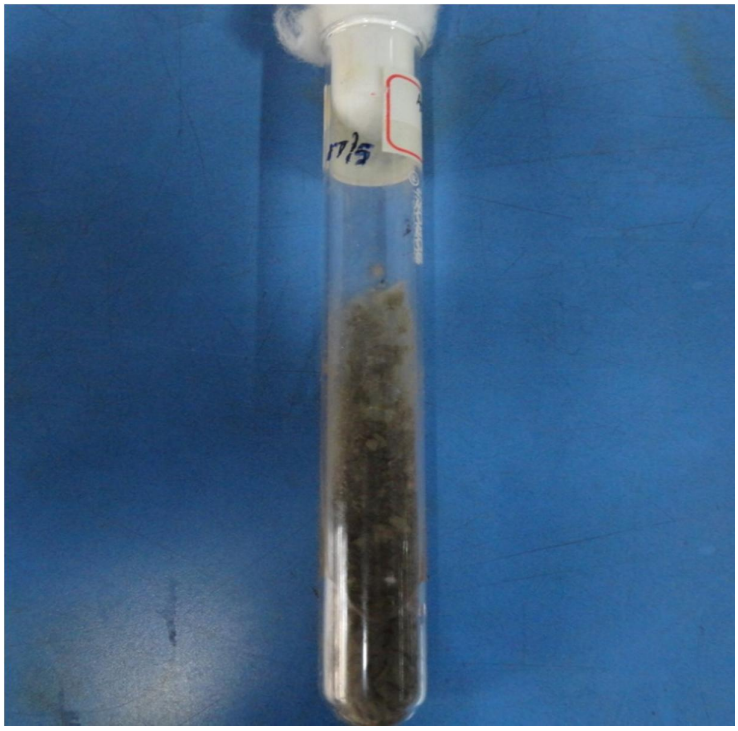
CULTURAL CHARACTERS OF FUSARIUM

The colonies are usually white in the early stages but often acquire buff coloration. As the colonies mature, a variety of coloured pigments ranging from yellow to red to purple are produced.

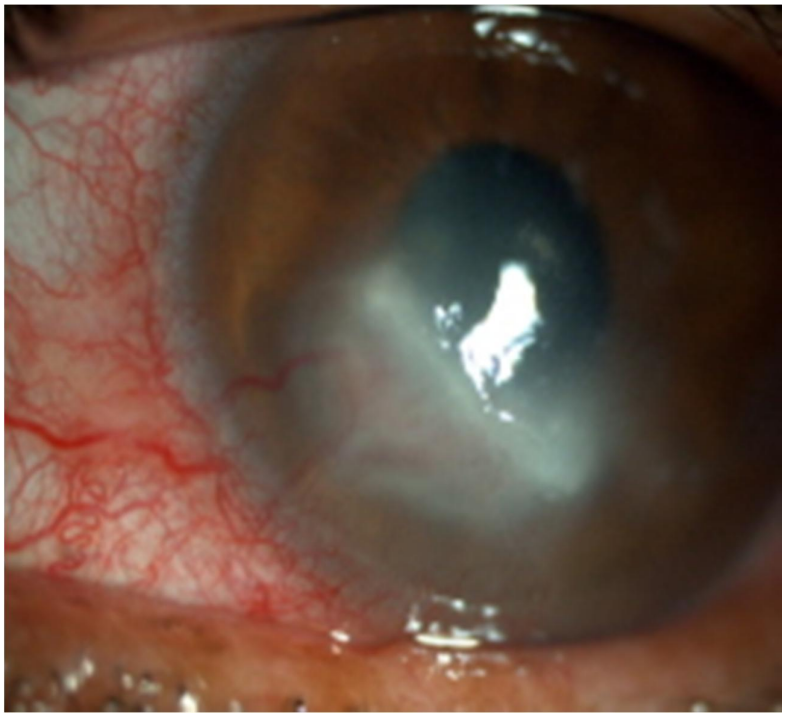
ASPERGILLUS FLAVUS



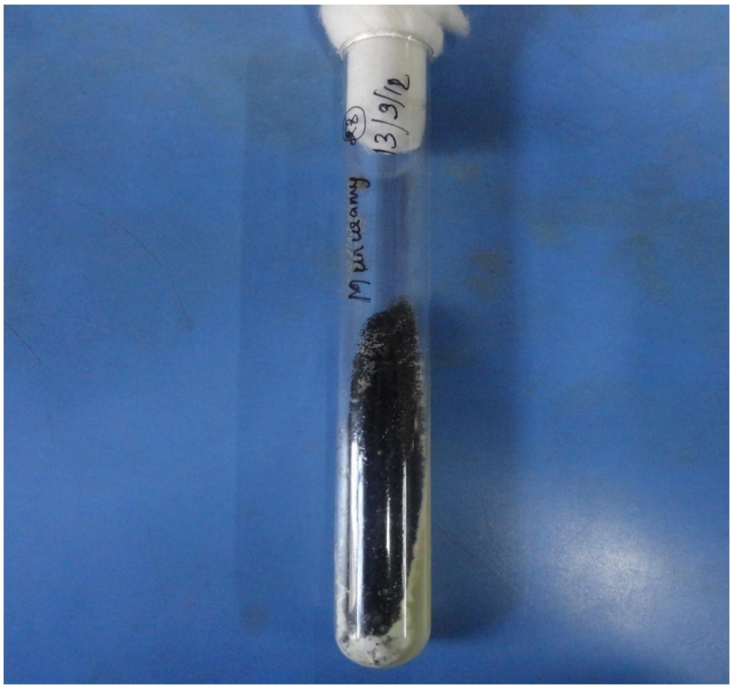
ASPERGILLUS FLAVUS IN SDA



ASPERGILLUS NIGER



ASPERGILLUS NIGER IN SDA



The pigments are seen on the undersurface of the colony. This is known as " **Reverse Pigmentation**".

ACREMONIUM

This species is devoid of macroconidia, produces **only microconidia**.

The conidiospores produce a sticky mucilaginous substance that holds the conidia together.

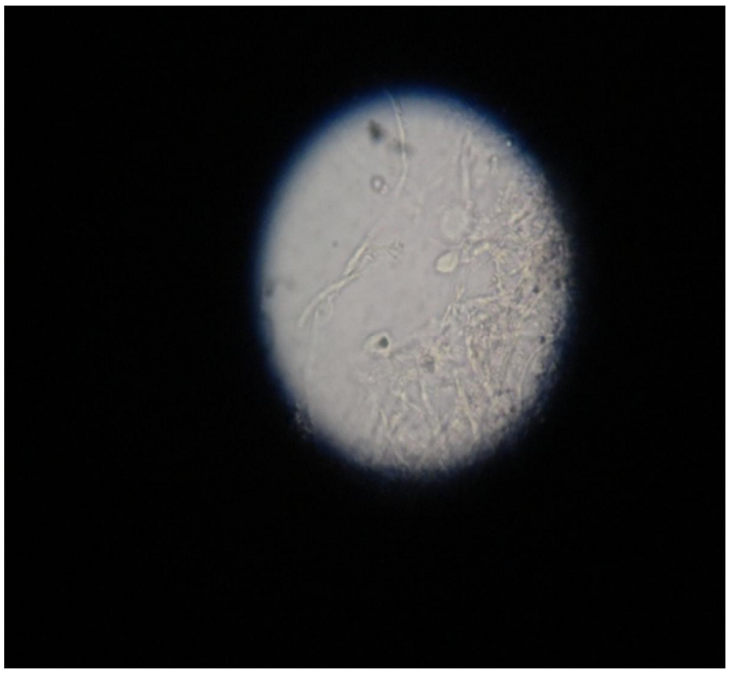
CULTURAL CHARACTERS

Young colonies of Acremonium are compact and moist but rapidly develop the typical **mold like appearance** with an overgrowth of white areal mycelia. Pigments varying from grey to white are seen and it is best appreciated by looking at the undersurface.

ASPERGILLUS

They are the easiest to identify if the spores are present. The Conidia-spores with its swollen terminal and (Vesicle) Surrounded by **flask- shaped strigmata**, each of which produce long chain of coccoid, conidia that radiate out from the vesicle. The dichomatous branching nature of the hyphae, is also diagnostic[40]

FUNGAL HYPHAE ON KOH WET MOUNT



SABAURAUDS DEXTROSE AGAR – CANDIDA ALBICANS

CULTURAL CHARACTERISTICS

Aspergillus is a large genus, but two are prominent. **Asp. fumigatus** colonies are white at first, but as spores are produced, the colonies become **velvety green** owing to the pigmentation of the conidia. It can tolerate very high temperatures and will grow invitro at 50° C**. Asp. Niger**. Colonies are also white during the initial growth phase but turn completely **black** as they sporulate.

YEAST FORMS

The yeast form is represents by Candida Sp. The presence of budding yeasts in a corneal scrapping is almost diagnostic for Candida.

Candida also produces psudohyphae and true hyphae and both the yeast form and hyphal form may be seen in corneal scrapings.

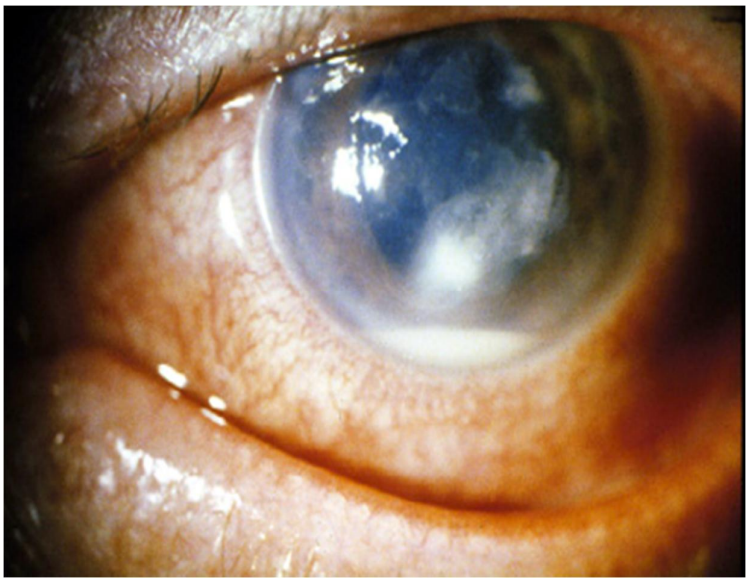
The hyphal form is considered invasive.

This is the most virulent stage.

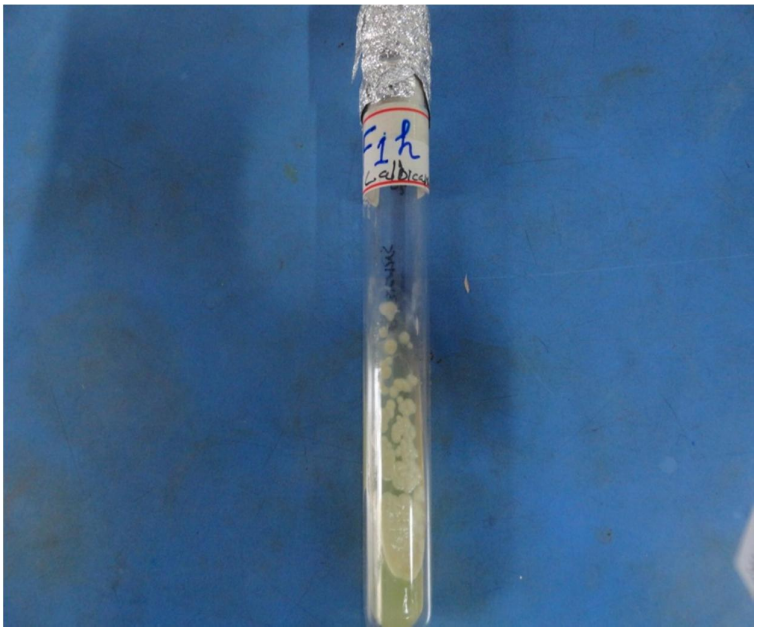
CULTURAL CHARACTERS

The typical candida colony grown on **SABOURAUDS DEXTROSE** Agar is white and opaque with smooth, flat, round contour and a pasty, soft consistency.

CANDIDA ALBICANS



CANDIDA ALBICANS IN SDA



The colonies are white to beige, opaque, smooth, with soft, pasty structure. Candida colonies have a **distinct odour**[37] which is useful identifying feature.

PATHOGENECITY

Wogan's review of mycotoxins lists a class of toxins. Over 200 mycotoxins were isolated from 50 Genera of pathogenic filamentous fungi and yeast. The important toxins are:

a) Trichothecenes (Fusarium and Acremonium)

b) Zearalenones

c) Aflotoxin (Asp. Flavus – liver carcinogen)

TOXIC ENZYMES:

a) Phospholipidases

b) Proteases

c) Pseudocollagenases

The Trichothecenes are cytotoxic and cytostatic. In low doses they cause prolonged inflammation while in higher doses they cause tissue necrosis.

Fusarium produces at least 9 complex mycotoxins and destructive enzymes. The Pathogenicity of fusarium is attributed to its mycotoxins and to its ability to replicate at 37°C. There is also a tendency to multiply extensively without inducing much inflammatory response [32]. Fusarium has a tendency to invade the posterior chamber, lens, causing fungal glaucoma.

Acremonium and large number of other fungi produce extracellular proteases.

