"A ONE YEAR PROSPECTIVE CROSS SECTIONAL STUDY OF CLINICAL AND MICROBIOLOGICAL SPECTRUM OF CORNEAL ULCERS AT TERTIARY CARE HOSPITAL, BELGAUM"

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This is to certify that the dissertation entitled **"A ONE YEAR PROSPECTIVE CROSS SECTIONAL STUDY OF CLINICAL AND MICROBIOLOGICAL SPECTRUM OF CORNEAL ULCERS AT TERTIARY CARE HOSPITAL, BELGAUM"** is a bonafide research work done by **REG. NO.BK0111003**

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LIST OF ABBREVIATIONS

AC	-	Anterior chamber
AL	-	Adherent leucoma
CF	-	Counting fingers
DCT	-	Dacryocystitis
HMCF	-	Hand Movements Close to Face
KOH	-	Potassium hydroxide
MIC	-	Minimum inhibitory concentration
NSAID	-	Non steroidal anti inflammatory drug
PL	-	Perception of light
PMN	-	Polymorphonuclear cells
RVD	-	Retro viral disease
SD	-	Standard deviation
SDA	-	Sabouraud's dextrose agar

ABSTRACT

Background and Objectives

Burden of corneal blindness is 5.1 % worldwide according to WHO[2] and 9% in India [3]. 65-90% cases are secondary to bacterial infections[4-7] and 6-50% cases due to fungal infections[8]. For *Acanthamoeba* keratitis contact lens wear is the predominant risk factor in the developed world. However, in developing countries it is usually associated with exposure to contaminated water. The epidemiological data regarding prevalence and incidence of Acanthamoeba keratitis are lacking[38-45]. The aim of this study is to understand clinical and microbiological profile of corneal ulcers at a tertiary care centre.

Methods

The present one year randomized controlled trial was conducted in the Department of Ophthalmology, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum during the period of January 2012 to December 2012 on 45 patients diagnosed with microbial keratitis. After obtaining written informed consent corneal ulcer was examined at slit lamp to achieve clinical diagnosis and microbiological investigations in terms of Gram's smear, KOH preparation and inoculation of corneal scrapings on culture media like blood agar, chocolate agar, MacConkey's agar and Sabouraud's Dextrose Agar was done. Growth of organisms on different media were studied.

Results

Males accounted for 38(84.44%) and females 7(15.55%) out of total 45 patients enrolled in the study. Males were predominant with. M:F ratio of 5.42:1 in our study. Majority of the patients were from rural population 30 (66.66%) . Farmers accounted for 24(53.33%) of the study group, males were 22(57.89%) and females were 2(28.57%) . Out of 45 cases, patients with injury with a vegetative matter in the form of seeds, leaf, stems and sticks were found to be 35.55% and considered as a major risk factor associated with development of corneal ulcers. Majority of patients presented during first 10 days of the onset of symptoms 24(53.33%) and 27(60%) presented with an visual acuity of CF to HMCF, PL +. Streptococcus Pneumoniae 6/17 (35.29%) and Pseudomonas species 4/17(23.52%) were most common bacteria isolated where as Aspergillus sp positivity was 12/30(40%) followed by Fusarium 7/30(23.33%).

Conclusion and interpretation

Present study concludes microbiological investigations offer a great help in diagnosis of corneal ulcer and provide a guideline to initiate an early specific treatment.

Keywords

Microbial keratitis; Gram's stain; KOH stain;SDA; Corneal injury

CONTENTS

SL. NO.	TOPIC	PAGE NO.
1	INTRODUCTION	1
2	OBJECTIVES	3
3	REVIEW OF LITERATURE	4
4	METHODOLOGY	51
5	RESULTS	62
6	DISCUSSION	79
7	CONCLUSION	87
8	SUMMARY	88
9	BIBLIOGRAPHY	91
10	ANNEXURES	
	ANNEXURE I – CONSENT FORM	102
	ANNEXURE II – PROFORMA	106
	ANNEXURE III – PHOTOGRAPHS	114
	ANNEXURE IV – MASTER CHART	123
	ANNEXURE V - KEY TO MASTER CHART	124

LIST OF TABLES

TABLE. NO.	DESCRIPTION	PAGE NO.
1	Classification of bacterial organisms causing microbial keratitis	15
2	Classification of antibiotics based on Mechanism of Action	40
3	Classification of antifungals based on Mechanism of Action	46
4	Grading of Corneal Ulcer based on Jones Criteria	54
5	Ulcer Status	60
6	Sex Distribution	62
7	Age Distribution	63
8	Distribution based on the area of Residence	64
9	Distribution based on Occupation	65
10	Risk Factors- Ocular Trauma	67
11	Other Risk Factors	68
12	Distribution of Symptoms at Presentation	69
13	Duration of Symptoms at Presentation	70
14	Visual Acuity at Presentation	71

15	**Ulcer Characteristics at Presentation**	73
16	**Gram's Staining**	74
17	**Bacteria isolated on Culture**	74
18	**KOH Preparation**	75
19	**Fungal species isolated on Culture**	76
20	**KOH and Fungal growth**	77
21	**Clinical Diagnosis and Final Diagnosis**	77
22	**Antibiotic Sensitivity**	78

LIST OF GRAPHS

GRAPH NO.	DESCRIPTION	PAGE NO.
1	Sex Distribution	63
2	Age distribution	64
3	Distribution based on the area of residence	65
4	Distribution based on Occupation	66
5	Risk factors - Ocular trauma	67
6	Other risk factors	69
7	Distribution of Symptoms at Presentation	70
8	Duration of Symptoms at Presentation	71
9	Visual Acuity at Presentation	72

LIST OF FIGURES

FIGURE NO.	DESCRIPTION	PAGE NO.
1	**Microscopic structure of corneal layers on cross section at 160X**	4
2	**(a) Nonkeratinized stratified squamous epithelial cells by Confocal microscopy** **(b) Mosaic pattern of hexagonal cuboidal endothelial Cells**	5
3	**Life cycle of Acanthamoeba species**	30

LIST OF PHOTOGRAPHS

PHOTO NO.	DESCRIPTION	PAGE NO.
1	Procedure of corneal scraping	114
2	Laboratory armamentarium	115
3	Bacterial corneal ulcer with marked infiltration	115
4	Hypopyon corneal ulcer with chemosis	116
5	Fungal corneal ulcer with feathery margins	116
6	Perforation in a Bacterial corneal ulcer	117
7	**Epithelial defect with fluorescein staining**	117
8	**Corneal ulcer with both superficial and deep vascularisation**	118
9	**Endothelial plaque and hypopyon in polymicrobial keratitis**	118
10	Pseudomonas keratitis with ring infilteration and hypopyon	119
11	Superficial vascularisation in Curvuleria keratitis	119
12	Aspergillus Keratitis	120
13	Staphylococcal corneal ulcer with stromal infiltration	120
14	Aspergillus Niger growth on SDA	121
15	Candida sp on SDA	121
16	Streptococcus pneumoniae on Blood agar	122
17	Aureobassidium growth on blood agar	122

INTRODUCTION

Cornea is a strong refractive medium of eye owing to its transparent nature. Avascularity, peculiar arrangement of collagen lamellae, paucity of mitochondria in corneal cells, barrier functions of epithelium and endothelium are integral for maintaining integrity and transparency. In a southern India-based study, corneal blindness has been projected to grow from prevalence of 0.66% (2001) to 0.84% (2020), from unilateral cases[46].

Breach in continuity of corneal epithelium results in a formation of an epithelial defect which is associated with infiltration of corneal stroma altogether is called a corneal ulcer or keratitis[1]. It could be of infective or non infective etiology. Burden of corneal blindness is 5.1 % worldwide according to WHO[2] and 9% in India [3]. 65-90% cases are secondary to bacterial infections[4-7] and 6-50% cases due to fungal infections[8]. *Acanthamoeba* keratitis contact lens wear is the predominant risk factor in the developed world. However, in developing countries it is usually associated with trauma or exposure to contaminated water and epidemiological data regarding prevalence and incidence are lacking[38-45]. It is of utmost importance to identify the etiology and causative agent for correct diagnosis and treatment. **Trend of infective keratitis is changing from country to country hence identification of bacterial , fungal and protozoal species is of great importance for early and definitive management and in preventing profound visual loss by complications like visual loss, endophthalmitis, corneal perforation, adherent leucoma and corneal opacity[4,5,10-12,24,76].**

In the era of modern medical technology, various methods of investigations are available for corneal ulcers including; microbial smears and cultures, antibiotic sensitivity, confocal microscopy, corneal biopsy and polymerase chain reaction. Microbiological investigations are easy to perform, less invasive and cost-effective and also provide prompt diagnosis hence they are considered gold standard investigations[14] . Apart from its diagnostic value, corneal scraping allows improved antibiotic penetration and therapeutic debridement of necrotic tissue [14]. Due to identification of specific organism, most specific treatment drug can be incorporated which reduces irrational use of multiple medications, their ocular toxicity and emergence of resistance.

Microbial corneal ulcers (bacterial, fungal and protozoa) vary in their presentations and various times they show mixed clinical features hence pose a diagnostic dilemma.

Hence, the present prospective cross sectional study was undertaken to know the clinical pattern and microbiological spectrum of corneal ulcers for their successful diagnosis and management.

OBJECTIVES

1. **Primary objective :** The primary objective is to study clinical and microbiological spectrum of corneal ulcers at Tertiary care hospital , Belgaum.

2. **Secondary objective :** To identify risk factors, epidemiological characteristics and visual acuity pattern in microbial corneal ulcers

REVIEW OF LITERATURE

ANATOMY OF CORNEA[15,16]

Cornea , a principle refractive medium of eye is transparent, avascular, watch glass like structure. It forms the anterior one sixth of the outer fibrous coat of eyeball. The adult cornea appears as an ellipsoid being about 12 mm in horizontal meridian and 11 mm in the vertical. However, from the back, the cornea is circular with a diameter of nearly 11.5 mm, thicker at the periphery (0.67 mm) than at the centre (0.52 mm)[15].