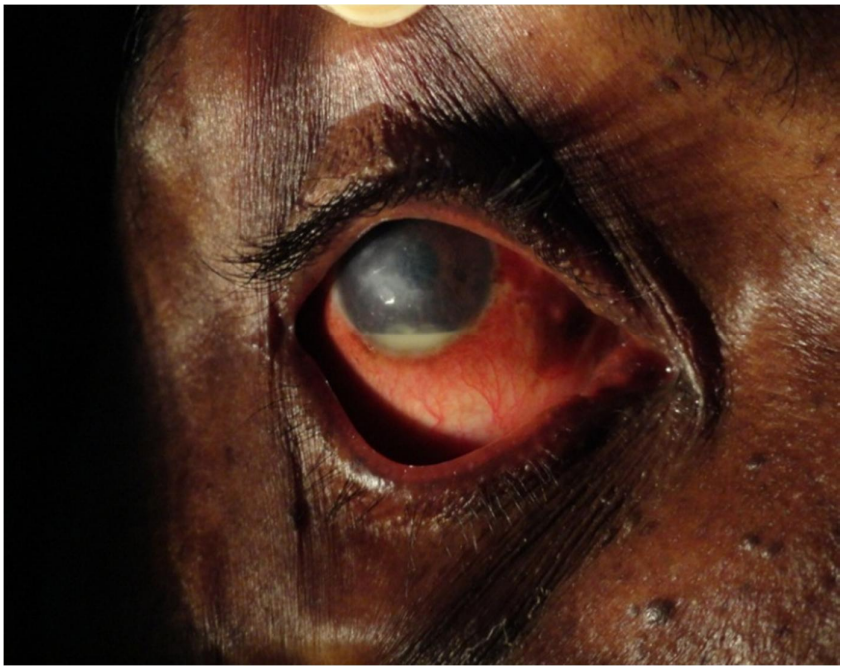
Candida produces proteolytic enzymes that cause lysis of the host tissue, which allows the candida to invade host tissue. Like bacteria, Candida Alb. can adhere to host cells, by a surface protein that binds to epithelial cell receptors.

Diamond and associates have demonstrated that a substance releases by the candida inhibit the adherence of neutrophils with Candida psudohyphae[4].

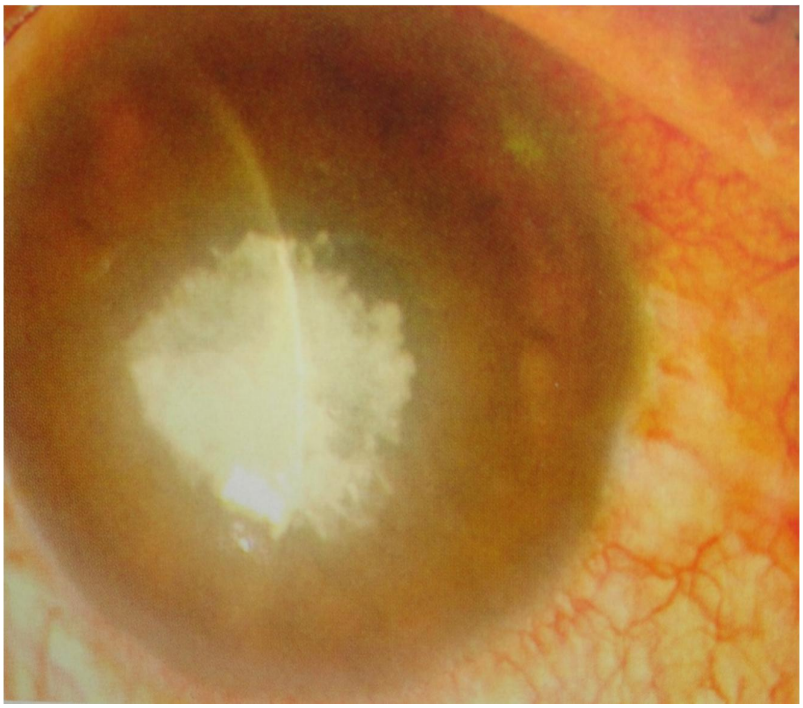
In general, the hyphae are too large to be phagocytosed by neutrophils.

While the Descemets membrane is usually impermeable to bacteria, the fungi can easily penetrate the descemets membrane and hyphae may be seen waiving into the anterior chamber[40].

FUNGAL CORNEAL WITH HYPOPYON



IRREGULAR FEATHERY MARGINS AND DRY TEXTURE OF FUNGAL KERATITS



CLINICAL FEATURES OF FUNGAL CORNEAL ULCER

Keratitis caused by Filamentous Fungi

More common in young individuals involved in out-door activities. Men usually predominate. There is always history of trauma with vegetable matter. Prior to the injury cornea is healthy. No evidence of ocular surface disorders like, dry eyes, herpes or neuroparalytic keratitis is present.

The incubation period is 24-48 hours. It may involve any area of the cornea.

Characteristic Features

a) Greyish, white infiltrate with hyphate margins elevated above the surface of the cornea, surrounded by satellite lesions.

b) Ulcer base may have a dry texture.

c) Ulcer margins are irregular and elevated and may demonstrate irregular fine linear, infiltration branching into the surrounding stroma.

d) Satellite lesions are discrete, stromal infiltrates that surround the ulcer and are separated by clear cornea.

e) Corneal endothelial plaques are seen. They are composed of inflammatory cells[20].

f) Immune rings may also be seen.

g) Hypopyon is a commonly associated feature even with a small ulcer and has no diagnostic value.

Any post traumatic ulcer, even if looks like a bacterial one and does not respond to antibiotics, should arouse the suspicion of a fungal etiology[24].

NON FILAMENTOUS KERATOMYCOSIS

This occurs commonly and exclusively in eyes with pre-existing corneal surface abnormalities like keratitis sicca, neuroparalytic keratitis, herpes affected eyes[39]. In all cases of non traumatic microbial keratitis, one must try to find out any ocular surface abnormalities, which predisposes this particular condition.

CHARACTERISTIC FEATURES

a) The Ulcer usually occurs at the area of exposure; at the junction of superior 2/3 and inferior 1/3.

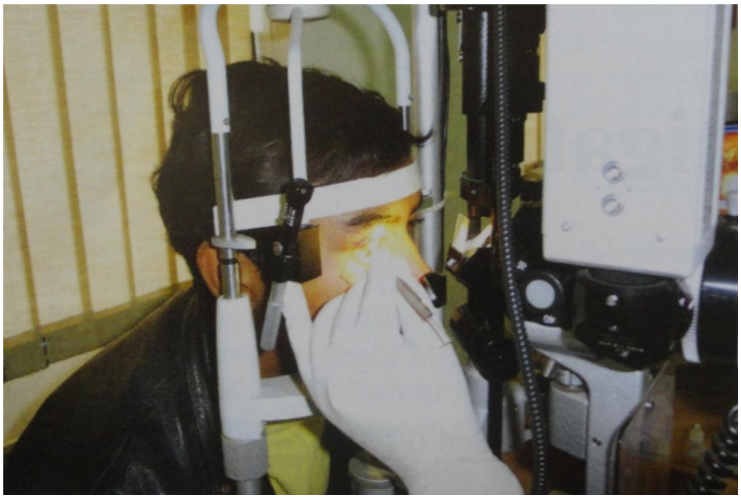
b) Keratitis is more localised; may have a "**collar button**" configuration, often with a small ulceration and an expanding, small discrete infilterate[22].

c) No hyphate margins seen; edges not feathery.

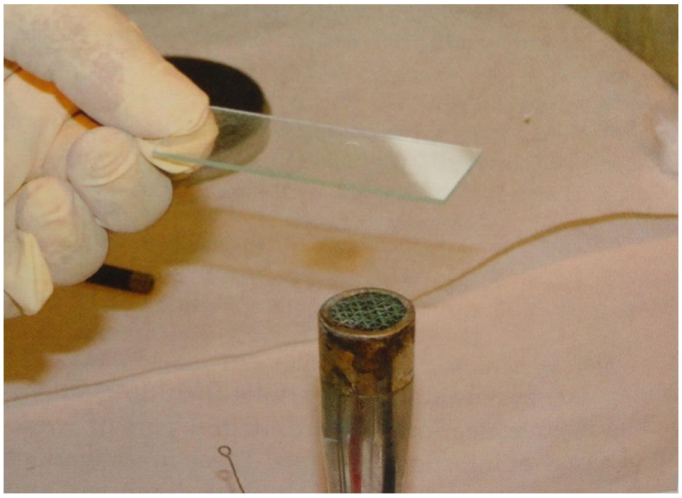
d) Satellite lesions are not usually seen.

KIMURA'S SPATULA

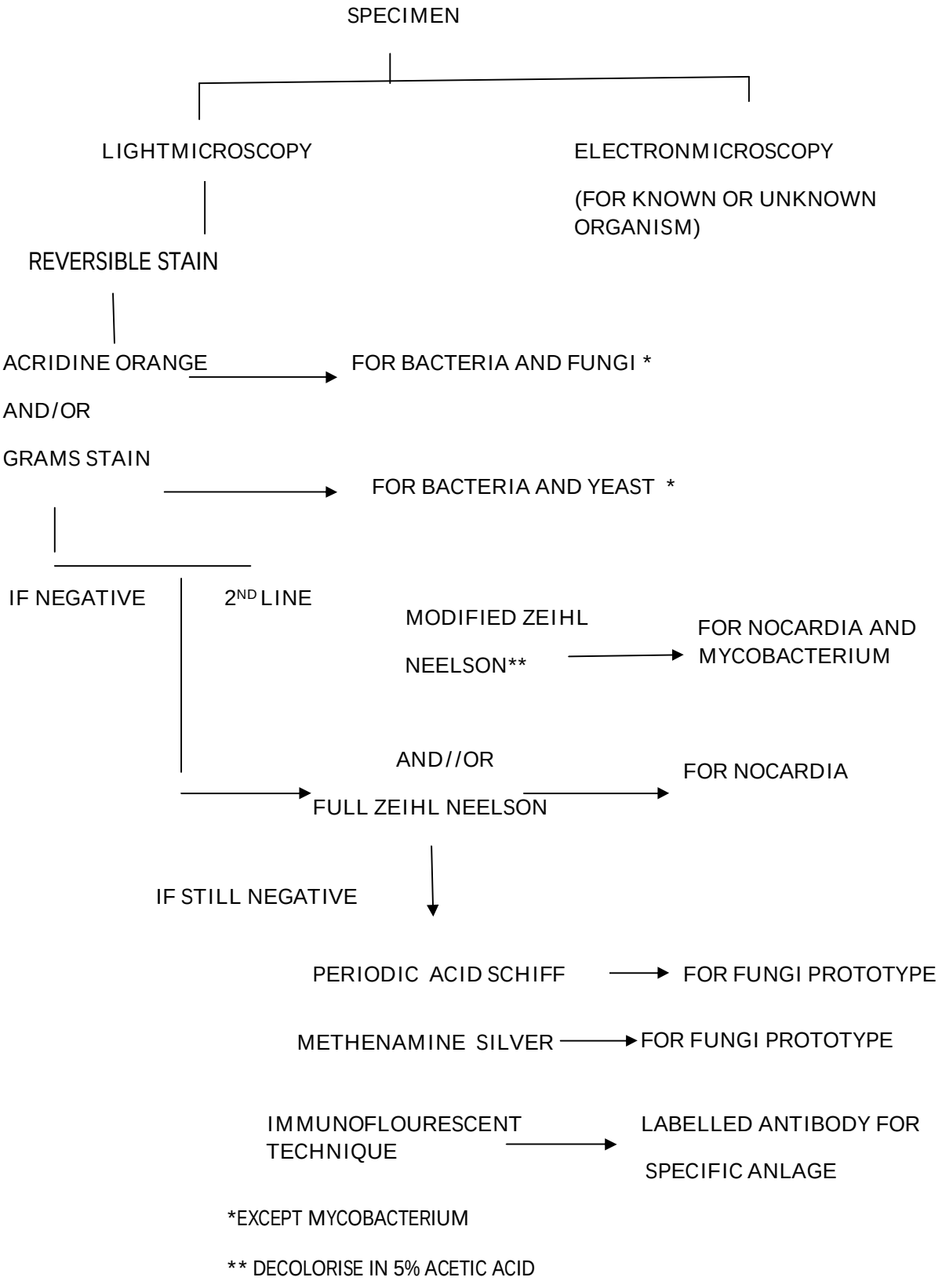
CORNEAL SCRAPING



FIXATION OF SMEAR BY HEATING



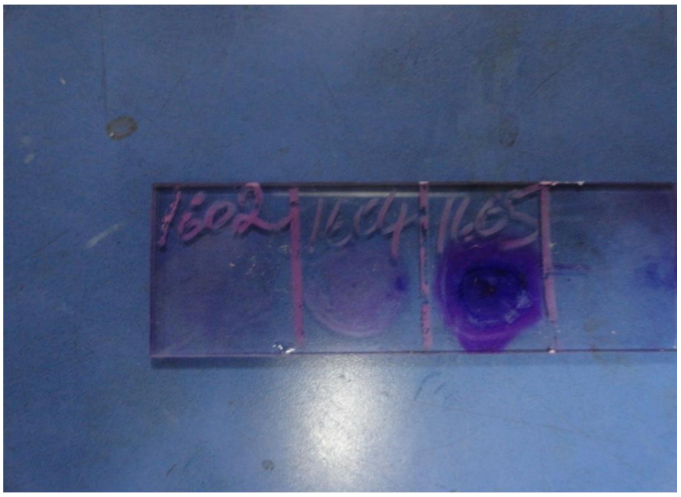
Laboratory Diagnosis Flow Chart



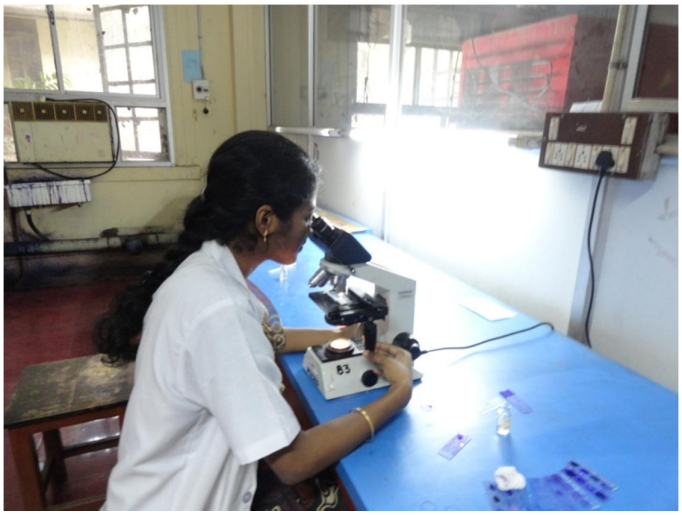
*EXCEPT MYCOBACTERIUM

** DECOLORISE IN 5% ACETIC ACID

GRAM STAIN



DIRECT MICROSCPOPE



LABORATORY TECHNIQUES

The aims of microbial keratitis should be the those as prepared by Jones (1981).

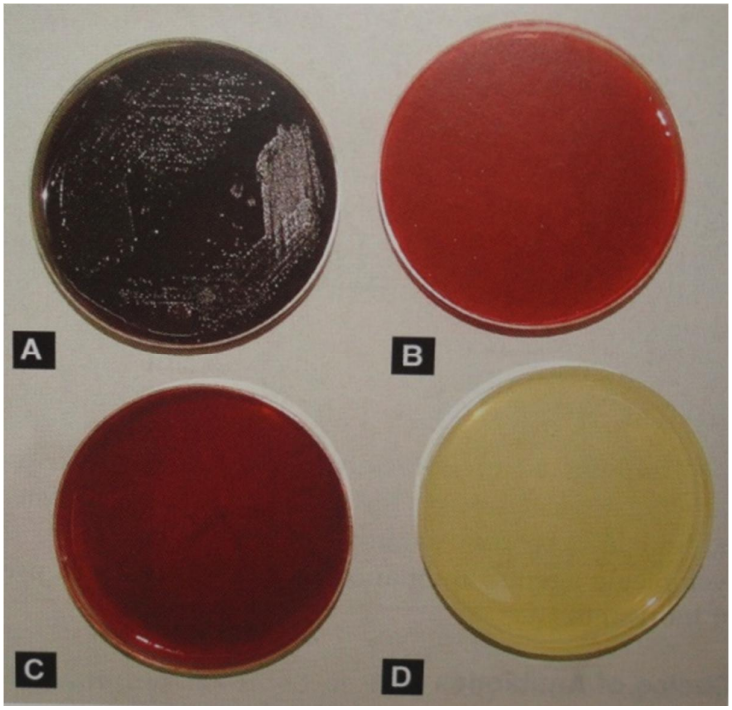
1. Make the diagnosis
2. Perform the necessary laboratory investigations
3. Start treatment
4. Review treatment in light of laboratory results
5. Stop treatment

DIRECT MICROSCOPY

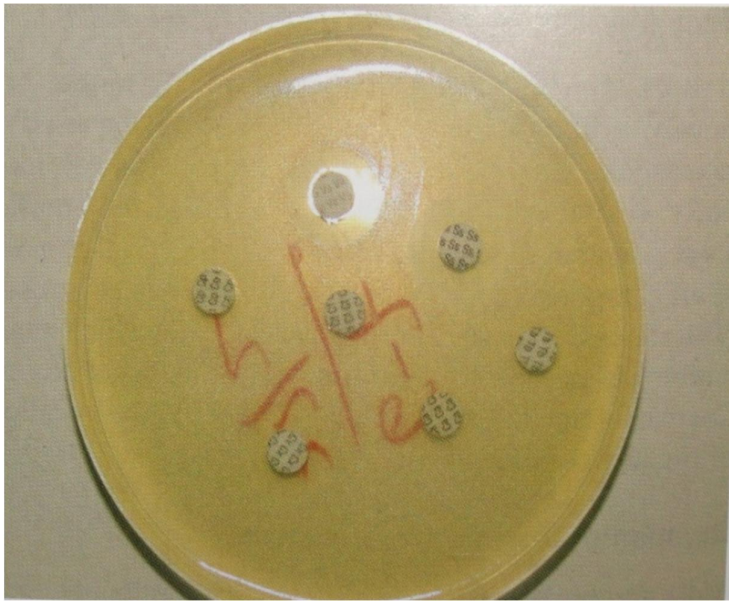
Recently Ficker et al (1981) sought to develop guidelines established by Jones (1981) and others for the investigation of corneal infections[12]. A minimum of atleast two smears for microscopy is advisable. Recently Holdren et al (1993) demonstrated a single end stain (periodic acid thiocarbahydrazide silver methenamine) which appear to have considerable promise as a broad spectrum stain to be used on its own; as yet its use has not been fully investigated.

1. Gram's stain
2. Geimsa stain
3. Acridine orange – requires fluorescent microscopy.

BLOOD AGAR AT ROOM TEMPERATURE(A)AND 37 DEGREE(B)CHOCOLATE AGAR(C) SABOURAUDS AGAR (D)



ANTIMICROBIAL SUSCEPTIBILITY TESTING



4. Calcoflour white

5. 10% potassium Hydroxide specific for fungi

6. Ink-potassium hydroxide specific for fungi

7. Grocott's methenamine Stain

8. Lactophenal cotton blue.

9. Modified Ziehl-Neelson/Full Zeihl Neelson for Nocardia and Atypical Mycobacteria.

10. Fluorescein – conjugated lectins – Concanavalin A, wheat germ agglutinin for trophozoite and cysts of Acanthamoeba.

11. Trichrome, wrights, PAS, Haematoxylin-Eosin-HPE sections to demonstrate Acanthamoeba.

12. Immuno fluorescent, immune peroxidise stains[33].

CULTURE MEDIA

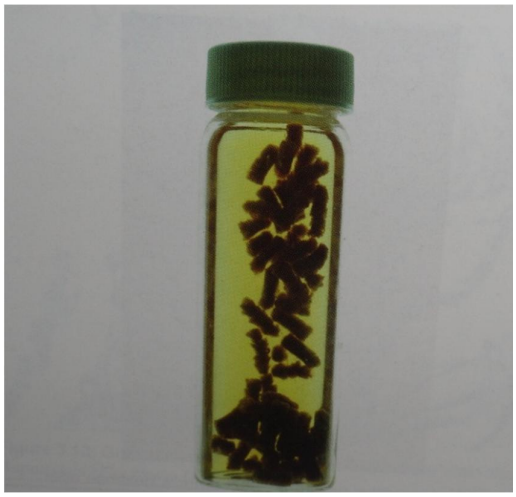
1. Blood agar for 48-hours at 37°C for bacteria and 25°C for fungi.

2. Chocolate agar for Neiserria and Hemophilus.

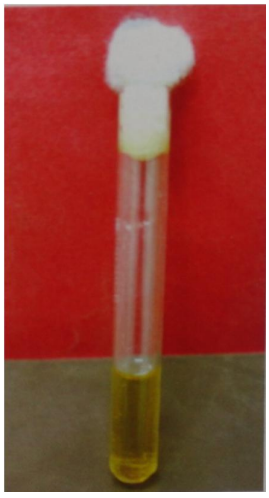
3. Sabourauds dextrose agar at 25°C – most sensitive for fungi.

4. Brain Heart infusion agar for 1 week at 37°C and 25°C

ROBERTSON COOKED MEAT MEDIUM



THIOGLYCOLATE BROTH



LOWENSTEIN JENSEN MEDIUM

5. Thioglycollate broth for 1 week at 37°C and 25°C

6. Reduced blood agar for anaerobes.

7. Lowenstein – Jenson Media for Mycobacteria.

8. Non nutrient E.coli seeded agar and Horse blood agar for Acanthamoeba.

CULTURE TECHNIQUES

Corneal material is best obtained under the magnification of the slit lamp. After instilling topical proparacaine, a kimuras platinum spatula is scraped over the base and edge of the ulcer with moderate firm strokes. The lids and lashes are avoided. Multiple areas of the ulcer are scraped.

CULTURE INTERPRETATION

The majority of aerobic bacteria responsible for keratitis appear on the standard media within 48 hours, and it is not unusual for the pathogen to be recognized in 12 to 15 hours. All plates should be examined daily with magnification, the media should be examined 12 to 18 hours after inoculation. Growth outside the C streaks on solid media should disregarded as a contaminant and circled with a pencil[25]. If growth is detected, an estimate of the number of colonies should be made, with a

description of the colony morphology. Criteria for some authors have been the signs of clinical infection plus one of following

1. Growth of an organism in two or more media
2. Confluent growth of a known ocular pathogen in one solid medium
3. Growth in one medium of an organism identified on routine stain

Jones offers the following criteria Clinical signs of infection plus

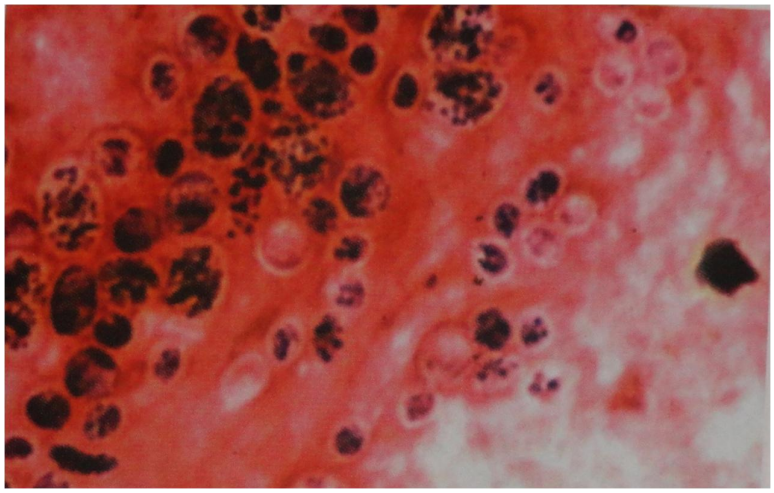
- Isolation of bacteria (10 or more colonies) on one solid medium and one additional medium
- Or isolation of fungi (any detectable growth) on any two media or one medium in the presence of a positive smear.

Candida-smooth flat colony that are pasty milky white and resemble bacterial colonies especially at 35°C with yeast like odour.

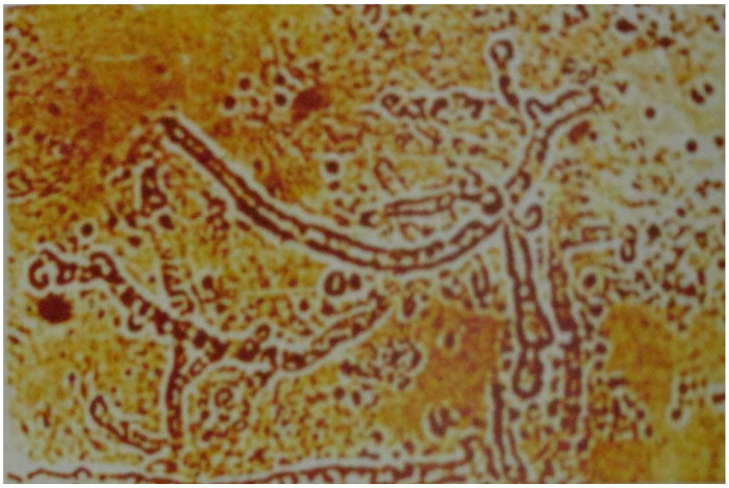
Filamentous fungi –appear as aerial colonies on solid colonies on solid media as a feathery mycelium in liquid medium. Some colonies are pigmented especially on the undersurface of the colony (**reverse pigmentation**).

Fusarium is white in its early stages but acquires a buff colouration as the colony matures.

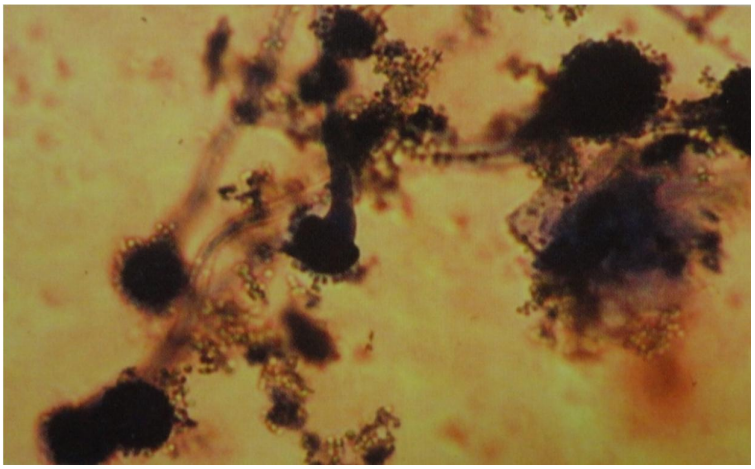
GRAM STAINS SHOWS YEAST CELLS



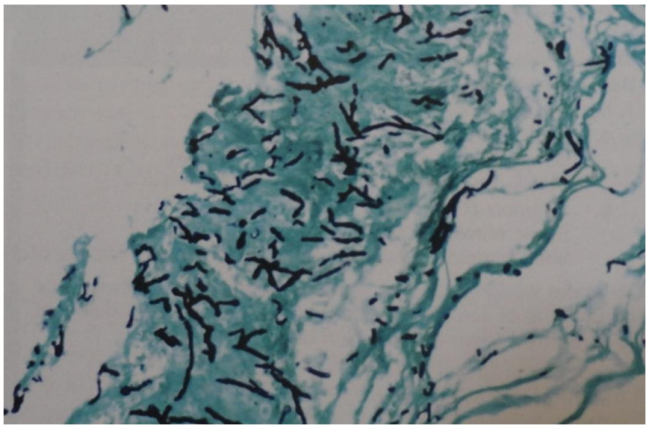
PSEUDO HYPHAE ON KOH WET MOUNT



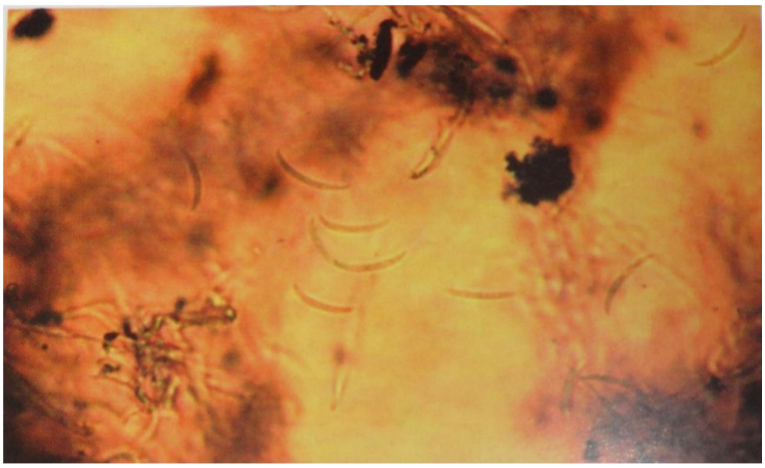
ASPERGILLUS FUMIGATUS



GROCOTT_GOMORI METHENAMINE SILER NITRATITE SHOWING ASPERGILLUS



FUSARIUM SPECIES



Aspergillus fumigatus is white initially but later turns velvety green, Aspergillus nigrans later turns black when sporulation begins, Acremonium is compact early later develops a typical woody appearance.