The cornea has refractive index of 1.376. The concave posterior surface of the cornea faces the aqueous which has lower refractive index 1.336.The refractive power of this surface is –5.8 diopters giving the entire cornea a refractive power of +43 diopters, out of the total 58 diopters refractive power of the eye[15].

Cornea is covered by precorneal tear film which is 7 µm thick made up of outer lipid, middle aqueous and inner mucin layer[16].

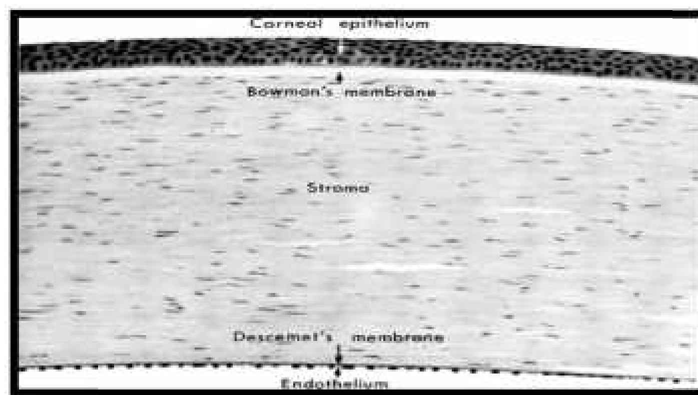


Figure 1. Microscopic structure of corneal layers on cross section at 160X

Layers of the cornea

Epithelium: Consists of five or six layer of stratified squamous non keratinized epithelium resting on a very delicate argyrophilic basement membrane. It is about 50-90 micron in thickness. The deeper layer is basal cells which stand in palisade manner in perfect alignment on a basement membrane.

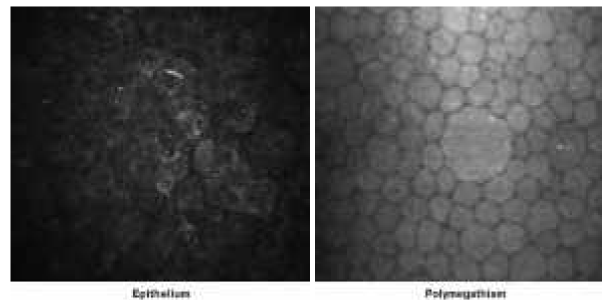


Figure 2. (a) nonkeratinized stratified squamous epithelial cells by Confocal microscopy (b) Mosaic pattern of hexagonal cuboidal endothelial cells

Bowman's membrane: Acellular membrane measures 8-14 microns in thickness composed of collagen I and III. Collagen fibrils are smaller and measure between 240 Å and 270 Å. Towards the periphery, it becomes thinner and arrangement of fibres looser and collagen gradually merges with that of conjunctiva. Bowman's membrane once destroyed will not regenerate and thus gives rise to permanent opacity.

Stroma: It is 0.5 mm in thickness. It is composed of modified connective tissue of which the constituents have very nearly the same refractive index so that in the perfectly fresh condition, it is difficult to make out any indications of structure. Among the lamellae of the cornea are a considerable number of fixed cells, corneal

fibroblasts or keratocytes, wandering macrophages. Occasionally, lymphocytes or polymorphonuclear leucocytes are seen.

Descemet's membrane: It is a strong, homogenous and very resistant membrane. It is 10 to 12 micron thick and sharply defined from the stroma. It has anterior banded(1/3) and posterior non banded(2/3) zones. In the periphery, it bends form Schwalbe's line to which the trabecular meshwork is attached anteriorly. Any cause of raised Intraocular pressure leads to descematocele formation in corneal ulcers

Endothelium: It is the most posterior layer of the cornea and consists of a single layer of cuboidal cells with 4-6 micron thickness. Endothelium has Na^+K^+ATPase activated pump hence maintains the clarity by preventing water imbibation. With slit lamp, endothelium can be visualized in mosaic pattern. Specular microscopy helps to evaluate the endothelial status and count allowing an objective estimation of the endothelial cell loss. Adult endothelial cell count ranges from 1400-2500 cells/ mm^2 when this cell count falls below 400 cells/ mm^2 corneal edema occurs.

NORMAL WOUND HEALING

Corneal epithelium is a mass of three complex phenomena which are interactive feedback mechanisms that maintain cell death, proliferation and cell thickness. X phenomenon explains proliferation of basal cells, Y explains centripetal mass movement of peripheral cells and Z is loss of cells due to apoptosis. [17,19]

Corneal abrasion[17]

An abrasion results from an injury that removes some or all of the layers of epithelium but leaves the Bowman's membrane intact[18]. The wound heals by epithelial sliding and mitotic proliferation and without a scar formation.

After about an hour of lag period, the normal epithelium from the edge of the abraded area flattens and lamellipodia, filopodia and ruffles develop in basal cells so they slide inward to cover the gap. If the entire corneal epithelium is abraded, the gap can be covered by sliding of conjunctival epithelium in 48 to 72 hours. At first the epithelium is thinner than normal, but mitotic cell division restores rapidly the normal thickness. Over a period of weeks to a few months, the conjunctival epithelium takes on completely the morphological characteristic of the corneal epithelium. Mitotic multiplication of the epithelium restores the normal thickness.

Superficial defect[17]

A superficial defect results from an injury that removes epithelium and Bowman's membrane with or without a few anterior layers of stroma. The wound healing takes place exactly as in an abrasion except that mitotic multiplication results in a focus of thicker than normal epithelium called an epithelial facet. An epithelial facet can be seen with the slit lamp as a tiny, focal separation the two anterior most zones of corneal relucency. This type of lesion results most frequently from a small, superficially embedded foreign body.

No attempt is made to repair Bowman's membrane or any superficial stroma that may be involved.

Deep defect[17]

A deep defect results from an injury that removes epithelium, Bowman's membrane and at least the anterior quarter of the stroma. Healing takes place mainly by epithelial sliding and corneal thinning results. The epithelium from the edge of the injured area flattens and slides over the wound, thus attempting to fill the gap promptly.

Mitotic multiplication of the epithelium results in a normal thickness or a slightly thicker than normal epithelium but cannot restore the normal curvature of the anterior corneal surface. Thus a concavity of the anterior corneal surface results in corneal thinning.

Definition of Corneal Ulcer:

Corneal ulcer is defined as a loss of the corneal epithelium with underlying stromal infiltration and suppuration associated with signs of inflammation with or without hypopyon[20].

Corneal ulcer is a nonspecific term, and include both non infectious and infectious keratitis cases, although more precise term such as microbial keratitis is gaining acceptance. Microbial keratitis is a common, potentially sight threatening ocular infection caused by viruses, bacteria, fungi or parasites[17]. Viral keratitis and Acanthemeba keratitis present with specific clinical features but corneal ulcers with fungal and bacterial etiologies create a dilemma for diagnosis and management.

Protective mechanisms of eye[16] :

1) Eye is protected by microbial invasion by bony and membranous structure of orbit.
2) Blink reflex protects cornea by intrusion of any flying foreign body, dust or organic matter.
3) Conjunctiva and tear film act as biological barrier against microbial infections. Conjunctiva is sterile at birth but rapid colonization of conjunctiva and adenexa occurs after birth by saprophytic bacteria[31]. The frequency of organisms in normal conjunctival flora include Staphylococcus albus in 85%, diptheroids in 50%, Staphylococcus aureus in 20% and Proteus in 3% and gram negative rods in 5% of the cases[31]. The presence of non pathogenic micro organisms in the normal, uninfected conjunctival sac, provides a constant reservoir of potentially pathogenic bacteria capable of causing serious ocular infections once the normal protective mechanisms of the cornea are breached[31]. Some organisms such as Neisseria gonorrhea, Neisseria meningitides, Corynebacterium diptheriae, Listeria, and Shigella can directly penetrate an intact epithelium[27].
4) Lacrimal apparatus help to drain away small foreign bodies, dust particles and desquamated epithelial cells hence keep the cornea free from microbes.
5) Mucus layer of tear film has lysozyme, immunoglobulins, lactoferrin , betalysin and complements to combat infections in the eye
6) Conjunctiva associated lymphoid tissue produces antibody response whenever eye is exposed to particular antigen and provides humoral immunity.

Predisposing factors[16,17]

Exogenous factors

1. Corneal trauma with vegetative organic and non organic matter.
2. Foreign body like dust, stones, soil, metal objects, sticks etc.
3. Contact lens wearers are more prone to bacterial infection, especially with Gram-negative organisms. The contact lens induces hypoxia, trauma, increases corneal temperature and decreases tear flow over the corneal surface. The adhering of microorganisms (*Staphylococci, Moraxella, Candida*) to the contact lens and epithelium is aided by mucus and proteins. The risk of developing corneal infections is 9–15 times greater with overnight use of contact lenses compared to daytime use.
4. Topical drugs like indiscriminate use of antibiotics and corticosteroids.

Endogenous factors

1. Lacrimal disorders such as dry eyes and dacryocystitis.
2. Lid disorders such as blepharitis, entropion, ectropion, lagophthalmos etc.
3. Conjunctival infections like trachoma, vernal catarrah, cicatricial pemphigoid
4. Corneal diseases such as bullous keratopathy, corneal abrasions, chemical injuries, herpetic eye disease, neurotrophic keratitis.

Systemic factors

Extensive burns, alcoholism, debilitating diseases, rheumatoid arthritis, diabetes mellitus, drug addictions, acquired immunodeficiency syndrome, previous viral conjunctivitis , ocular surgeries like pterygium excision, photorefractive keratectomy and laser in situ keratomileusis are considered predisposing factors for secondary invasion by microbes.