MICROSCOPIC MORPHOLOGY

Fusarium species are characterised by distinctive, large, banana shaped conidia, which are produced an short lateral hyphae or conidiophores. Aspergillus has a conidiophores with swollen terminal end (vesicle) surrounded by **flask-shaped sterigma**[38], it also has dichotomous branching. Similar laboratory findings are found with Penicillium.

Fungi can additionally be identified by biochemical tests (especially yeasts) germs tube production, immunodiffusion, counter immunoelectrophoresis, and enzyme linked immune sorbent assay (ELISA).

CORNEAL BIOPSY

Indications

- In some instances even repeated scrapes may not yield positive culture.
- When there is continued failure of the patients reponse to medical therapy.
- Deep infection in stroma with intact or uninvolved overlying epithelium
- Long standing acanthamoeba keratitis with cysts in deeper stroma.

*Discontinue antibiotics 48 hours before a deep biopsy.

*have donor corneal material for a tectonic graft should perforation occur.

The biopsy should include the edge of the ulcer and the tissue deep to the apparent extent of the infiltrate.

Advantage: Provides a much larger inoculum for culture and tissue for histological examination.

MANAGEMENT OF CORNEAL ULCER

1.General treatment

Keratitis is particularly liable to occur in persons whose general health is poor. In such cases, it should be improved by attention to diet, fresh air and hygienic surroundings. Vitamins may be given by mouth or by injection. Any other disease which may be the causative factor, such as variola or acne rosacea should be treated and foci of injection in any part of the body should receive attention.

2.Pre-existing local conditions

Foreign bodies on the cornea should be removed. Conjunctivitis of any kind should be treated by appropriate method and dacryocystitis or mucocele should be treated.

3.Local treatment of ulcerative keratitis

Atropine: is the essential local treatment of keratitis. Atropine sulfate may be used every 4 hrs. in the form of 1% drops or 1% ointment. Some uveal irritation is present in most cases of keratitis and atropine acts by paralysing the clliary muscle and thereby putting the intrinsic muscles of the eye at rest[9].

Protection of the eye is the second local necessity in the treatment of keratitis. When ulceration of cornea is present, a pad and bandage is essential except in the presence of conjunctivitis.

Irrigation with bland lotions, such as normal saline or 5% sodium bicarbonate is useful to cleanse the eye and in ulcerative cases to encourage the flow of antibiotics.

Antibiotics: are of great value locally after an ulcerative keratits has supervened, although they reach their highest valve in the prevention of condition after injury of the cornea and by treating established conjunctivitis.

The frequent use of fortified (high concentration) drops is the most effective way of maintaining a high and a sustained level of antibiotics at the site of infection and therefore a delivery system of choice[36].

Frequency of instillation: For the first few days, the drops are instilled at half hourly intervals round the clock. If two different antibiotics are used, one can be given on the hour and the other on the half hour. However, prolonged use of potentially toxic agents, such as idoxuridine and gentamicin may delay epithelial wound healing.

Subconjunctival injections: Sub conjunctival injections provide a high but transient peak of antibiotic at the site of injection and must be

used with topical topical therapy. In relatively mild cases, their use is unnecessary but their use should be considered for moderate to severe infections particularly when visual axis is threatened. The injections are given at 24 hrly intervals for about 5 days.

Promotion of reepithelization :

In eyes with stromal thinning, it is important to encourage healing of any associated epithelial defects. This is because stromal thinning seldom progresses in the presence of an intact epithelium.

The 3 main ways in which epithelial healing can be encouraged are:

1) **Lubrication**: Artificial tears and ointments are useful irrespective of the presence or absence of keratoconjunctivitis sicca If possible, tear substitutes, containing toxic 9benzalkonium chlorite) or sensitizing (thiomersol) preservatives should be avoided.

2) **Lid Closure**: Although this is particularly useful with exposure and neurotrophic keratopathy, it is also useful in eyes with persistent epithelial defect from other causes. The lids can be temporarily closed with a piece of tape extending horizontally[27]. Patients with corneal anaesthesia or exposure keratopathy due to facial nerve palsy may require tarsorrhaphy.

3) **Bandage soft contact lenses**: Protect the regenerating corneal epithelium from the constant rubbing action of eyelids

4) **Collagen shields** have been used to promote epithelial healing and to deliver drugs.

Prevention of perforation:

1) Tissue adhesive glue: such as isobutyl cyanacrylate, is useful in limiting stromal ulceration and prevention ulceration. It can be used to seal small perforation.

2) Conjunctival flap: The cornea may be completely or partially covered by a conjunctival flap if corneal ulceration is progressive and unresponsive to other measures[30]. This procedure is particularly suitable in cases of chronic unilateral diseases in which the prognosis for useful vision is poor due to associated vascularisation or scarring.

3) Ascorbate: both systemic and topical, may be helpful in reducing stromal ulceration in eyes with severe alkali burns.

4) Management of opacities: Small peripheral opacities are left untreated.

   Big central opacities by keratoplasty.

When a leucoma entirely occludes the pupillary area an optical iridectomy may be performed.

ADJUNCTIVE THERAPY

- Cycloplegics – to increase comfort and decrease formation of posterior synechiae.
- Mucopurulent and necrotic debris should be removed daily, removing microbilogical or antigenic load, facilitates antibiotic penetration.
- Entropion , ectropion, trichiasis, lagophthalmos should be corrected.
- Punctual occlusion or lateral tarsorrhaphy in patients with keratitis sicca.
- Intraocular Pressure should be monitored and treated.
- Cautery using chemicals – pure carbolic acid, 20% zinc sulphate, strong alcoholic solution of iodine.
- Heat cautery.
- Lacrimal passages must be evaluated.

TREATMENT OF FUNGAL KERATITIS

Classification

- Polyenes- Large- Amphotericin –B, Natamycin – Small – Nystatin.
- Imidazoles – ketaconazole, Miconazole, Clotrimazole, Itraconazole, fluconazole.
- Flourinated Pyrimidines –Flucytosine.

Fungus	**Topical**	**Subconjunctival**	**Systemic**
Filamentous	Natamycin (5%) 50 mg/ml	Miconazole (1%) 5-10 mg	Micanozole 30mg/kg/day i.v
Yeast	Amphotericin B (0.15%)	Miconazole (1%) 5-10 mg	Flucytosine 150 mg/kg/day orally

5% Natamycin suspension is initial drug of choice for keratitis by filamentous fungi or yeast. In severe keratitis additional treatment with Micanozole should be considered. Deep keratitis with threatened perforation or endophthalmitis oral flucystosine should be added if the clinical findings or Smear suggest yeast keratitis[7]. Under the same conditions i.v. micanozole should be considered if the clinical picture or smear suggests a filamentous fungi. Debridement is valuable and should accompany the drug therapy.

Clinical signs of improvement includes

1. Rounding up of the perimeter of the ulcer.
2. Decrease in stromal edema and infiltration
3. Epithelium grows over the defect.
4. Decrease in anterior chamber reaction and hypopyon.

TREATMENT OF ACANTHAMOEBA KERATITIS

1. Aromatic diamidines (pentamidine, hydroxyl stilbamidine, propamidine isethionate)
2. Aminoglycosides (neomycin,paromomycin)
3. Imidazole (clotrimazole,ketaconozole,micanozole)
4. Polymyxin B
5. Polymeric biguanides

SURGICAL MANAGEMENT OF CORNEAL ULCER

The role of surgery may be diverse and as follows:

1. Aid in medical management

    a. By increasing drug penetration

    b. By bringing in blood vessels in the form of conjunctival flaps.

2. Stabilise the corneal epithelial surface by conjunctival flaps.

3. Excise the infected corneal tissue and eliminate or reduce the microbial load.

4. Tectonically support the globe.

    The various modalities of treatment available in such cases are:

    - **Removal of epithelium**

    - **Conjunctival flaps**

    - **Tissue adhesives** for perforations upto 2mm along with bandage contact lens, left in place until it loosens spontaneously are bed becomes vascularised.

- **Penetrating keratoplasty** : The indications for penetrating keratoplasty are
    - Perforation
    - Descemetocele or impending perforation
    - Continued progression despite maximal medical treatment
    - Post infectious corneal scar.

NON-HEALING CORNEAL ULCER

CAUSES AND TREATMENT OF NON-HEALING CORNEAL ULCER

1. **Local**

    a. Improper antibiotics

    b. Persistence of irritant factor

        i. Neglected foreign body

        ii. Trichiastic cilia

    c. Persistent source of infection

        i. In the lid – chronic blepharitis.

        ii. In the conjunctiva – trachoma

        iii. In sac chronic Dacryocystitis

    d. Corneal anaesthesia : neurotrophic keratits, corneal exposure

        i. proptosis,

        ii. Lagophthalmos

    e. Secondary glaucoma

2. Systemic Cause

a. Malnutrition. Vit. A deficiency.

Vit. C deficiency.

b. Diabetes Mellitus

Management of non-healing ULCER

1.Remove any irritant factor

-Trichiastic Cilia

-foreign body

2.Dacryocystectomy in ulcers associated with dacryocyctitis

3.Cauterization of ulcer – Electrocautery, Galvanocautery,

Chemical cautery, pure carbolic acid,

Absolute alcohol, 7% iodine and 5% potassium iodine in 100% spirit may be used. The cornea should be dried with blotting paper and whole area of the ulcer touched with sharp pointed stick which has been dipped in the caustic. The blotting paper should then be reapplied to prevent cauterizing fluid from being spread over the cornea by any flow of tears[29]. Atropine is then instilled and pad and bandage applied. This

procedure may cause considerable pain and discomfort lasting for several hours.

It is therefore advisable, when possible to recommend 12 hrs in bed and suitable analgesics after the cauterisation has been affected. Contraindicated in neuroparalytic keratitis.

4. Paracentesis of cornea:

May on occasion be valuable in those cases associated with secondary glaucoma which cannot be controlled by oral diamox. The liberation of the aqueous and sometimes removal of hypopyon allows a new flow of aqueous, probably carrying antibodies and the condition may improve greatly.

5. **Tarsorrhaphy**

Done in resistant ulcers.

Neurotrophic Keratitis

Neuroparalytic keratitis

6. **conjunctival hooding**: Healthy tissue releases biogenic substances done in resistant marginal ulcers.

7. **PERITOMY**: Excision of conjunctiva at the limbus in case of Mooren's ulcer and persistent vascularisation

8. **β irradiation**: done in Mooren's ulcer, senile keratitis

9. **Bandaging soft contact lens**

10. **Cryotherapy**: - has been tried in Herper simplex keratitis and also in acanthamoeba keratitis [28].

11. **Therapeutic Keratoplasty**

NEWER METHODS IN THE DIAGNOSIS OF INFECTIOUS KERATITIS

Methods for detection of specific nucleic acid sequences of infectious agents

Most agents known to cause infectious disease contain a DNA or RNA fingerprint specific for that agent, partial sequence data are available for many agents involved in ocular infections and it is now possible to chemically or biologically construct complementary replicas of the nucleic acid and use these to probe for the presence of suspected infectious agent – **Nucleic Acid Probe Technique**.

Polymerized chain reaction is based on using specific oligonucleotide probes to detect unique sequences of DNA or RNA following amplification of target sequences in vitro[34].

Advantages

- Can be applied theoretically to all organisms.Can diagnose genetic diseases. Safety of working with inactive DNA/RNA

Disadvantages

- Too cumbersome, time consuming, expensive equipment, radioactive material.

PART-II

AIM OF THE STUDY

(i) To determine the frequency of occurance of microbial keratitis in patients presenting at Stanley Medical College, Ophthalmology Department.

(ii) To find the correlation between the aetiological agents of Microbial Keratitis and the age, sex and occupation of the patients and trauma and other pre disposing factors for microbial keratitis.

(iii) To evaluate the presence of certain clinical features as aids to diagnosis of microbial keratitis.

(iv) To evaluate the use of direct microscopic staining techniques and culture media as aid to the diagnosis of microbial keratitis.

(v) To isolate the common causative organisms of microbial keratitis among patients presenting to our department.

MATERIALS & METHODS

The study was performed on 150 patients presenting with suspected microbial keratitis at Ophthalmology Department in Stanley Medical College Hospital.

Clinical Criteria for selection of the patients.

Patients with

- ❖ Epithelial defect
- ❖ Epithelial defect with stromal infilteration
- ❖ Corneal Ulcer

(i) A Brief History High Lighting

- **a.** History of Trauma
- **b.** Traumatising agent
- **c.** Duration of Ulcer
- **d.** Occupation

(ii) History of contact lens use

(iii) Other systemic diseases

(iv) SLIT LAMP Examination of the Ulcer

Microbiological work up

Topical anaesthesia 4% lignocaine instilled on the affected eye

Conjunctival Smear – Any mucus wiped away from the surface of the Ulcer

Corneal smear – Margin and base scraped with kimura spatula, 11-blade or 21G Hypodermic needle

Smear to be made on glass slide for gram stain and microscopy.

Stains and Culture Media Used

(i) Gram / Giemsa Stained Smear for identification of organisms.

(ii) 10% KOH wet preparations for fungal hyphae.

(iii) Lactophenol cotton blue stained film for Acanthamoeba Cyst.

(iv)Calcoflour White (CFO) Fungal filaments

Cysts of Acanthamoeba[14]

Culture on

Blood Agar

Sabourauds dextrose Agar

Non Nutrient Agar with Overlay of E.coli

So according to the clinical features and direct microscopy the results obtained were analysed and tabulated.

INCLUSION CRITERIA

- Both male and female patients
- Ulcer following injury with vegetative matter
- Ulcer following Trauma
- Ulcer following fall of foreign body into the eye.
- Ulcers in patients with eyelid abnormalities.
- Ulcers in patients using contact lens.

EXCLUSION CRITERIA

- Patient already on treatment.
- Old healed ulcer.
- Children less than 3 years of age.
- Ulcer due to other causes.

RESULTS

AGE INCIDENCE

Highest incidence was found in between 3rd and 5th decade. The following table shows age incidence of corneal ulcers in our study.

Table – 1: AGE INCIDENCE

Age	No. of Cases	Percentage
4 – 10	9	6.00%
11- 20	14	9.3%
21-30	17	11.3%
31-40	32	21.3%
41-50	31	20.6%
51-60	28	18.6%
60 and above	19	12.6%

Table – 2: SEX INCIDENCE

Sex	No. of Cases	Percentage
Male	105	70.00%
Female	45	30.00%

Table -3 : OCCUPATION

Occupation	No. of Patients	Percentage
Agriculture	79	52.69%
Coolie	20	13.33%
Housewife	14	9.33%
Mechanic	14	9.33%
Student	9	6.00%
Children	7	4.66%
Govt. Servant	5	3.33%
Merchant	2	1.33%

Table – 4: NATURE OF INJURY

Nature of Injury	No. of Cases	Percentage
Metallic Foreign Body	44	29.33%
No Injury	35	23.34%
Chemical/Dye	15	10.00%
Thorn	12	8.00%
Sand	10	6.66%
Stick	10	6.66%
Plant stem	10	6.66%
Cow tail /animal matter	7	4.66%
Paddy husk	7	4.66%

Table – 5 :HYPOPYON

Hypopyon	No. of Cases	Percentage
With Hypopyon	52	34.66%
Without Hypopyon	98	65.35%

Table – 6: ASSOCIATED SYSTEMIC CONDITIONS

Systemic conditions	No. of Cases	Percentage
Diabetes Mellitus	35	23.33%
Malnutrition	7	4.68%
Tuberculosis	4	2.67%

Table – 7: ASSOCIATED OCULAR CONDITIONS

	No. of Cases	Percentage
Dacryocystitis	20	10.67%
Bullous Keratopathy	1	0.68%
Keratoplasty	1	0.68%
Exposure Keratitis	6	4.00%

Table – 8: MICROBIOLOGICAL STUDY IN 150 CASES OF CORNEAL ULCERS :

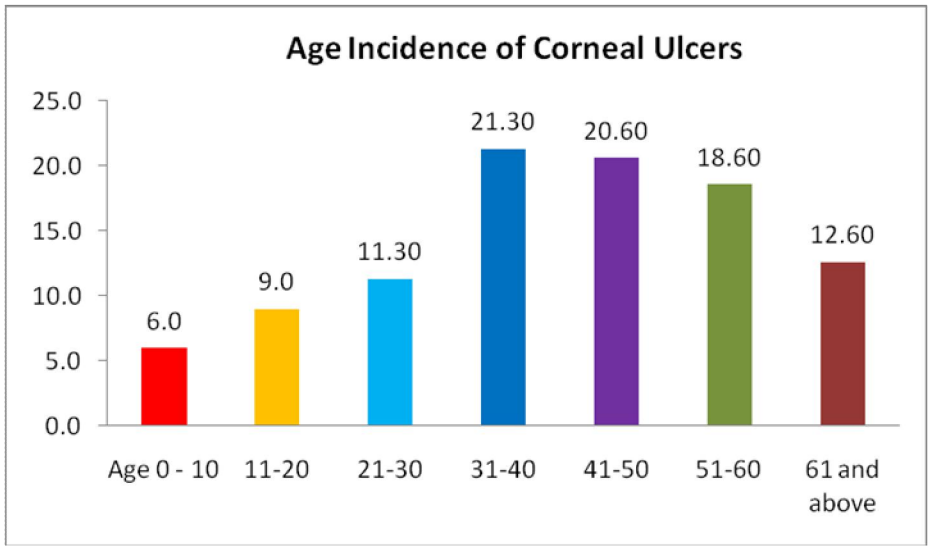
Organisms	No. of Cases	Percentage
Bacteria alone	49	32.66%
Fungi alone	48	32.00%
No organisms	30	20.00%
Acanthamoeba	06	04.00%
Bacteria + Fungi	17	11.33%

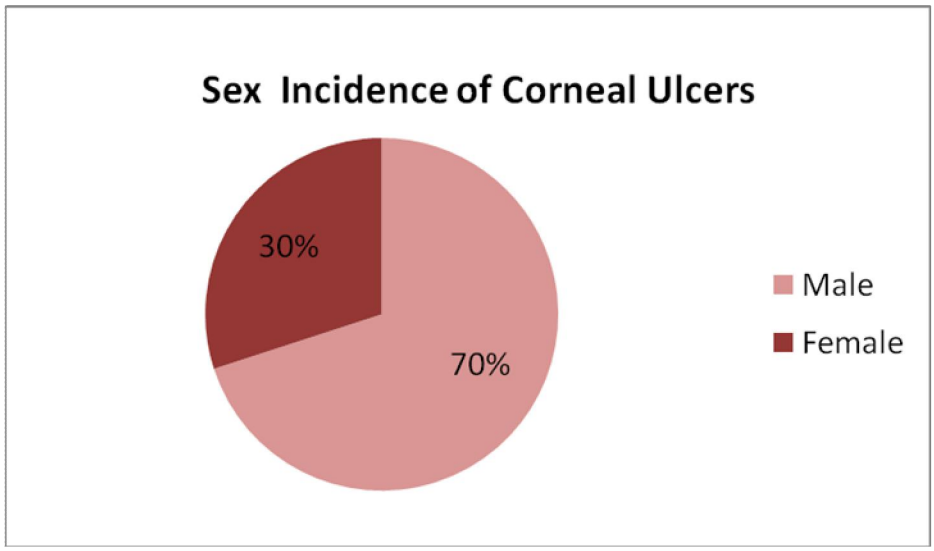
Table – 9: BACTERIA ISOLATED FROM CORNEAL ULCERS

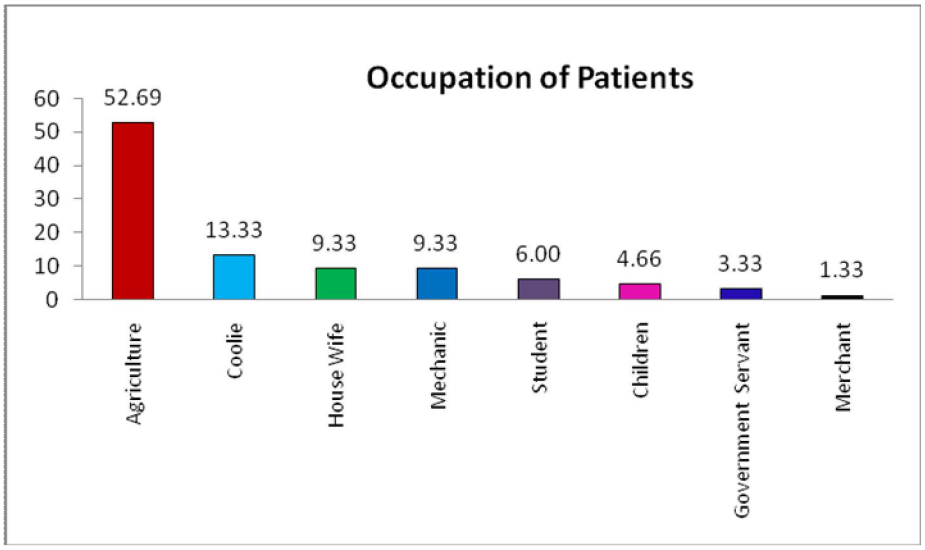
Type of Bacteria	No. of cases	Percentage
Staphylococcus Aureus	12	8.00%
Pseudomonas Aeruginosa	11	7.34%
Staphylococcus Albus	6	4.00%
Klebsiella Species	5	3.34%
Pneumococci	4	2.67%
E.Coli	2	1.34%
Diphtheroids	1	0.67%

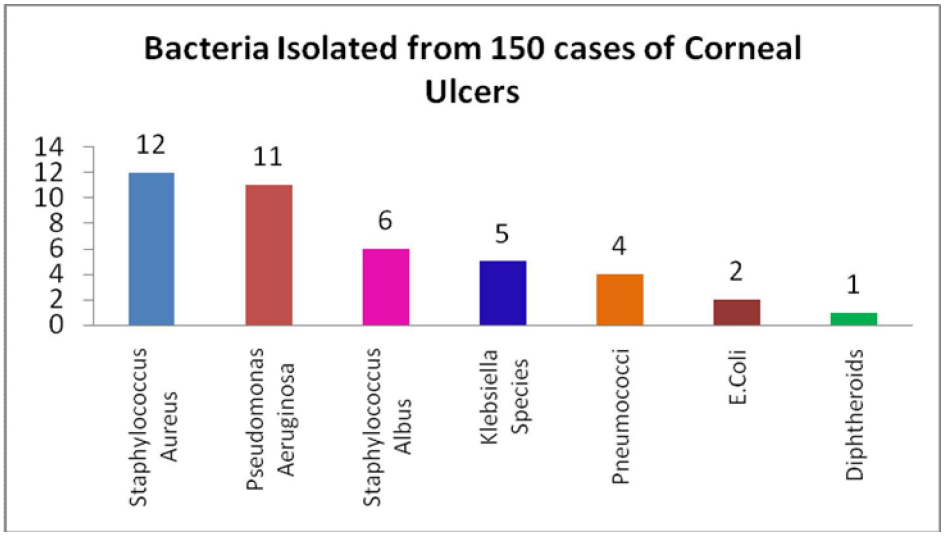
Table – 10: FUNGI ISOLATED FROM CORNEAL ULCERS

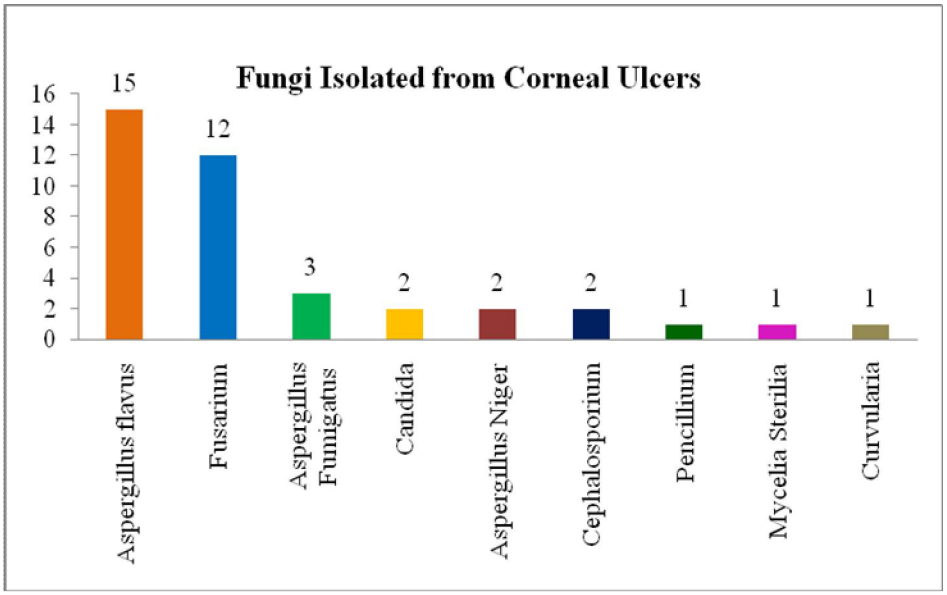
Type of Fungi	No. of cases	Percentage
Aspergillus flavus	15	10.00%
Fusarium	12	8.00%
Aspergillus Fumigatus	3	2.00%
Candida	2	2.00%
Aspergillus Niger	2	1.34%
Cephalosporium	2	1.34%
Pencillium	1	0.67%
Mycelia Sterilia	1	0.67%
Curvularia	1	0.67%

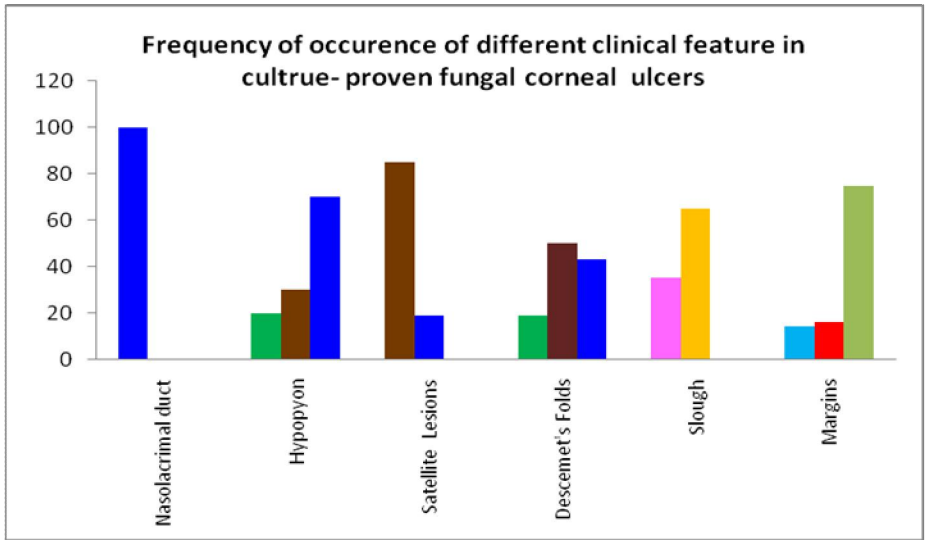

Age Incidence of Corneal Ulcers
25.0
20.0
15.0
10.0
5.0
0.0
6.0
9.0
11.30
21.30
20.60
18.60
12.60
Age 0 - 10
11-20
21-30
31-40
41-50
51-60
61 and above