Pathogenesis of corneal ulcer[16,22-24]

This involves the following stages.

Stage of infiltration

In this stage corneal ulcer results when the balance between the cornea is disrupted and the protective mechanisms are compromised. The organisms adhere to damaged epithelium, invades stroma and begins to replicate by binary fission. The host inflammatory response is initiated by the polymorphonuclear cells (PMNs) which arrive via tear film initially and later from the vascular limbus. These PMNs are secreted in response to the corneal insult which secrete various lytic enzymes such as collagenase, elastase and cathepsin causing destruction of the cornea. The PMN response is a two edged sword-both often adding to corneal stromal destruction and helping to contain the infection and promote sterilization of the corneal ulcer.

Stage of progressive ulceration

If the infection is not controlled, the inflammatory reaction progresses relentlessly and results in tissue loss. There may be associated hypopyon which is

usually sterile as the organism does not ordinarily penetrate an intact Descemet's membrane.Progression of such ulcerative process can lead to corneal perforation.

Stage of Regression

This stage is characterized by decrease in ocular symptoms. The infiltrates decrease in size and the ulcer becomes more demarcated. Necrotic areas may slough off, mimicking progression even though the inflammation is regressing.

Healing stage

In the healing stage, the necrotic stroma is replaced by the scar tissue laid down by fibroblasts which are transformed histiocytes and keratocytes. The scar never becomes transparent, although its density decreases with time especially in children and young adults. Vascularisation occurs towards the ulcer site, which further promotes healing as a result of influx of fibroblasts and antibodies. When the healing is complete, the vessels regress and become "ghost vessels" which may be visualized by an indirect illumination.

SEQUELAE AND COMPLICATIONS [16,24]

Sequelae can vary in severity from corneal scarring to perforation, endophthalmitis, and loss of the eye.

Corneal Opacification

Depending on the depth of the corneal ulceration, different types of corneal opacities may occur that is, nebular involves less than 50% corneal involvement, macular is > 50% and <75% corneal involvement or leucomatous, when >75% of corneal is involved.

Descemetocele

Ulcers due to pneumococci extend rapidly in depth, to involve entire thickness of the cornea sparing the Descemet's membrane.This membrane offers resistance to the inflammatory process, but is unable to withstand the intraocular pressure and therefore herniates through the corneal ulcer as a transparent membrane called as descemetocele or keratocele.

Perforation

On perforation aqueous escapes and the intraocular pressure falls to the atmospheric levels. Subsequently, the lens iris diaphragm moves forward and adheres to back of the cornea. Due to decreased intraocular pressure, the pain is alleviated; extension of the ulcer decreases and the process of scar formation is initiated.

When perforation is small, the iris is plugged to the back of the cornea and adhesions from the iris gets organized and the scar tissue is formed which is called as *"pseudocornea"*. The plastered iris at the back of the cornea, allows anterior chamber to form. When perforation is large, the iris prolapses out through the perforation; in long standing cases of prolapsed fibrin and exudates deposition occurs on the surface. Thus, adherence of iris tissue to the back of cornea, subsequent to a perforated corneal ulcer, is called as a *corneo-iridic scar*.

Ectatic Cicatrix

Due to the presence of anterior synechiae and plugging of the iris, adherent leucoma forms, often leading to secondary glaucoma. The cicatricial tissue is too

weak to support this raised intra ocular pressure and hence cicatrix becomes ectatic. An ectatic cicatrix into which the iris is incarcerated is called *anterior staphyloma*.

Corneal Fistula

If perforation occurs near the pupillary margin corneal fistula forms due to continuous drainage of aqueous from it.

Hemorrhage

The sudden decrease in the intraocular pressure dilates the intraocular blood vessels, which may rupture causing an intraocular hemorrhage. It may even lead to expulsive hemorrhage.

Endophthalmitis

Organisms, may gain access to the interior of the eye as a result of perforation and cause purulent iridocyclitis, endophthalmitis and even panophthalmitis.

Bacteriology

Table 1. Classification of bacterial organisms causing microbial keratitis[16]

Aerobic bacteria	**Anaerobic bacteria**
Gram positive cocci	Gram positive cocci
Micrococcaceae	*Peptococcaceae*
Staphylococcus aureus	Peptococcus
Staphylococcus epidermidis	Peptostreptococcus
Streptococcaceae	
Streptococcus pneumoniae	
A and B hemolytic streptococci	
Enterococcus	
Gram positive bacilli	Gram positive bacilli
Bacillaceae	*Propionibacterium acne*
Bacillus cereus	Actinomyces
Bacillus subtilis	Clostridium
Corynebacteria diphtheria	
Corynebacteria xeroses	
Listeria monocytogenes	
Gram negative bacilli	Gram negative bacilli
Pseudomonaceae	Fusobacterium
Pseudomonas aeruginosa	Bacteriodes
Acinetobacter	
Azotebacter	

Enterobacteriaceae	
Klebsiella	
Serratia	
Proteus	
Citro bacter	
Enterobacter	
Gram negative diplococcic	*Gram negative cocci*
Neisseria	Veillomella
Gram negative diplobacilli	*Spirochaetales*
Moraxella	Treponema
Gram negative coccobacilli	Leptospina
Hemophilus	
Gram positive filaments	
Mycobacterium (Non tuberculosis)	
Nocardia	

SPECIFIC ORGANISMS

Staphylococcus organisms[16,17]

The presence or absence of coagulase enzyme divides the Staphylococci into two broad groups. The coagulase positive and coagulase negative Staphylococci. Staphylococcal ulcers are more often found in compromised corneas like bullous keratopathy, dry eyes, chronic herpetic keratitis, atopic disease and rosacea keratitis.

Streptococcus pneumococci

Important cause of bacterial keratitis in cases of chronic dacryocystitis.[17,26]

Streptococcus viridans keratitis is characterized by a distinct, non-inflammatory, indolent crystalline keratopathy. This entity is typically seen after penetrating keratoplasty.

Gram positive aerobic bacilli

Bacillus Cereus have been associated with keratitis following foreign body injury.[25] *Bacillus cereus*, a large aerobic Gram-positive rod is extremely virulent. It causes rapid and devastating bacterial keratitis, starting as a ring infiltrate in the cornea away from the site of the injury and rapidly progresses to abscess formation often with corneal perforation.

Pseudomonas species

It is the most common Gram negative and highly virulent organism causing corneal ulcers. Pseudomonas species has also been increasingly isolated more so in daily or extended wear soft contact lens wearers.[26]

Anaerobes

Anaerobes and higher order bacteria cause ulcers following corneal injuries with contaminated soil.[27] Actinomyces species, a filamentous bacteria is an important cause of canaliculitis.[17]

Nocardia species inhabits the soil and cause indolent ulceration. Mycobacterium fortuitum also inhabits the soil and causes indolent ulcerations with “cracked windshield” appearance.[16]

Non-spore forming Anaerobic Organisms

Non-spore forming anaerobic organisms should be suspected as a cause of keratitis following human or animal bites. They are associated with extensive necrosis of the tissue, gas formation in tissue or foul discharge. The most frequently isolated anaerobes are Peptostreptococcus, Peptococcus, Propionibacterium, Bacteroides and Fusobacterium species.[27]

Non-tuberculous Mycobacteria

Non-tuberculous mycobacteria, including Mycobacterium fortuitum, M. chelonae and M. avium intracellular are emerging as causes of indolent keratitis especially after surgical procedures such as laser-in-situ keratomileusis.[29]

GENERAL CLINCIAL FEATURES[17,22, 24]

Most patients with bacterial keratitis manifest with decreased visual acuity, photophobia, pain, redness and swelling. Discharge is usually absent in keratitis unless it is associated with purulent conjunctivitis.

Slit lamp examination reveals a non-specific conjunctival response (mainly papillary response) and conjunctival chemosis, if a significant iritis is present, a circumlimbal ciliary flush may be seen. The corneal epithelium become ulcerated and the stroma exhibits variable degree of gray white infiltration. The cornea will be edematous, with visible folds in descemet’s membrane. Fine keratic precipitates are

found on the endothelium. Anterior chamber reaction exhibits variable degrees of cells and flare. A hypopyon may be present which in bacterial keratitis, is typically sterile .It is relatively more common in ulcers caused by Streptococcus pneumoniae and Pseudomonas.

SPECIFIC CLINICAL FEATURES[16,17,22,24]

Staphylococcus

They are typically localized round or oval ulcers with distinct borders surrounded by grayish white zone of infiltration. The ulcer develops more in depth than in width and may cause intra-stromal abscess which may lead to perforation. Corneal ulcers produced by non-aureus strain tends to be more indolent with less infiltration and anterior chamber reaction.

Streptococcus pneumoniae

Pneumococcal ulcers tends to remain localized or may have a tendency to spread in one direction, usually centrally (Ulcer serpens). Fibrin deposition may be seen on endothelial side of the ulcer and a deep stromal abscess may form, with intervening stroma being relatively clear. There is marked anterior chamber reaction with hypopyon formation. Perforation is more common with pneumococcal ulcers.

Pseudomonas species

It is the most common gram negative and highest virulent organism causing corneal ulcers clinically. It causes a rapidly spreading central or paracentral ulcer and is associated with dense stromal infiltration. The surrounding cornea is edematous which gives a characteristic ‘ground-glass’ appearance .If untreated, the ulcer spreads

circumferentially to form a ring ulcer within 48 to 96 hours .There may be, soupy melting of the cornea with greenish mucopurulent discharge with eventual perforation within two to five days of onset of infection.

Gram negative rods

Moraxella ulcer: It is indolent with mild to moderate anterior chamber reaction .Oval and central corneal ulcer mostly remain localized but has deep penetration and may progress to endothelial decompensation.

Clostridium species: Gas bubbles may be visualized in anterior chamber , corneal stroma or under epithelium.

Klebsiella, Escherichia coli and Proteus cause indolent ulcer commonly seen in cases of trauma, malnourished debilitated patients and alcoholic patients.

Epidemiology of bacterial keratitis

Gonzales *et al* has documented that incidence of corneal infection in Madurai District, south India was estimated to be 11.3 per 10 000 population or at least 10 times more frequent than the incidence of similar infections in an age and sex adjusted population in the USA[20].

Madurai study in south India has shown that out of 167 isolates (79.0%) were Gram positive and 35 (21.0%) were Gram negative. *Streptococcus pneumoniae* was the most commonly isolated bacterial organism representing 74 (44.3%) of all positive bacterial cultures. *Corynebacterium xerosis* (12.5%) *Staphylococcus epidermidis* was cultured from 17 patients (10.2%) and *Staphylococcus aureus* from nine patients (5.4%)[20]. *Pseudomonas* sp. was isolated from 24 cultures (14.4%) and

was the most frequently occurring Gram negative organism. .Of these 21 cultures, seven were pure, 6 were mixed with other bacteria, and 8 had mixed bacterial and fungal isolates[20].

Staphylococcus epidermidis (42.3%) was found to be most common cause of bacterial keratitis in a 10 year retrospective study at Tertiary care hospital at Hydarabad, India. (82.1%) were gram-positive and (17.9%) were gram-negative isolates out of 5897 keratitis patients[47].

Basak SK et al[49] conducted a study at Barrackpore, West Bengal in between 1 January, 2001 to 31 December, 2003 at a tertiary care eye institute suggested that Staphylococcus aureus, representing (42.6%) of all the bacterial culture (P< 0.0001) was most common cause of bacterial keratitis followed by Pseudomonas sp 63 (21.1%) and %. Mixed infections both by bacteria and fungi were found to be (9.5%).

A prospective study of suppurative keratitis was conducted in Ghana and southern India between June 1999 and May 2001 and concluded that *Pseudomonas* species were the commonest bacterial isolates from corneal ulcers in Ghana (52.5%), Streptococci (20%) and staphylococci (10%) were commonly associated with corneal infection. In India streptococci accounted for 46.8% of corneal ulcers followed by staphylococci (26.8%) and pseudomonas (14.9%)[20,35]. Mycology[16,56-61]

I Filamentous fungi

1. Septate	**2. Nonseptate**
(a) Moniliaceae (non pigmented)	
Acremonium	Rhizopus
Acrostalagmus	Absidia
Arthrobotrys	Mucor
Arthrographis	
Aspergillus	
Aureobasidium	
Beauvaria	
Botryodiplodia	
Botrytis	
Calcarisporium	
Cladosporium	
Colletotrichum	
Cylindrocarpon	
Drechslera	
Epidermophyton	
Exserohilum	
Fonsecaea	
Fusarium	
Fusidium	
Geotrichum	
Glenospora	
Graphium	

Helminthosporium

Hormodendrum

Metarhizium

Microsphaeropsis

Microsporum

Nigrospora

Paecilomyces

Penicillium

Periconia

Phaeoisaria

Phoma

Pithomyces

Rhizoctonia

Scedosporium

Scopulariopsis

Stachybotrys

Syncephalastrum

Tetraploa

Trichoderma

Trichophyton

Trichosporon

Verticillium

Volutella

(b) *Dematiaceae* (pigmented)

Alternaria

Aureobasidium

Bipolaris

Curvularia

Exserohilum

Lasiodiplodia

Phialophora

Torula

II Yeasts

Candida

Monilia

Oospora

Pichia

Rhodosporidium

Rhodotorula

Saccharomyces

Sporotrichum

Torulopsis

Trichosporon

Ustilago

III Dimorphic Fungi

Blastomyces

Cryptococcus

Sporothrix

Epidemiology of Mycotic keratitis[6,8,12,21,56-61]

At least 70 genera of filamentous fungi (56), yeasts (11), and dimorphic fungi (3) have been reported to cause human keratitis. Of importance to ophthalmologists are the differences in geographic prevalence, risk factors, pathogenesis, distinctive signs of keratitis, and antifungal susceptibility of filamentous fungi and yeasts.

Fungi account for less than 5% of cases of microbial keratitis in developed countries and 10% to 15% or more in developing countries. Prevalance of fungal keratitis is 44% in south India, 17% in Nepal, 36% in Bangladesh, 37.6% in Ghana and 35% in Florida.

In India most common isolated organism was Aspergillus species (27-42%), Fusarium species(6-32%) and Penicillium (2-29%).

Basak SK[49] et al conducted a study in WestBengal in year 2001-2003 and found 623 fungal isolates out of 1198 with 59.8% Aspergillus sp, 21.2% Fusarium sp and 10.1% were Penicillium sp, Candida sp (1.12%) and pigment-producing dematiaceous fungi (1.78%) .

A prospective study of suppurative keratitis was conducted in Ghana and southern India between June 1999 and May 2001 and total 1090 patients with

suppurative keratitis were enrolled in the study; 290 in Ghana and 800 in India and concluded that fungal keratitis accounted for 38.6% in India and 36.2% in Ghana and mixed bacterial and fungal infection occurred in 5.5% in India and 1.4% in Ghana[20,35].

A retrospective review[7,76-81] of all culture-proven fungal keratitis seen from 1999 -2002, Bharathi MJ[33] et al reported incidence of fungal keratitis in Madurai district was 34.4%. Incidence of mycotic corneal ulcers were found to be 7.3% in North India, 32% in East India,38.9% in West India and 32% - 39.8% in South India. This regional variation could be because fungal keratitis is expected to be more common in the tropical and subtropical regions than in the temperate regions.

Clinical features[16]

Fungal keratitis present with insidious onset gradually progressive loss of vision, pain , photophobia and manifest with a grayish white or yellowish white epithelial defect and zone of infiltration ; soft, creamy and raised exudates ; hyaphate and feathery margins, rough-dry texture, thick yellowish-white immobile hypopyon. Special features like immune ring, endothelial plaque, posterior corneal abscess and satellite lesions are hallmark of fungal keratitis.

Specific fungi[16]

Dematiacious Fungi

Brown pigmentation is a key feature to these type of fungi due to melanin metabolism alteration. Other features are superficial keratitis , low virulence of organism and less anterior chamber reaction. Intact epithelium with deep stromal Infiltrate may also be a presentation in fungal keratitis.

Fusarium species

Severe keratitis with deep extensions and early perforation are features of fusarium solani keratitis.

Aspergillus species

Aspergillus fumigates, flavus and niger are common species associated with moderate type of keratitis and responsive to antifungal agents.

Yeast

“collar button” central corneal ulcer is typical of candida albicans. These ulcers are small to begin with and rapidly increases in size with expanding deep stromal infiltrate mostly seen in eyes with previous corneal diseases, patients with immunosuppresion and Diabetes mellitus.

Parasitology[62]

CLASSIFICATION OF MEDICALLY IMPORTANT PARASITES

1.PROTOZOA	**2.HELIMINTHS(METOZOA)**
Sarcodina (Amoebae)	***Platyhelminthes***
(a) Genus, Entameba:	**Trematodea:**
E.g. Entameba histolytica	**(a) Genus Schistosoma**
(b) Genus Endolimax	**E.g. S. mansoni**
E.g. Endolimax nana	**(b) Genus Fasciola**

(c) Genus Iodameba

E.g. Iodameba butchlii

(d) Genus Dientmeba

E.g. Dientameba fragilis

Mastigophora (Flagellates)

(a) Genus Giardia

E.g. G. lamblia

(b) Genus Trichomonas

E.g. T. vaginalis

(c) Genus Trypanosoma

E.g. T. brucci

(d) Genus Leishmania

E.g. L. donovani

Sporozoa

(1) Genus Plasmodium

E.g. P. falciparum

(2) Genus Toxoplasma

E.g. T. gondi

E.g. F. hepatica

Cestoda:

(a) Genus Diphylobotrium

E.g. D. latum

(b) Genus Taenia

E.g. T. saginata

(c) Genus Echinococcus

E.g. E. granulosus

(d) Genus Hymenolepsis

E.g. H. nana

Nemathelminthes:

(a) Intestinal Nematodes

E.g. A. lumbricoides

(b) Somatic Nematodes

E.g. W. bancrofti

(3) Genus Cryptosporidum

E.g. C. parvum

(4) Genus Isospora

E.g. I. beli

Ciliates

E.g. Balantidium coli

Acanthemeba[63]

Acanthamoeba is an ubiquitous free-living protozoa of the subphyla Sarcodina because of the wide spread distribution of Acanthamoeba, human contact with the organism is inevitable and frequent. Most common cause of keratitis asoociated with contact lens wear.

Life cycle [16,62]**:**

Acanthamoeba organisms exist in two forms, the active trophozoite(14-40μ) form and the resting cystic(12-16μ) form. The cystic form is dormant and is resistant to desiccation, extreme temperature and chemicals. The cysts do not exhibit any movements and may remain viable for many years until they are exposed to a food source, where they again assume the trophozoite form. Humans may come to contact with the Acanthamoeba while swimming in lakes, swimming pools, in seawater and through the mud from fresh water and salt water falling into the eye. Direct exposure may incorporate the amoeba into the cornea and may lead to corneal infection.