Sex Incidence of Corneal Ulcers
30%
70%
Male
Female


Occupation of Patients
60
50
40
30
20
10
0
52.69
13.33
9.33
9.33
6.00
4.66
3.33
1.33
Agriculture
Coolie
House Wife
Mechanic
Student
Children
Government Servant
Merchant

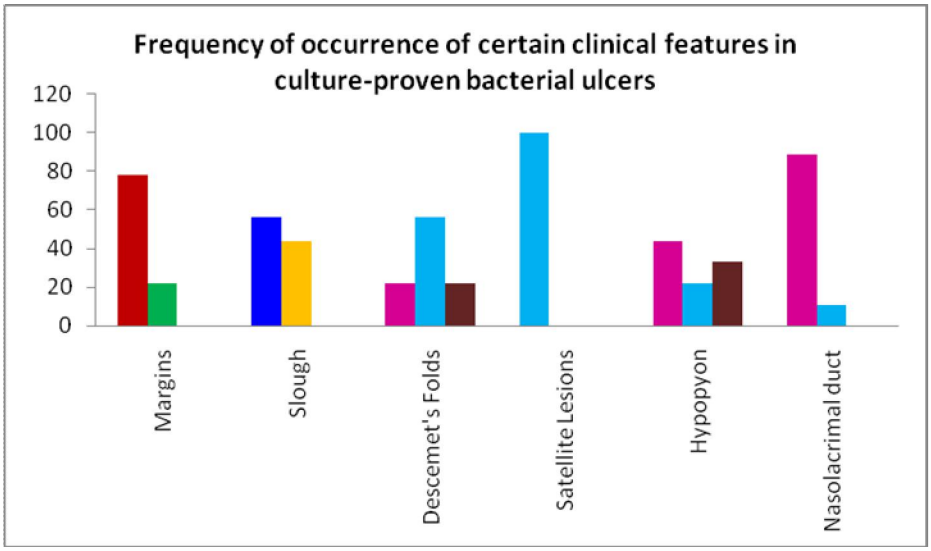

Bacteria Isolated from 150 cases of Corneal Ulcers
14
12
10
8
6
4
2
0
12
11
6
5
4
2
1
Staphylococcus Aureus
Pseudomonas Aeruginosa
Staphylococcus Albus
Klebsiella Species
Pneumococci
E.Coli
Diphtheroids


Fungi Isolated from Corneal Ulcers
16
14
12
10
8
6
4
2
0
15
12
3
2
2
2
1
1
1
Aspergillus flavus
Fusarium
Aspergillus Fumigatus
Candida
Aspergillus Niger
Cephalosporium
Penicillium
Mycelia Sterilia
Curvularia



Blue - Present
Brown – Absent
Yellow – Raised
Light Green – Well defined

Green - Count not been seen
Pink - Flat
Light Blue - Total Ulcer
Red - Serrated



Red – Well defined
Dark Blue – Raised
Pink – Present
Brown – Could not be seen

Green - Serrated
Yellow - Flat
Light Blue - Absent

COMPARISON WITH OTHER STUDIES

A Study conducted by M.Srinivasan, Christine A Gonzoles, Celine George et al., Epidemiology and Aetiology of Ulceration in Madurai, Arvind hospital South India, IJO, 2009[21].

Table 11: FUNGI ISOLATED FROM CORNEAL ULCERS

Type of Fungi	No. of cases	Percentage
Fusarium	18	20%
Aspergillus Niger	14	15.55%
Aspergillus flavus	10	11.11%
Cephalosporium	6	6.66%
Pencillium	4	4.44%
Candida	2	2.22%

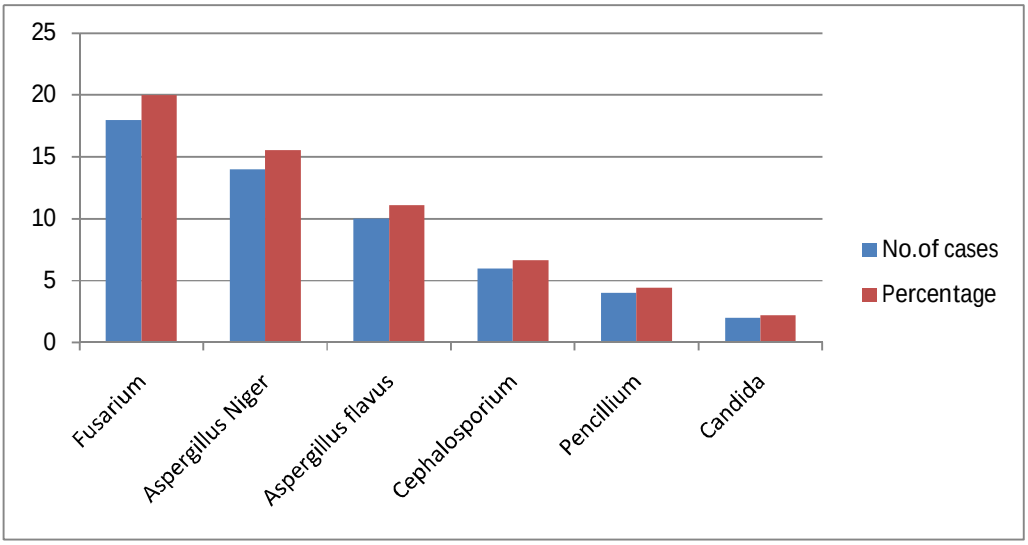
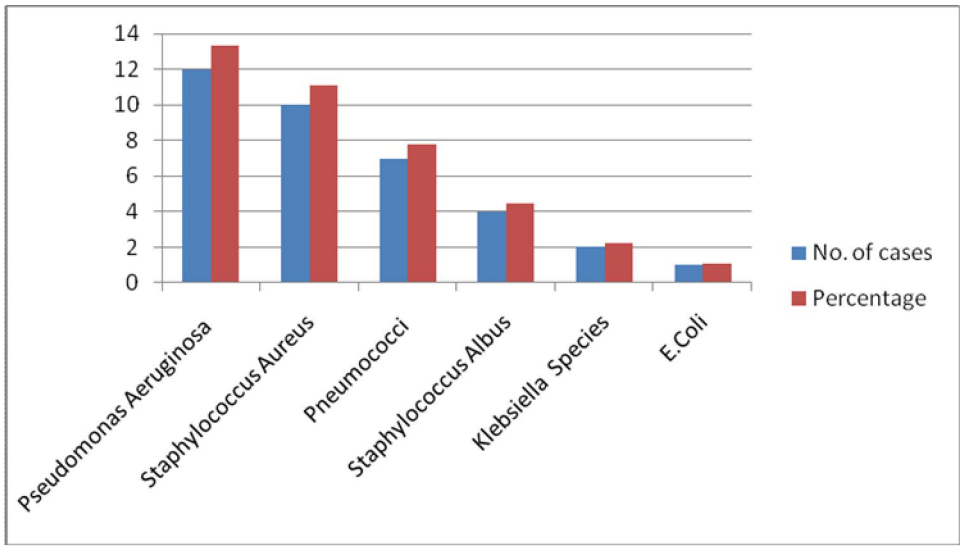


Table -12: BACTERIA ISOLATED FROM CORNEAL ULCERS

Type of Bacteria	No. of cases	Percentage
Pseudomonas Aeruginosa	12	13.33%
Staphylococcus Aureus	10	11.11%
Pneumococci	7	7.77%
Staphylococcus Albus	4	4.44%
Klebsiella Species	2	2.22%
E.Coli	1	1.11%



According to this study the incidence of fungi corneal ulcer 60% and bacterial corneal ulcer is 40% among fungus fusarium was the commonest organism and among bacteria Pseudomonas Aeruginosa was the commonest organism causing keratitis.

Spectrum of microbial keratitis in South Florida, liesegang TJ et all, American journal of Ophthalmology 2010[29].

Table -13: BACTERIA ISOLATED FROM CORNEAL ULCERS

Type of Bacteria	No. of cases	Percentage
Staphylococcus Albus	24	24%
Staphylococcus Aureus	17	17%
Pseudomonas Aeruginosa	12	12%
Pneumococci	6	6%
Klebsiella Species	1	1%

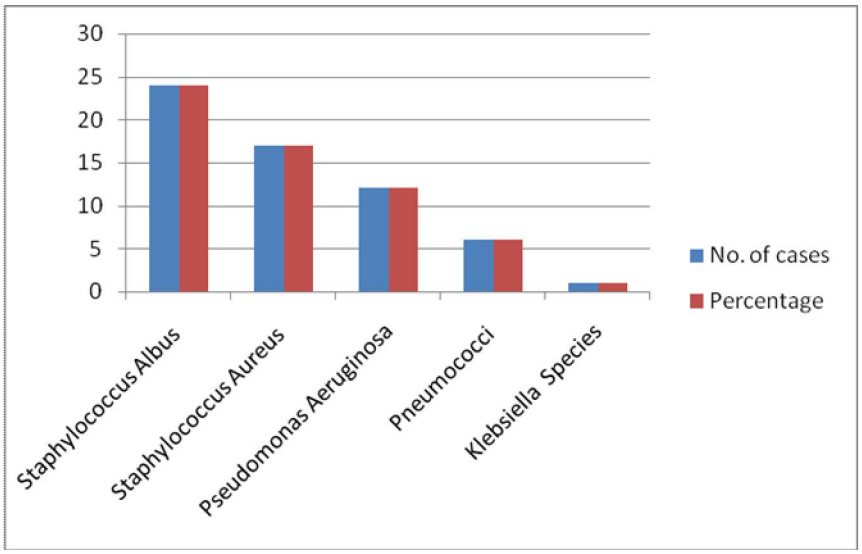
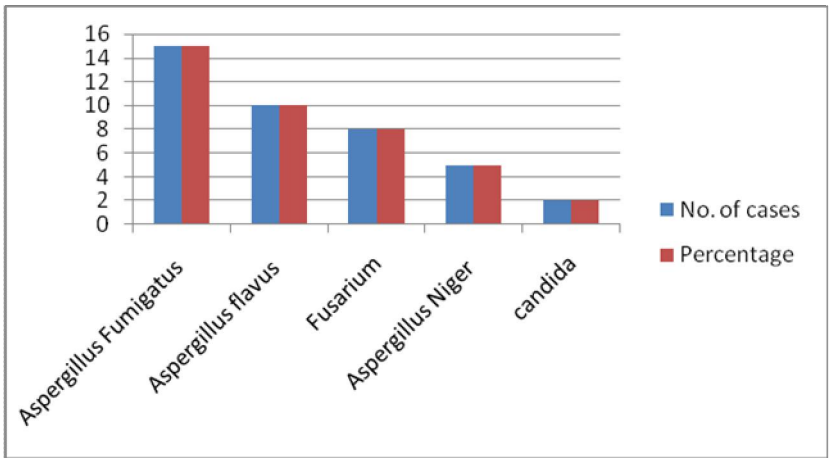


Table -14: FUNGI ISOLATED FROM CORNEAL ULCERS

Table – 14		
Type of Fungi	**No. of cases**	**Percentage**
Aspergillus Fumigatus	15	15.00%
Aspergillus flavus	10	10.00%
Fusarium	8	8%
Aspergillus Niger	5	5%
candida	2	2%



According to this studythe incidence of bacterial ulcer was 60% and the incidence of fungal ulcer was 40%. Staphylococcus Albus was the commonest organism among bacteria and Aspergillus Fumigatus was the commonest organism in fungal corneal ulcer.

But in our study in an area surrounded by industries with minimum agriculturists the incidence of bacteria and fungus were almost equal. The incidence of bacterial ulcer was 27.33% and fungal was 26.67%.

SUMMARY

1) 50 healthy controls were investigated for normal conjunctival bacterial and fungal flora. Bacterial growth was found in 56% of healthy conjunctival sacs. No fungal growth was found in normal conjunctival sac. Coagulase negative staphylococci was the commonest organism isolated being present in 56% of cases.

2) 150 patients of corneal ulcer were studied and microbiological investigations were done for both bacteria and fungi and acanthamoeba. Patients included both male and female of various age groups.

3) Age Incidence:- Highest incidence was found in between 30-50 years.

4) Sex Incidence:- 70% of patients were males.

5) Occupation:- Maximum number of patients were agriculturists. They constituted 52.69% of patients.

6) History of Injury:- History of injury with metallic foreign body was found in 29.33% of cases. Injury with vegetable matter was 25.98%. No injury was elicited in 23.34% of patients. History of injury with chemicals was elicited in 10% of cases.

7) Diabetes mellitus was the main systemic predisposing factor which constituted 23.33% of patients in the age group of 35-55 years. Dacryocystitis was the main associated ocular condition being present in 10.67% of cases. Malnutrition was associated with 4.68% in the age group of 4-12 years.

8) Bacteria were isolated from 32.66% of cases. Fungi were isolated from 32% of cases. Thus, there was almost equal incidence of bacterial and fungal ulcers. Acanthamoeba were isolated from 4%

9) Staphylococcus aureus was the commonest organism (8%) isolated coagulase negative staphylococcus (7.34%)

10) Aspergillus flavus was the commonest fungi (10%) isolated followed by cephalosporium (8%).

11) All the cases were treated with appropriate antibiotics antifungal with loading dose :

Every minute for five minutes

Every 5 minutes for half an hour

Every half an hour for 6 hours

Every hourly for 24 hours

Maintenance dose

6 times daily until the ulcer resolves

Mydriatic cycloplegics were also given. 0.15% Amphotericin B anterior chamber wash also given for a case of fungal corneal ulcer, responded well after therapy . Secondary glaucoma was treated with acetazolamide 250 mg bd. Dacryocystectomy was done in 10.67% of cases.