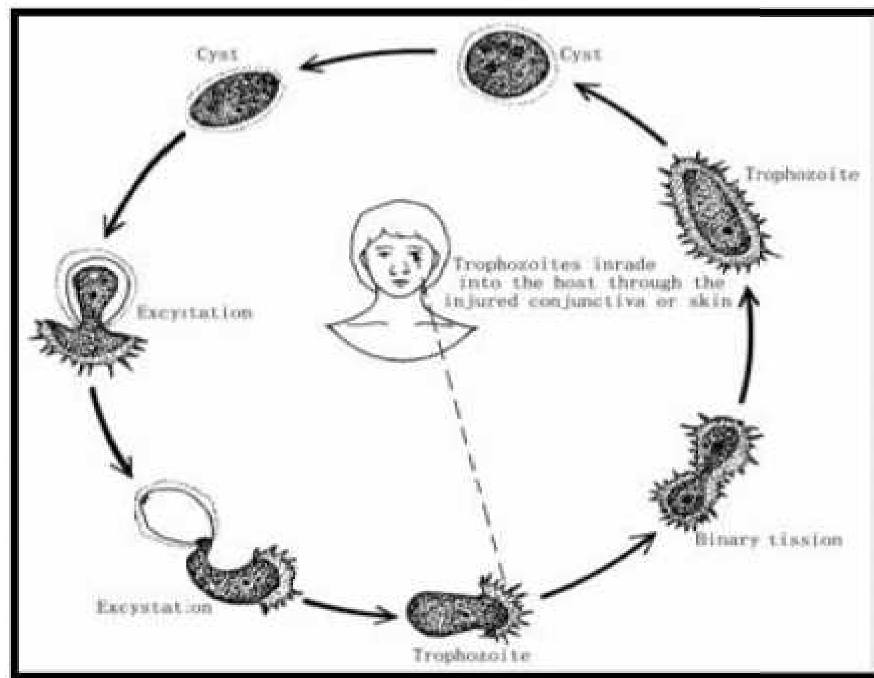


Figure 3. Life cycle of Acanthamoeba species[62]

Epidemiology of Acanthamoeba Keratitis

The incidence of Acanthamoeba keratitis was found to be 0.34 to 1.4% in various Indian studies. Risk factors are mainly contact lens use, contaminated water and homemade solutions for contact lenses and corneal trauma.[16]

In UK and Hongkong incidence of Acanthamoeba keratitis was estimated to be 0.33 per 10,000 in hydrogel contact lens wearers per year[50].

Out of 5897 keratitis patients, reterospective 10 year study at L.V. Prasad Eye Institute, Hyderabad, India, suggested that *Acanthamoeba* keratitis accounted for 2.4% cases and major risk factor is contact lens wear[47].

S. Basak et al reported incidence of *Acanthamoeba keratitis* (0.49%) in a study of 1198 patients of West Bengal in year 2001-2003 and showed that most common risk factor is ocular injury[49].

Clinical features[63,16] : Severe-moderate pain, decreased vision, redness, ocular irritation with foreign body sensation , severe photophobia due to irritation of corneal nerves, mucous discharge and lacrimation. Cornea shows epithelial stippling with microcystic edema. Coarse-opaque streaks with fine epithelial-subepithelial opacities and dendritiform lesions. Moore et al described radial keratoneuritis is a presenting sign is characterized by linear, radial, branching infiltration along long corneal nerves in stroma , it begins paracentrally and extend to limbus in a radial pattern.

LABORATORY DIAGNOSIS OF MICROBIAL KERATITIS

A complete laboratory workup is very essential owing to considerable overlap in the clinical appearance of corneal ulcers due to various microorganisms.[32]

The first step is to obtain samples from eyelids and conjunctiva using calcium alginate swabs moistened with nutrient broth of both the eyes (infected and non-infected).[32]

Corneal scraping from the ulcer area is performed, under local anaesthesia; Ulcer is scraped from the leading edge and base, under magnification (binocular loupe, slit lamp, operating microscope) using a blunt cataract knife, Beaver blade No. 64, Bard parker blade No. 15.[20,33,34] A prospective comparative evaluation of Bard parker blade No. 15 and calcium alginate swab for collecting the corneal material, showed that there is no significant difference in microbial yield between the two methods[36] contrary to that reported earlier.[37]

Material is "C-streaked" on culture plates. These include fresh blood agar, chocolate agar and sabouraud's agar media. The scraped material is also smeared on two glass slides, one for KOH wet mount and the other for Gram's stain.[20,33-35] If the patient is already on treatment then stop treatment 12 hours prior to culture to enhance recovery of organism. Beyond its diagnostic value, corneal scraping may accelerate disease resolution by enhancing antibiotic penetration and therapeutic debridement of necrotic tissue.[17,24]

In case of contact lens keratitis, contact lens, case and solution should also be sent for microbiologic examination.[28]

Microbial cultures are considered significant when there is growth of the same organism on two or more media, confluent growth at site of inoculation on one solid medium, growth in one medium with consistent direct microscopy findings or growth of the same organism on repeated corneal scraping.[20,33-35]

If infectious keratitis is suspected clinically and twice repeated microscopic evaluation of smears and culture results are negative and no clinical improvement is noted on the initial broad spectrum antibiotic therapy, corneal biopsy is recommended. The biopsied tissue is bisected, half being sent to microbiology laboratory for homogenization and culture, and the remaining half is placed in 10% buffered formalin to be transported to a pathology laboratory.[32]

Morphology of bacteria on Gram's stain[31]

- *Staphylococcus***:** consists of gram positive cocci arranged in grape like clusters.
- *Streptococcus***:** it consists of gram positive cocci arranged in chains or pairs.

- *Haemophilus:* it consists of gram negative , rod shaped cells
- *Pseudomonas:* consists of non fermentative gram negative rods.
- *Enterococci:* consists of gram positive cocci arranged in chains or pairs.
- *Corynebacterium diphtheria:* gram positive rods
- *Listeria monocytogenes:* gram positive bacilli
- *Bacillus:* gram positive rods
- *Neisseria and Brahnmella:* gram negative cocci with bean shaped diplococcic, 0.6-1 micrometer in size
- *Enerobacteriaceae(E.*coli, klebsiella, proteus, salmonella, shigella and yersinia) : Gram negative bacilli
- *Bacteriodes and fusobacterium:* gram negative bacilli

Colony characterstics of bacteria[31]

- *Staphylococcus aureus:* typically small colonies, often less than 1mm diameter.
- *Streptococcus pneumonia:* frequently cultured on blood agar. Some species can break down erythrocytes to produce a clear zone around the colonies.
- *Haemophilus influenzae:* these organisms grow on enriched chocolate agar.
- *Pseudomonas aeruginosa*: several species secrete soluble pigments, some of which are only fluorescent and may be seen with the aid of ultraviolet lamp.
- *Enterococci*: the colonies are similar to Streptococcus.
- *Neisseria species* : translucent colonies appear on modified Thayer martin medium after 48 hours of inoculation

- *Corynebacterium diphtheria*: grow on liquid medium like serum broth and loeffler's serum slope ; solid media like blood tellurite agar, Mcleod's media and Hoyle's media in 18-24 hours.
- *Diphtheriods*: grow on ordinay media like blood agar and chocolate agar
- *Bacillus* : colonies are 2-3mm in diameter, grayish white, raised with frosted appearance on non nutrient agar and non-hemolytic colonies in blood agar.
- *Salmonella*: They grow on ordinary culture media and in MacConkey's agar and produce small, circular, translucent and colorless colonies at 37˚celcius.
- *Shigella*: 2mm diameter, circular, convex, smooth and translucency colonies on MacConkey's agar.

Chocolate agar is used for growing fastidious bacteria, such as *Haemophilus influenzae, Moraxella* species, *Neisseria gonorrhoeae* and *Streptococcus pneumoniae*.

Morphology of fungi[31]

Fungal cells are made up of chitin, glucans, mannans and ergosterol which are lysed by KOH 20% stain. They are seen in capsulated cyst like cells or hyphaete structures with branching patterns and conidiospores at terminal ends.

Mucor: ribbon like nonseptate hyphae

Cephalosporium: septate fungi with unbranched tapering conidiophores with elliptical conidias

Fusarium: septate mycelium with single branching conidiophores with ball shaped microconidia

Penicillium: septate mycelium and flask shaped phalides.

Aspergillus species: non pigmented mycelium 3-5 microns in diameter with dichotomous branching and irregular outline

Candida species: spherical or ovoid budding cells of 2×5 microns in size with pseudohyphae and blastospores

Culture characteristics of fungi[31,64]

Culture plates like SDA, blood agar and chocolate agar are inoculated with corneal scraping and read after 12-24 hours for following features of growth to identify the species of fungi.

- Form - circular, filamentous, etc.
- Size – The diameter of the colony. Tiny colonies are referred to as punctiform
- Elevation
- Margin/border
- Surface - smooth, glistening, rough, wrinkled, or dull.
- Opacity - transparent (clear), opaque, translucent
- Colour - (pigmentation)

Aspergillus flavus : colonies grow on BA and SDA by 48 hours. But the typical yellowish green granular colonies seen on SDA on Day 2 are not seen on BA until Day 5[64].

Penicillium species: they grow rapidly on SDA at room temperature with velvety white appearance later turn into powdery and blue green.

Fusarium species: grow at room temperature with woolly appearance and later become orange or lavender coloured.

Cephalosporium: it grows on SDA at room temperature and produce white, grey or rosy colonies.

Mucor : it grows on SDA not containing cycloheximide as a constituent produce growth of nonseptate hyphae in 3-4 days.

Morphology of Acanthamoeba[31,16]

Acanthamoeba can be identified by different staining methods like Hematoxylin and eosin, Gram's stain, Giemsa-Wright, Calcoflour white, Methylene blue, Congo red , Janus green, Lugol solution , acridine orange and KOH stain.

Type of stain	*Staining of Trophozoite*	*Staining of Cyst*
Giemsa wright	**purple**	**purple**
Calcoflourwhite	**Red brown**	**Apple brown**

Bharathi *et al* [33], concluded that a KOH smear is of greater diagnostic value in the diagnosis of fungal keratitis, *Nocardia* keratitis and *Acanthamoeba* keratitis

Corneal ulcers already treated with antibiotics show growth of Gram-negative bacteria, fungi and *Acanthamoeba* more commonly than fresh, non-treated cases as reported by Rodman *et al* [66].

Culture characterstics of Acathamoeba

NNA with E.coli / Aerobacter aerogenes/ Klebsiella pneumonia/ Xanthomonas maltophilia is a growth medium for Acanthamoeba. Growth occurs within 1-3 days. The incubated nonnutrient agar plates (37 degree C) are screened daily for signs of *Acanthamoeba* growth under the low-power (X 10) objective using a light microscope. Amoebal migration tracks, bacterial clearing show where the amoeba have been grazing and amoebal trophozoites or cysts were considered to be positive for *Acanthamoeba* growth.[16]

SDA inoculated at 27ºC and 5% sheep blood agar at 37ºC are also inoculated for its optimal growth.

OTHER INVESTIGATIVE MODALITIES

Anterior chamber paracentesis[51]

Anterior chamber (AC) paracentesis is done when there is strong clinical suspicion of endophthalmitis. In addition, progressive corneal damage and persistent hypopyon are also indicative of this procedure. The aspirate is collected with the help of sterile tuberculin syringe and 22 gauge needle. The AC is tapped via the limbus. The needle should be removed before the specimen is submitted in order to decrease the danger to laboratory personnel. However, the nozzle of the syringe should be sealed with a sterile rubber bung and the whole set should be transported immediately to the laboratory for processing.

Corneal biopsy[51]

When the culture of scrapings of a progressive, non-responding corneal ulcer is negative, histological examination of biopsy specimen is indicated. Superficial keratectomy or corneal biopsy specimen may be obtained by a trephine or a sharp blade by free lamellar dissection for immunohistochemical and light microscopic examination. This approach is especially useful for the detection of fungi and *Acanthamoeba* in deep ulcers and could be excisional or incisional. Excisional biopsy is performed for peripheral lesions while incisional biopsy is done in cases where the visual axis is spared. A 1-mm margin of macroscopically uninvolved tissue should be included, where possible, to ensure that the active edge is sampled.

Confocal microscopy[51]

In vivo confocal microscopy provides a real-time and non-invasive method for identifying corneal pathogens in early stages of infection. Compared to the slit-lamp, confocal microscopy offers better resolution and contrast. Thus, confocal microscopy can be used as a complement to slit-lamp biomicroscopy in diagnosis of the cause of infectious keratitis. Images of fungal structures are obtained immediately and allow early treatment to be started, before laboratory investigations conclude on the definitive diagnosis. They also play a useful role in rapid, non-invasive, *in vivo* detection of *Acanthamoeba* cysts and trophozoites in the cornea and in monitoring the efficacy of amebicide treatment.

Polymerase chain reaction[51]

Polymerase chain reaction (PCR) has been shown to detect small amounts of microbial DNA and hence improve therapeutic efficacy. Direct PCR amplification

and sequencing of bacterial genes encoding the small subunit of ribosomal RNA (16S rDNA) without prior cultivation allow the identification of fastidious or unculturable bacteria. PCR is a promising technique as a means to diagnose fungal keratitis , viral and acanthamoeba keratitis.

TREATMENT

Bacterial Keratitis

The objective of therapy in bacterial keratitis is to rapidly eliminate the infective organism, reduce the inflammatory response, prevent structural damage to the cornea, and promote healing of the epithelial surface.[17]

Until the results of the definitive cultures are available, Gram's stain is a quick and helpful tool for initiating a rational antibiotic therapy. Gram's stain may identify pathogen in upto 75% of the mono bacterial keratitis and 37% of the polybacterial cases.[16,17] If the gram stain is equivocal or there is uncertainty in interpretation of diagnostic smears, broad spectrum antibiotic coverage should be initiated in the initial treatment of all cases of severe suppurative microbial keratitis.[17,30] The initial management of cases of bacterial keratitis includes the use of medical therapy.

Medical management

A study[32] identified two different approaches in the initial treatment of microbial keratitis. The 'Shotgun therapy' in which the choice of a combination of fortified antibiotics, used as intensive initial therapy based on local epidemiological information regarding likely infecting organisms and the 'Specific therapy', in which intensive treatment with a single antibiotic is directed by the results of the

microbiological investigation. With shotgun therapy, complete antibiotic cover is not possible and treatment toxicity is more likely, whereas specific therapy risks disease progression if microbiological investigations are incomplete or misleading. A compromise approach is often adopted in which shotgun therapy is continued until clear evidence of the infecting agent and its antimicrobial sensitivities emerges to direct specific therapy.

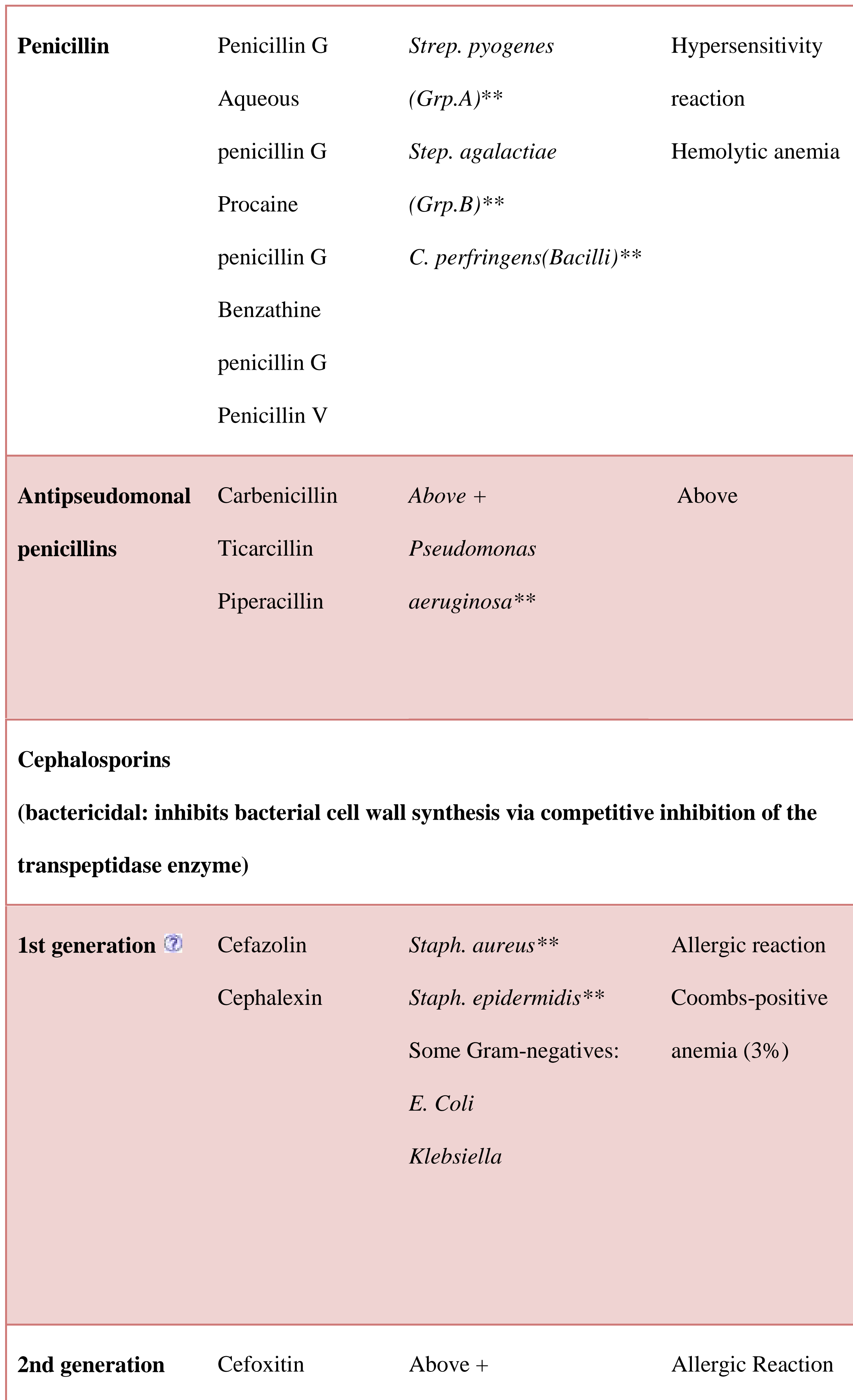
- Combination therapy: Cephalosporins + aminoglycosides etc
- Monotherapy: 2nd ,3rd and 4th generation Fluoroquinolones, Chloremphenichol etc. are braod spectrum antibiotics.