12.Acanthamoeba ulcers were treated with combination therapy of topical

0.02% polyhexamethylene biguanide

0.02% chlorhexidine

0.01% propamidine(Brolene)

1% neomycin

Applied every hour for 72-96 hours

2 hourly for 2-4 hours

Tapered to qid dose for 6-12 months

CONCLUSION

Corneal ulcer is an important ocular emergency, in which if treatment is started with adequate and appropriate antibiotics can prevent impairment of vision. Early diagnosis and knowledge of agents involved are of utmost importance to start appropriate therapy. Each case should be treated after investigating into exact cause of the disease.

Any patient with corneal trauma and patients with history of contact lens use should be followed closely and such a patient should be viewed with high index of suspicion for infective keratitis and acanthamoeba keratitis, because of potential for devastating consequences from delayed diagnosis.

In a tropical country like India where agriculture constitutes the main occupation, corneal infection is one of major ophthalmic problems. They commonly occur in elderly persons pursuing agricultural occupation and who are often subjected to debilitating conditions which predispose them towards infections. They should be educated regarding protection of eyes during work and to approach an ophthalmologist as soon as they sustain injury. This could help in starting early treatment which in turn prevents loss of vision.

ANNEXURE

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PROFORMA

Name : Date: Address :

Age :

Sex :

O.P. No. : Social Status :

Complaints : Pain/Watering/Redness/Photophobia/ D.V.

and

Duration :

Past History : Injury Nutritional Def.:

History of Previous Surgeries

TB/Syphilis/Leprosy/Diabetes

Occupational/ History :

Family History :

General Examination :

Local Examination R.E. L.E.

Lids

Conjunctiva

Cornea

- Size
- Shape
- Colour
- Margin
- Depth
- Exudate Covering the base of the Ulcer
- Hypopyon
- Flare
- Cells
- Keratic precipitates

Iris

Pupil

Lens

Fundus examination

Vision

Without glasses :

With glasses :

Tension digitally :

Special Investigation : R.E. L.E.

Staining : Flourescein

Conj. smear KOH

Gm. Stain

Culture Bacterial

Fungal

Duct

Blood Sugar

KEY TO MASTER CHART:

AGB	**–**	**Apple Green Bifringence**
GPC	**-**	**Gram Positive Cocci**
GNC	**-**	**Gram Negative Cocci**
GNB	**-**	**Gram Negative Bacilli**
GPB	**-**	**Gram Positive Bacilli**
NG	**-**	**Nisseria Gonorrhea**
St.alb	**-**	**Staphylococcus albus**
St.aur	**-**	**Staphylococcus aureus**
Kleb	**-**	**Klebsiella**
PS.aer	**-**	**Pseudomonas auerginosa**
FUS	**-**	**Fusarium**
G	**-**	**Good**
S	**-**	**Sensitive**
R	**-**	**Resistance**

PL	**-**	**Perception of Light**
DM	**-**	**Diabetes Milliteus**
M	**-**	**Male**
F	**-**	**Female**
Gr	**-**	**Grade**
DV	**-**	**Defective Vision**
HM	**-**	**Hand Movements**
Li.Ed	**-**	**Lid Edema**
LC	**-**	**Lens Changes**
CCC	**-**	**Circum Corneal Congestion**
PCO	**-**	**Posterior Capsular Opacification**
Mon	**-**	**Months**
N	**-**	**Normal**
Red	**-**	**Reduced**
St	**-**	**Stage**

MASTER CHART

Sl.No.	Name	Age/Sex	Occupation	Eye affected	Injury	Hypopyon	Previous Medication	Diabetes Mellitus	Dacryocystitis	KOH	Gram's Stain	Calcoflour White	Culture	Response to Treatment
1	Jayamani	40 /M	Agriculture	L	+	+	-	-Ve	-Ve	-Ve	GPC	-Ve	St. Alb	G
2	Selvam	50/M	Mechanic	L	+	+	-	+ve	-Ve	-Ve	GNB	-Ve	Ps. Aer	G
3	Saroja	40/F	Agriculture	L	+	-	-	-Ve	-Ve	-Ve	PUS CELLS	-Ve	NG	G
4	Ramanav	30/M	Coolie	R	+	+	-	-Ve	+ve	-Ve	GPC	-Ve	St. Alb	G
5	Munuswamy	70/M	Agriculture	R	+	+	-	+ve	-Ve	+ve	HYPHAE	-Ve	NG	G
6	Marathy	29/F	Agriculture	L	+	-	-	-Ve	-Ve	+ve	-ve	-Ve	NG	G
7	Sundarambal	65/F	Agriculture	L	+	-	-	+ve	-Ve	+ve	GPC	-Ve	St. Aur	G
8	Sakthivel	40/M	Agriculture	R	+	-	-	-Ve	-Ve	-Ve	GNB	-Ve	E.Coli	G
9	Ramamoorthy	55/M	Agriculture	R	+	+	Ab	+ve	-Ve	-Ve	GNB	-Ve	Kleb	P
10	Kanimozhi	19/F	Student	L	+	+	-	-Ve	-Ve	-Ve	GPC	AGB	St. Aur	G
11	Anthony	58/M	Agriculture	L	+	-	-	+ve	-Ve	-Ve	HYPHAE	-Ve	Myc.st	P
12	Nanjappan	80/M	Agriculture	R	+	-	-	-Ve	-Ve	+ve	-ve	-Ve	NG	P
13	Muniammal	60/F	Coolie	R	-	-	Ab	+ve	-Ve	-ve	-ve	-Ve	NG	G
14	Mahalingam	62/M	Coolie	R	-	-	_	-Ve	-Ve	-ve	-ve	-Ve	NG	G
15	Munirathnam	30/M	Coolie	L	+	-	-	-Ve	+ve	-ve	-ve	-Ve	NG	G
16	Prabhu	20/M	Student	L	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
17	Munuswamy	70/M	Agriculture	R	+	-	-	+ve	-Ve	-ve	-ve	-Ve	NG	G
18	Ponnusamy	50/M	Agriculture	L	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
19	Shivaji	40/M	Agriculture	R	+	+	-	-Ve	+ve	+ve	GNB	-Ve	Kleb	G
20	Joseph	30/F	Coolie	L	-	+	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
21	Dhana Lakshmi	27/M	House Wife	L	+	-	-	-Ve	+ve	-ve	-ve	-Ve	NG	G
22	Sujatha	60/M	House Wife	R	+	-	-	+ve	-Ve	-ve	GPC	-Ve	St.aur	G
23	Nagammal	55/F	House Wife	L	+	-	-	-Ve	-Ve	-ve	GNB	-Ve	Ps. Aer	G
24	Poongavanam	70/M	Agriculture	L	+	+	-	+ve	-Ve	-ve	GPC	-Ve	St. aur	p
25	Ranjan	70/M	Agriculture	R	+	-	-	+ve	-Ve	+ve	GNB	-Ve	Kleb	G
26	Balasundaram	16/M	Student	L	+	-	-	-Ve	-Ve	+ve	GNB	-Ve	E.Coli	G
27	Rajendran	60/M	Limeseller	R	+		-	+ve	-Ve	+ve	GPC	-Ve	Fus+Stalb	G
28	Yashodha	30/F	House Wife	L	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
29	Ramesh	30/M	Coolie	L	+	-	-	-Ve	+ve	-ve	-ve	-Ve	NG	P
30	Rani	30/F	House Wife	R	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
31	Natarajan	45/M	Mechanic	L	+	-	-	+ve	-Ve	-ve	-ve	-Ve	NG	G
32	Muniammal	40/F	Coolie	R	-	+	-	-Ve	-Ve	-ve	GPC in pairs	-Ve	Pneumo	G
33	Shanmugam	35/M	Agriculture	L	+	+	-	-Ve	-Ve	-ve	GPC	-Ve	St. Alb	G
34	Rajarathinam	20/M	Agriculture	R	+	-	-	+ve	+ve	Yeast Forms	-ve	-Ve	NG	G
35	David Raj	40/M	Coolie	R	+	+	-	-Ve	-Ve	+ve	-ve	-Ve	NG	P
36	Aisha	20/F	Student	L	+	-	-	-Ve	+ve	-ve	-ve	-Ve	NG	G
37	Muniammal	50/F	House Wife	L	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	St.aur	G
38	Buhari	40/M	Mechanic	R	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	St.aur	G
39	Jamuna	30/F	House Wife	L	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
40	Devagi	22/F	House Wife	R	+	-	-	-Ve	+ve	-ve	-ve	-Ve	NG	G
41	Durai	34/M	Agriculture	L	+	+	-	-Ve	-Ve	-ve	-ve	-Ve	NG	P
42	Chinnammal	33/F	Agriculture	R	+	+	-	-Ve	-Ve	-ve	GNB	-Ve	Kleb	G
43	Shanthi	45/F	Coolie	L	+	+	Ab	-Ve	-Ve	-ve	-ve	-Ve	NG	G

Sl.No.	Name	Age/Sex	Occupation	Eye affected	Injury	Hypopyon	Previous Medication	Diabetes Mellitus	Dacryocystitis	KOH	Gram's Stain	Calcoflour White	Culture	Response to Treatment
44	Dhanam	35/F	Agriculture	L	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
45	Quillammal	22/F	Student	R	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	St.aur	G
46	Ramalingam	22/M	Student	R	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
47	Shankar	34/M	Agriculture	L	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
48	Anjalai	30/F	Agriculture	L	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	NG	P
49	Shanthi	36/F	Agriculture	R	+	+	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
50	Jaya	80/F	Agriculture	L	+	-	-	+ve	-Ve	-ve	PUS CELLS	-Ve	NG	G
51	Rajeswari	43/F	Agriculture	R	+	-	-	-Ve	-Ve	+ve	GNB	-Ve	Ps. Aer	G
52	Ganesan	46/M	Coolie	L	+	+	-	-Ve	-Ve	+ve	GPC IN PAIRS	-Ve	Pneumo	P
53	David	55/M	Agriculture	R	-	+	-	+ve	-Ve	+ve	-ve	-Ve	NG	P
54	Jeenath	42/F	Agriculture	L	+	-	-	-Ve	-Ve	+ve	-ve	-Ve	St.aur	P
55	Kuppayee	40/F	Agriculture	R	+	-	-	-Ve	-Ve	-ve	PUS CELLS	-Ve	NG	G
56	Gunaneethi	32/M	Agriculture	L	+	+	-	-Ve	-Ve	-ve	-ve	-Ve	St.aur	G
57	Chokkammal	27/F	House Wife	L	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	NG	P
58	Kallappan	44/M	Mechanic	R	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
59	Saroja	43/F	House Wife	L	+	-	-	-Ve	-Ve	+ve	GNB	-Ve	Ps. Aer	G
60	Sara	31/F	Coolie	R	+	-	-	-Ve	-Ve	+ve	-ve	-Ve	NG	G
61	Gnanam	31/M	Agriculture	L	+	+	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
62	Periyanayagi	51/F	Agriculture	R	+	+	-	+ve	-Ve	-ve	-ve	-Ve	St.aur	P
63	Geetha	32/F	House Wife	L	+	+	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
64	Mychel	43/M	Agriculture	L	+	+	-	-Ve	-Ve	-ve	-ve	-Ve	NG	P
65	Indrani	30/F	Agriculture	R	-	-	-	-Ve	-Ve	+ve	GNB	-Ve	Ps. Aer	P
66	Alathamma	45/F	Agriculture	L	+	-	-	+ve	-Ve	+ve	GPC IN PAIRS	-Ve	Pneumo	G
67	Balasundaram	29/M	Agriculture	R	+	+	-	-Ve	-Ve	-ve	GNB	-Ve	Kleb	G
68	Senthamarai	18/F	Agriculture	L	+	+	-	-Ve	-Ve	-ve	GNB	-Ve	E.Coli	G
69	Mariappan	27/M	Coolie	R	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	Asp.Fla	G
70	Banumathi	35/F	Mechanic	L	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
71	Anurekha	30/F	Agriculture	L	+	+	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
72	Uma	32/F	House Wife	R	+	-	-	-Ve	-Ve	-ve	GNB	-Ve	E.Coli	P
73	Lavanya	18/F	Student	L	+	+	-	-Ve	+ve	+ve	-ve	AGB	Asp.Fla	G
74	Keerthi	60/F	Agriculture	R	+	+	-	-Ve	-Ve	-ve	-ve	-Ve	Asp.Fla	G
75	Priya	12/F	Student	L	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	Asp.Fla	P
76	Jayabalan	42/M	Agriculture	R	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
77	Nagalingam	52/M	Agriculture	L	-	+	-	+ve	-Ve	-ve	GNB	-Ve	Ps. Aer	G
78	Tamarai	32/F	Agriculture	R	+	-	-	-Ve	+ve	-ve	GNB	-Ve	Ps. Aer	G
79	Angammal	40/F	Agriculture	L	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
80	Prasad	33/M	Coolie	R	+	+	-	-Ve	+ve	-ve	-ve	-Ve	NG	P
81	Rajammal	53/F	House Wife	L	+	+	-	+ve	-Ve	-ve	-ve	-Ve	St. aur	G
82	Vittabai	52/M	Coolie	R	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	Peni	P
83	Mani	43/M	Coolie	L	+	+	-	-Ve	-Ve	-ve	-ve	-Ve	NG	P
84	Shivanandan	36/M	Agriculture	R	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	Fus	G
85	Raja	49/M	Agriculture	L	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	Fus	G
86	Lakshmi	36/F	Agriculture	R	+	-	-	-Ve	-Ve	+ve	-ve	-Ve	Fus	G
87	Ramadas	38/M	Agriculture	L	+	-	-	-Ve	-Ve	+ve	GPB chinese Letter p	-Ve	Diphtheroids	G
88	Moorthi	26/M	Coolie	L	+	+	-	-Ve	+ve	+ve	-ve	-Ve	Asp.Fla	G
89	Devi	20/F	Student	L	+	+	-	-Ve	-Ve	-ve	-ve	-Ve	Asp.Fla	G
90	James	56/M	Agriculture	R	+	-	-	+ve	-Ve	-ve	-ve	-Ve	Asp.Fla	G