Table 2. Classification of antibiotics based on mechanism of action

Antibiotic Grouping By Mechanism of Action[67]	
Cell Wall Synthesis	Penicillins Cephalosporins Vancomycin Beta-lactamase Inhibitors Carbapenems Aztreonam Polymycin Bacitracin
Protein Synthesis Inhibitors	<u>Inhibit 30s Subunit</u> Aminoglycosides (gentamicin) Tetracyclines

	<u>Inhibit 50s Subunit</u> Macrolides Chloramphenicol Clindamycin Linezolid Streptogramins
DNA Synthesis Inhibitors	Fluoroquinolones Metronidazole
RNA synthesis Inhibitors	Rifampin
Mycolic Acid synthesis inhibitors	Isoniazid
Folic Acid synthesis inhibitors	Sulfonamides Trimethoprim

Inhibits Cell Wall Synthesis			
Penicillins **(bactericidal: blocks cross linking via competitive inhibition of the transpeptidase enzyme)**			
Class/Mechanism	*Drugs*	*Indications (**Drug of Choice)*	*Toxicity*



Penicillin	Penicillin G Aqueous penicillin G Procaine penicillin G Benzathine penicillin G Penicillin V	*Strep. pyogenes* *(Grp.A)*** *Step. agalactiae* *(Grp.B)*** *C. perfringens(Bacilli)***	Hypersensitivity reaction Hemolytic anemia
Antipseudomonal **penicillins**	Carbenicillin Ticarcillin Piperacillin	*Above +* *Pseudomonas* *aeruginosa***	Above
Cephalosporins **(bactericidal: inhibits bacterial cell wall synthesis via competitive inhibition of the transpeptidase enzyme)**			
1st generation	Cefazolin Cephalexin	*Staph. aureus*** *Staph. epidermidis*** Some Gram-negatives: *E. Coli* *Klebsiella*	Allergic reaction Coombs-positive anemia (3%)
2nd generation	Cefoxitin	Above +	Allergic Reaction

	Cefaclor Cefuroxime	Gram-negative	
3rd generation	Ceftriaxone Cefotaxime Ceftazidime Cefepime (4th generation)	Above + Gram-negative *Pseudomonas*	Allergic Reaction
Other Cell Wall Inhibitors			
Vancomycin (bactericidal: disrupts peptioglycan cross-linkage)	Vancomycin	MRSA** PCN/Ceph allegies** *S. aureus* *S. epidermidis*	Red man syndrome Nephrotoxicity Ototoxicity
Beta-lactamase Inhibitors	Clavulanic Acid Sulbactam Tazobactam	*S aureus*** *S epidermis*** *E.Coli*** *Klebsiella***	Hypersensitivity Reaction Hemolytic anemia
Carbapenems	Imipenem (+ cilastatin) Meropenem Doripenem Ertapenem	Broadest activity of any antibiotic (except MRSA, Mycoplasma)	
Polymyxins	Polymyxin B Polymyxin E	Topical Gram-negative infections	

Bacitracin	Bacitracin	Topical Gram-positive infections	
Protein Synthesis Inhibition			
Anti-30S ribosomal subunit			
Aminoglycosides (bactericidal: irreversible binding to 30S)	Gentamicin Neomycin Amikacin Tobramycin Streptomycin	Aerobic Gram-negatives *Enterobacteriaceae* *Pseudomonas*	Nephrotoxicity Ototoxicity
Tetracyclines (bacteriostatic: blocks tRNA)	Tetracycline Doxycycline Minocycline Demeclocycline	*Rickettsia* *Mycoplasma* *Spirochetes* (Lyme's disease)	Hepatotoxicity Tooth discoloration Impaired growth Avoid in children < 12 years of age

Anti-50S ribosomal subunit			
Macrolides (bacteriostatic: reversibly binds 50S)	Erythromycin Azithromycin Clarithromycin	*Streptococcus* *H. influenzae* *Mycoplamsa pneumonia*	Coumadin Interaction (cytochrome P450)
Chloramphenicol	Chloramphenicol	*H influenzae*	Aplastic Anemia

(bacteriostatic)		Bacterial Meningitis Brain abscess	Gray Baby Syndrome
DNA Synthesis Inhibitors			
Fluoroquinolones **(bactericidal: inhibit DNA gyrase enzyme, inhibiting DNA synthesis)**			
1st generation	Nalidixic acid	*Steptococcus* *Mycoplasma* *Aerobic Gram +*	Phototoxicity Achilles tendon rupture Impaired fracture healing
2nd generation	Ciprofloxacin Norfloxacin Enoxacin Ofloxacin Levofloxacin	As Above +*Pseudomonas*	as above
3rd generation	Gatifloxacin	As above + Gram-positives	as above
4th generation	Moxifloxacin Gemifloxacin	As above + Gram-positives + anaerobes	as above

Table 3. Classification of antifungals based on mechanism of action[67]

Polyenes(break fungal cell wall by increasing permeability act on ergosterol)	**Large polyenes** **Nystatin, Amphoteracin B 0.15%** (Candida, Cryptococcus, Histoplasma, Aspergillus) **Small polyenes** **Natamycin 5%**(Aspergillus,Fusarium,Candida)
Azoles	**Imidazoles** (Candida, Aspergillus,Fusarium,Cephalosporium Cryptococcus) **Miconazole 1%, Ketoconazole 5%, Clotrimazole 1%** **Triazoles** (Candida, Aspergillus, Histoplasma, Blastomycosis) **Fluconazole 0.2%, Itraconazole 1%, Voriconazole 1%**
Pyrimidines	**Flucytosine 1-5% eye drops for Candida sp.**
Silver compounds	**Silver sulfadiazine for Aspergillus and Fusarium sp.**

Acanthamoeba Keratitis[70,82]

Propamidine 0.1%, Neomycin 1%, Chlorhexidine 0.02% , ketoconazole 5%, Itraconazole 1 % and Polyhexamethylene biguanide 0.02% are tried in cases of Acanthamoeba keratitis. But no drug has achieved a 100% success in treatment since

the cystic dormant phase in corneal stroma is difficult to iradicate. Furthermore Neomycin has high hypersensitivity rate hence it is no longer used nowadays. Imidazoles also act on trophozoite form of acanthamoeba so should not be used as monotherapy. Hence despite availability of drugs treatment is often unsatisfactory.

ADJUNCTIVE THERAPY[24,32]

- Topical cycloplegic agents (Atropine, homatropine, Cyclopentolate etc) administered to relieve ciliary spasm, alleviate pain and prevent the synechiae formation.

- Antiglaucoma drugs: (topical - adrenergic blocker or oral carbonic anhydrase inhibitors). They have a significant role in cases with descemetocele or impending perforation.

- Therapeutic soft contact lens: may be a useful adjunct to assist epithelial healing. Antibiotics should continue over the lens. Caution should be exercised as infection may occasionaly complicate therapeutic contact lens.

- Collagenase inhibitors like ethylene diamine tetra acetic acid (EDTA) (0.05% eye drops), Acetylcysteine 20% or heparin 2% have been shown to be effective. However they are seldom used.

- Analgesics to relieve pain.

SURGICAL MANAGEMENT[48]

Purpose of surgical treatment are…

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 where the integrity is threatened as in cases of thinning or perforation of the cornea.

The various modalities of treatment available in such cases are:

A. Removal of epithelium and anterior lamellar keratectomy

B. Conjunctival flaps

C. Tissue adhesives

D. Penetrating keratoplasty

A. Epithelial Removal and Anterior Lamellar Keratectomy

This modality of treatment is useful particularly in cases of fungal keratitis. Regular debridement of the base of the ulcer helps in elimination of organisms and necrotic material. This procedure facilitates penetration of antifungal drugs. This can be done under topical anaesthesia leaving a margin of 1 to 2 mm at the limbus with a no.15 Bard-Parker blade.

Anterior lamellar keratectomy helps in removal of the thick mat of fungal filaments on the cornea and facilitates increased drug penetration in cases of dematiaceous fungal filaments. Anterior stromal corneal infiltrates can also be ablated with excimer laser for therapeutic purposes.

B. Conjunctival Flaps

Conjunctival flaps help in achieving a stable epithelial surface in cases of persistent or recurrent epithelial defects and progressive ulceration especially in viral keratitis. These are particularly helpful in chronic peripheral disease where the flap does not encroach onto the visual axis . In peripheral fungal corneal ulcers, the blood vessels brought in by conjunctival flaps help in healing of the ulcer. A superficial lamellar keratectomy with removal of necrotic stroma is to be done with anchoring of a thin conjunctival flap over the ulcerated site.

C. Tissue Adhesives

Tissue adhesive (cyanoacrylates) helps in supporting corneal thinning and sealing corneal perforation upto 2mm. Cyanoacrylate adhesive is bacteriostatic for gram-positive bacteria .Necrotic stroma or epithelium and other debris must be removed from the base of the ulcer before the adhesive is applied. Usually a bandage contact lens must be fitted after the application. The adhesive is left in place until it loosens spontaneously, or the bed becomes vascularized or keratoplasty is performed. This modality of treatment has a valuable role in the management of infectious keratitis.

VI. D. Penetrating Keratoplasty

The indications for penetrating keratoplasty are (a) perforation, (b) descemetocele or impending perforation , (c) continued progression despite maximal medical treatment, (d) post-infectious corneal scar. The results of keratoplasty in acutely infected or inflamed eyes are relatively poor, the risk of rejection and glaucoma greater especially in larger grafts. At least 0.5 mm of clear tissue all around the infected area is to be excised to decrease the incidence of recurrence. Postoperative antimicrobial treatment

is to be continued. In fungal keratitis, postoperative topical steroids are to be used with caution. Surgery when performed with 8 mm or smaller diameter donor grafts had better results than larger grafts. Hence, penetrating keratoplasty is to be considered early when fungal ulcers do not respond to antifungal medication. The results of penetrating keratoplasty for *Acanthamoeba* keratitis are poor and surgery is to be considered only in patients with gross corneal thinning or perforation

METHODOLOGY

The present study was conducted in the Department of Ophthalmology, KLES Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre, Belgaum during the period of January 2012 to December 2012.

Study design

One year Prospective Cross sectional study

Source of Data

Patients with clinical features of microbial corneal ulcers attending Out Patient and In patient Department at Department of Ophthalmology, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum attached to Jawaharlal Nehru Medical College, Belgaum.

Method of data collection

A data collection instrument was used in which data was obtained by

1. Interview of patient
2. Hospital records
3. Clinical and Microbiological laboratory examination

Study Period

One year from January 2012 to December 2012.