Sl.No.	Name	Age/Sex	Occupation	Eye affected	Injury	Hypopyon	Previous Medication	Diabetes Mellitus	Dacryocystitis	KOH	Gram's Stain	Calcoflour White	Culture	Response to Treatment
91	Desammal	40/F	House Wife	R	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	Asp.Fla	P
92	Evarnamma	46/F	Agriculture	L	+	+	-	-Ve	-Ve	-ve	-ve	-Ve	Asp.fum	G
93	Gopal	29/M	Agriculture	L	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	NG	P
94	Malliga	36/F	Agriculture	R	+	+	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
95	Kallasamy	40/M	Coolie	R	+	-	-	-Ve	-Ve	-ve	HYPHAE	-Ve	Asp.fla	P
96	Ramaiah	51/M	Agriculture	L	+	-	-	+ve	+ve	-ve	GNB	-Ve	Ps. Aer	G
97	Manickam	61/M	Agriculture	L	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
98	Veerapandian	27/M	Agriculture	R	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	NG	P
99	Amrutham	20/M	Student	R	+	+	-	-Ve	-Ve	-ve	-ve	-Ve	Asp.fla	G
100	Sampoorna	18/F	Coolie	L	+	+	-	-Ve	+ve	-ve	-ve	AGB	Asp.fla	G
101	Vijayakumar	37/M	Agriculture	R	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	Asp.fla	P
102	Anothni	35/M	Agriculture	L	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	Asp.fla	G
103	Kondaiah	40/M	Agriculture	R	-	+	-	+ve	-Ve	-ve	-ve	-Ve	Asp.fla	G
104	Ramya	20/F	Student	L	+	-	-	-Ve	-Ve	-ve	HYPHAE	AGB	Asp.fla	G
105	Muthulakshsmi	35/F	Agriculture	R	+	-	-	-Ve	-Ve	+ve	-ve	-Ve	Fus	P
106	Ramaiah	40/M	Coolie	L	+	+	-	-Ve	-Ve	+ve	-ve	-Ve	Fus	G
107	Poongudi	28/F	Agriculture	R	+	-	-	-Ve	+ve	-ve	-ve	-Ve	Fus	G
108	Thangammal	43/F	Agriculture	L	+	+	-	+ve	-Ve	-ve	-ve	-Ve	NG	G
109	Yuvan	21/M	Student	L	+	+	-	-Ve	-Ve	-ve	HYPHAE	-Ve	Asp.fum	P
110	Pachiammal	41/F	Agriculture	L	-	+	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
111	Kallappan	47/M	Agriculture	R	+	-		-Ve	-Ve	-ve	-ve	-Ve	NG	G
112	Sultan	31/M	Mechanic	R	+	-	-	-Ve	-Ve	-ve	HYPHAE	-Ve	Asp.fum	P
113	Rasheetha	33/F	Coolie	L	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
114	Nazhir	46/M	Coolie	L	+	-	-	+ve	-Ve	-ve	-ve	-Ve	NG	P
115	Murugan	38/M	Agriculture	R	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
116	Krishnan	41/M	Agriculture	R	+	-	-	+ve	-Ve	-ve	-ve	-Ve	NG	G
117	Nalini	36/F	Agriculture	L	+	+	-	-Ve	+ve	-ve	-ve	-Ve	NG	G
118	Alamelu	28/F	Agriculture	L	+	+	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
119	Allirani	38/F	Agriculture	R	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	Asp.fla	G
120	Vinayaga	53/M	Coolie	R	+	-	-	+ve	-Ve	-ve	HYPHAE	-Ve	Asp.Fla	P
121	Baskar	42/M	Mechanic	R	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	Ceph	G
122	Loganathan	40/M	Agriculture	L	+	+	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
123	Karthikeyan	38/M	Agriculture	R	+	+	-	-Ve	+ve	-ve	-ve	-Ve	NG	G
124	Yashodha	38/F	House Wife	L	+	-	-	-Ve	+ve	-ve	-ve	-Ve	NG	P
125	Venkatesan	50/M	Agriculture	R	+	+	-	+ve	-Ve	-ve	GNB	-Ve	Kleb	G
126	Saravanan	35/M	Agriculture	L	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	Ceph	G
127	Murugeswari	46/F	Agriculture	R	+	+	-	+ve	-Ve	-ve	-ve	-Ve	Ceph	P
128	Manickandan	37/M	Agriculture	L	+	+	-	-Ve	-Ve	+ve	GPC	-Ve	St. Alb	G
129	Srinivasan	43/M	Agriculture	R	+	-	-	+ve	-Ve	+ve	-ve	-Ve	Fus	P
130	Velu	40/M	Agriculture	L	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	Fus	P
131	Kanniappan	47/M	Agriculture	L	+	-	-	+ve	-Ve	-ve	-ve	-Ve	NG	G
132	Asif	42/M	Agriculture	L	+	+	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
133	Suriya Kala	30/F	House Wife	R	+	-	-	-Ve	-Ve	-ve	-ve	AGB	Ceph	G
134	Subbamma	35/F	House Wife	R	+	-	-	-Ve	-Ve	-ve	GPC IN PAIRS	-Ve	Pneumo	P
135	Narasamma	46/F	Coolie	L	+	-	-	+ve	-Ve	-ve	GNB	-Ve	Ps. Aer	G
136	Sidhu	20/M	Student	L	+	-	-	-Ve	-Ve	-ve	GPC	AGB	St.Auer	G
137	Veerappa	53/M	Agriculture	R	+	+	-	+ve	-Ve	-ve	-ve	-Ve	NG	G

Sl.No.	Name	Age/Sex	Occupation	Eye affected	Injury	Hypopyon	Previous Medication	Diabetes Mellitus	Dacryocystitis	KOH	Gram's Stain	Calcoflour White	Culture	Response to Treatment
138	Muniyandi	47/M	Agriculture	R	+	-	Ab	-Ve	-Ve	-ve	GNB	-Ve	E.Coli	P
139	Mayavathi	35/F	Agriculture	L	-	-	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
140	Govindaraj	53/M	Agriculture	L	+	+	-	+ve	-Ve	-ve	-ve	-Ve	Ceph	G
141	Kasikumar	23/M	Student	R	+	+	-	-Ve	-Ve	-ve	-ve	-Ve	Ceph	G
142	Abdul Zuban	48/M	Agriculture	L	+	-	-	+ve	-Ve	-ve	GNB	-Ve	Kleb	G
143	Muniraj	28/M	Agriculture	L	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	Fus	G
144	Kamalamma	30/F	Agriculture	R	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
145	Raman	47/M	Agriculture	R	+	+	-	+ve	-Ve	+ve	GNB	-Ve	E.Coli	G
146	Arumugam	32/M	Mechanic	L	+	-	-	-Ve	-Ve	-ve	GPC	-Ve	St.Auer	P
147	Karthi	28/M	Coolie	L	+	-	-	-Ve	+ve	-ve	GPC	-Ve	St.Alb	G
148	Nataraj	31/M	Coolie	R	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G
149	Balan	23/M	Agriculture	L	+	-	-	-Ve	+ve	-ve	-ve	-Ve	NG	G
150	Gowtham	30/M	Agriculture	L	+	-	-	-Ve	-Ve	-ve	-ve	-Ve	NG	G

INSTITUTIONAL ETHICAL COMMITTEE, STANLEY MEDICAL COLLEGE, CHENNAI-1

Title of the Work : Clinical and Lab studies on suspected cases of Microbial Keratitis – a hospital based study

Principal Investigator : Dr.K.E. Keerthana

Designation : PG in MS(Ophtal)

Department : Department of Ophthalmology
Government Stanley Medical College,
Chennai-1

The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 13.07.2011 at the Modernized Seminar Hall, Stanley Medical College, Chennai-1 at 2PM

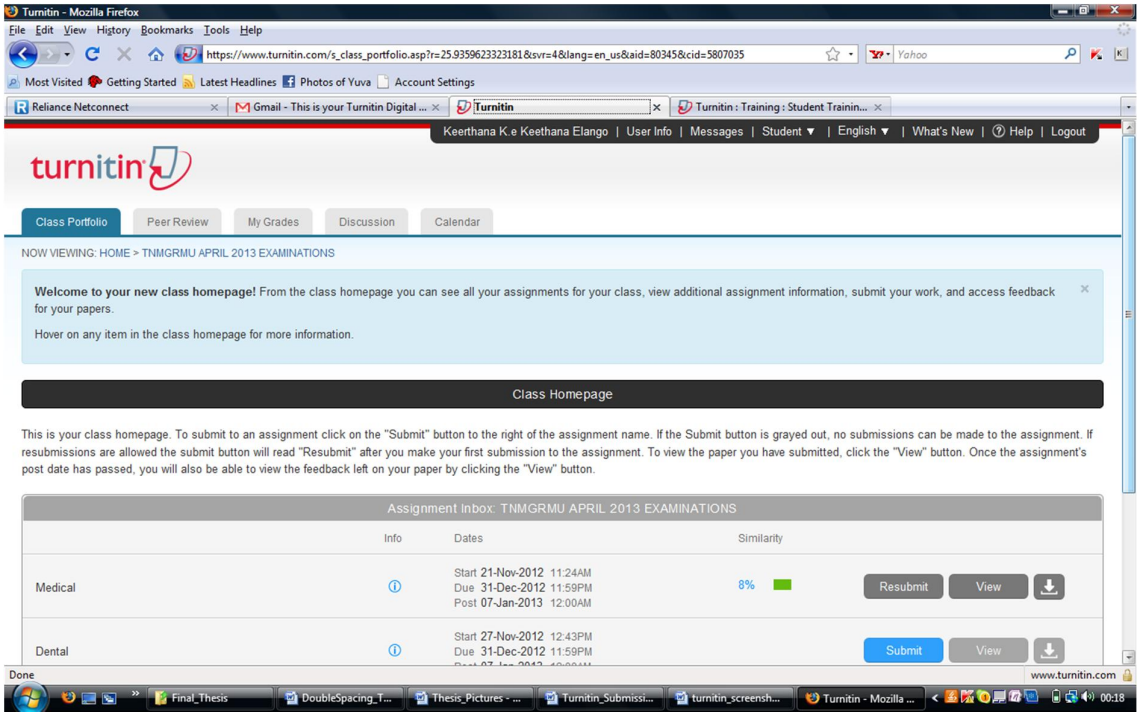
The members of the Committee, the secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The Principal investigator and their team are directed to adhere to the guidelines given below:

1. You should inform the IEC in case of changes in study procedure, site investigator investigation or guide or any other changes.
2. You should not deviate form the area of the work for which you applied for ethical clearance.
3. You should inform the IEC immediately, in case of any adverse events or serious adverse reaction.
4. You should abide to the rules and regulation of the institution(s).
5. You should complete the work within the specified period and if any extension of time is required, you should apply for permission again and do the work.
6. You should submit the summary of the work to the ethical committee on completion of the work.

13/3/12

MEMBER SECRETARY,
IEC, SMC, CHENNAI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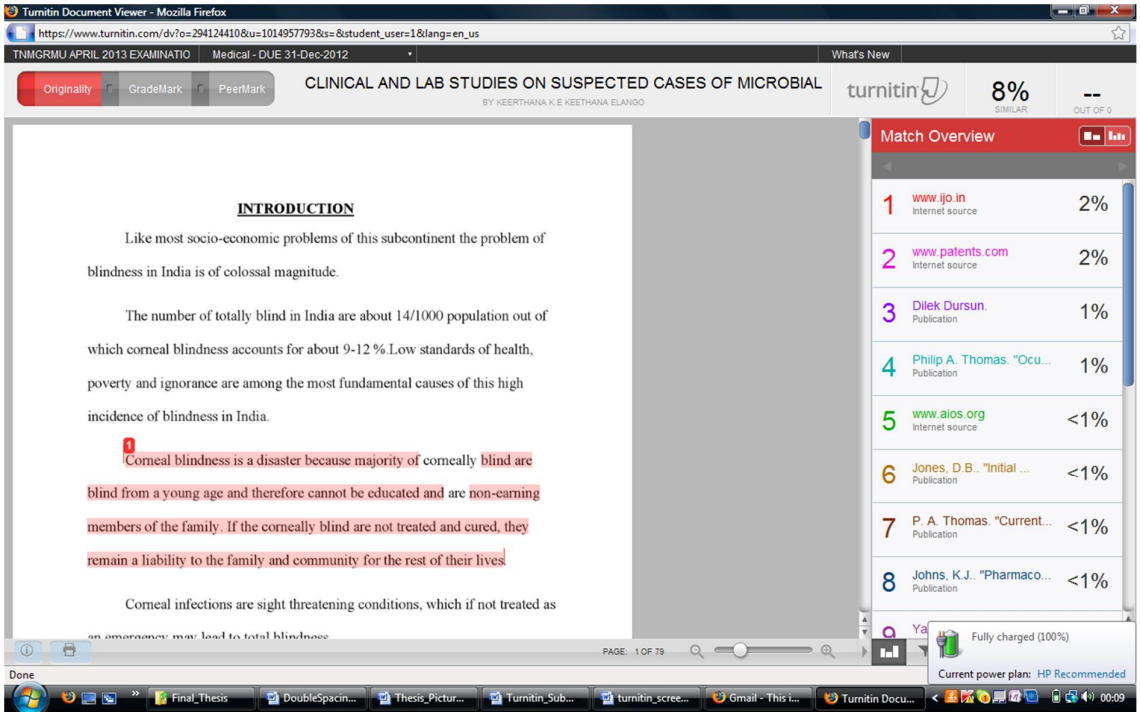
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INTRODUCTION
Like most socio-economic problems of this subcontinent the problem of blindness in India is of colossal magnitude.
The number of totally blind in India are about 14/1000 population out of which corneal blindness accounts for about 9-12 %.Low standards of health, poverty and ignorance are among the most fundamental causes of this high incidence of blindness in India.
1
Corneal blindness is a disaster because majority of corneally blind are blind from a young age and therefore cannot be educated and are non-earning members of the family. If the corneally blind are not treated and cured, they remain a liability to the family and community for the rest of their lives.
Corneal infections are sight threatening conditions, which if not treated as
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