Sample Size

Sample size for the study was calculated by following formula :

$$n = \frac{4pq}{d^2}$$

n = sample size

p = prevalence of major risk factor in previous studies i.e. 41.5%[83]

q = 100-p =59.5

d = absolute error i.e. 15%

sample size by this formula is 44

Selection criteria

Inclusion criteria

- All cases of clinically suspected microbial corneal ulcers (bacterial, fungal and protozoal)
- Fungal elements ,protozoa or bacteria seen on stained smear of corneal scrapings
- Growth of fungal elements, bacteria and protozoa on atleast one culture media

Exclusion criteria

- Viral keratitis
- Trophic ulcer associated with herpes zoster
- Mooren ulcer
- Chronic exposure keratitis
- Not willing to participate in the study

Procedure

Prior to the commencement of study ethical clearance was obtained from Institutional Ethics Committee. Based on the selection criteria, patients with suspected microbial corneal ulcers attending out patient department at Department of Ophthalmology, KLES Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre, Belgaum were screened for eligibility. The selected patients were briefed about the study nature and a written informed consent (Annexure I) was obtained. Data regarding demographic parameters such as age, sex, occupation, socio economic status and address were collected and detailed clinical and microbiological examination findings were recorded on predesigned and pretested proforma (Annexure II).

Ulceration was defined as a loss of the corneal epithelium with underlying stromal infiltration and suppuration associated with signs of inflammation with or without hypopyon.[20]

A detailed history was taken with special reference to;

- Name, age, sex, occupation, address, Socio-economic status of the patient
- Clinical information including duration and chronology of symptoms, previous treatment and investigations, predisposing factors and associated risk factors.
- The distant visual acuity was measured by the Snellen's chart and near visual acuity by Jaeger's chart .Complete initial ocular examination was carried out under slit lamp biomicroscope .The size of the ulcer was measured after

staining with wet sterile Sodium fluorescein 0.1% paper strip using the variable slit on the biomicroscope (coaxial retro illumination)with cobalt blue filter and recorded in millimeters, with special attention paid to the characteristics of ulcer in terms of:

- Site, size, shape, edges, margins, floor and depth of ulcer.
- Size and depth of the stromal infiltrate.
- Presence or absence of hypopyon and its measurement.
- AC reaction.
- Corneal edema, vascularisation
- Iris , pupil and lens details
- Associated risk factors such as blepharitis, dacryocystitis, dry eyes etc.

After detailed ocular examination, grading of the corneal ulcer was done using the criteria of Jones[21].

Feature	Non severe	Severe
Suppuration Area	6 mm	> 6 mm
Rate of progression	Slow, moderate	Rapid
Depth of ulcer	Superficial two-thirds	Deeper one-third
Scleral suppuration	Not present	May be involved
Perforation	Unlikely to occur	Present, imminent

Severe - when three or more of the criteria are met.

Clinical procedure

After explaining the procedure and obtaining written informed consent corneal scraping was performed under strict aseptic conditions using a sterile 15 No.(number) Bard Parker Blade.[20,33,34] The procedure was performed under magnification of a slit lamp following instillation of 0.5% Proparacaine. Scraping was taken from the base and progressive edge of the ulcer. Material obtained from scraping was inoculated onto the culture media in a "C-streaked" pattern. These media were 5 % sheep Blood agar, Chocolate agar and Sabouraud's dextrose agar without Cycloheximide.

The scraping was repeated several times using a fresh blade for each scrape. The scraped material was also smeared on two glass slides, one stained with Gram's stain and the other with 10% Potassium hydroxide (KOH) wet mount, for direct microscopic evaluation.[20,33,34] Smear reports were obtained from the laboratory as early as possible. Samples with a negative KOH wet mount result were considered to be bacterial in nature.

Technique of staining

Gram's staining[16,31]

Glass slide is pass through flame 2-3 times and allow it to cool then flood Crystal violet stain and allow it to remain on slide for 1minute. Rinse gently under tap water. Flood the slide with Gram's iodine solution for 1 minute. Rinse it thoroughly. Decolorize with decolorizer solution (alchohol acetone solution) until the colour stops running from smear. Rinse again under tap water. Flood the slide with Safranin stain and allow it to remain for 30 seconds. Rinse it with tap water. Allow it to dry. Slide is

the read under low power microscope for gram positive(purple) and gram neative (pink) bacteria, and yeast like gram positive fungi.

Principle: Crystal violet-Gram's iodine complex is formed within the cell of bacteria. Decolorizer removes this complex and hence bacteria with increased permeability of cell wall loose this complex and get stained by Safranin producing pink colour. Whereas bacteria with less permeability of cell wall will retain this complex within the cell and do not stain with Safranin impart purple colour.

10 % Potassium Hydroxide wet mount preparation(KOH stain)[16,31]

Corneal scraping was smeared on glass slide. 1-3 drops of 10% KOH solution placed on glass slide with coverslip. After a period of 10 minutes slide is observed under low and high power magnification to identify the type of fungal pathogen.

Principle: Fungal cell wall contains chitin and ergosterol and KOH lyse the cell wall by dissolving chitin and stains the fungi.

Laboratory procedures

Bacterial cultures were incubated aerobically at 37^0 C. Cultures on 5% sheep blood agar and chocolate agar were evaluated at 24 hours and 48 hours and then discarded if no growth was seen[67].

For fungal cultures materials were inoculated on to Sabouraud's dextrose agar (SDA) and incubated at room temperature at 27°C, examined daily and discarded at six weeks if no growth was present. Inoculated SDA media was observed daily for the first 7 days and on alternate days for next 7-21days for observing slow growing fungi.

Microbial cultures were considered significant only if growth of the same organism was isolated on both solid media, or there was semi-confluent growth at the site of inoculation (at least two "C" streaks) on one media with the identification of morphological characteristics of similar organism on Gram stain and KOH stain.[20,33-35] The specific identification of pathogens was based on microscopic morphology, staining characteristics and biochemical properties using standard laboratory criteria. After taking corneal scrapings patient was advised to put one drop of the broad spectrum antibiotic and antifungal medications that included 4th generation fluroquinolones and Natamycin 5% respectively.

Antibiotic sensitivity for bacterial keratitis was done using Kirby-Bauer disk diffusion method[34]. Bacterial growth is inoculated on Muller Hinton's Agar antibiotic disc like Chloramphenicol (30µg), Gentamicin (10µg), Ciprofloxacin (5µg), Lomefloxacin (10µg), Tobramycin (10µg) and Erythromycin (10µg) are applied and zone of inhibition around the disk gives an approximation of susceptibility or resistance of organism. All bacterial isolates were tested for their antimicrobial susceptibility by disc diffusion method and results of susceptibility were recorded as *Sensitive* or *Resistant* [69].

LPCB staining for isolation of fungal species[70]

Growth on fungal culture is used for lactophenol cotton blue(LPCB) staining. Lactic acid acts as a clearing agent and aids in preserving the fungal structures, phenol acts as a killing agent, glycerol prevents drying and cotton blue gives colour to the structures.

Technique : place a drop of LPCB on a clean glass slide. With a bent dissecting needle, remove small potion of colony from agar and place it in the drop of LPCB. With 2 dissecting needle gently tease apart the myecelial mass of the colony on the slide, cover with coverslip and observe under microscope under low power and high power.

For patients who were on prior antibiotic therapy, their antibiotics were stopped for a duration of 12-24 hours, and then their corneal scrapings were undertaken.

If the ulcer failed to respond to the initial therapy or showed signs of progression at the end of 72 hours, the study medication was discontinued and an alternate therapy was instituted. These outcomes were considered as treatment failure.

The patency of the nasolacrimal duct was evaluated by Lacrimal sac syringing in all cases, and patients with nasolacrimal duct obstruction underwent dacryocystectomy.

Documentation of the corneal ulcer was done by taking anterior segment photographs and using detailed schematic drawings.

Frequency of drug administration

The patients were advised to put one drop of Moxifloxacin each hour round the clock for the initial 48 hours. If a favourable clinical response was observed at Day 3, the medication was tapered. On the third day, one drop was instilled every hour by day and every two hours at night. For days four and five, one drop was instilled every two hours by day, and every four hours at night. For days six and seven, one drop was used every four hours. After Day seven, the drops were tapered

to every six hours, and stopped when clinically appropriate. If a favourable response is not seen or culture report shows a growth and organism is identified then treatment is changed based on antibiotic sensitivity.

Adjunct medications were prescribed such as cycloplegics (homatropine bromide 2%), antiglaucoma medications and oral NSAIDs.

Dosing regimen for Natamycin 5% is kept at hourly for 3 days followed by reduction of dosage to 3 hourly for 7 days and tapered over 3-4 weeks. Once a fungal species is identified combination therapy with azoles and imidazole are added. Adjunctive treatment with broadspectrum antibiotic (Chloremphenichol or Moxifloxacin) antiglaucoma medications, cycloplegics, NSAIDs and Vitamin C is continued for 1-2 weeks.

Treatment protocol for Acanthamoeba keratitis include Chlorhexidine 0.02-0.04% or Propamidine 0.1% with 4th generation fluoroquinolones to prevent secondary or concurrent bacterial infection. Both the eye drops should be put hourly for 3 days and 2 hourly for 2 weeks then tapered to 4hourly for 6-12 months.

Table 2. Definition of Outcome and Ulcer status

Ulcer status	Definition
Healing ulcer	Ulcer/infiltrate decreasing in size by 20% but not completely re-epithelialized , roundening of edges, reduced infiltration, reduced AC reaction
Treatment failure	Ulcer/infiltrate of same size at the end of 72 hours
No change in ulcer	
worsening ulcer	Ulcer/infiltrate increasing in size or evidence of complications like spread of infection, endophthalmitis, ulcer perforation and adverse drug reaction
Healed ulcer	Complete re-epithelialization with no fluorescein staining of cornea

Treatment was discontinued if the ulcer seemed to be healed early.

Criteria for favourable clinical response :[48]

1. Blunting of ulcer margin
2. Improvement in signs of inflammation
3. Reduction in infiltrate size and oedema
4. Reduction in AC reaction
5. Decreased pain and other symptoms by patient.

Statistical analysis

The data obtained was tabulated on Microsoft excel spreadsheet and analyzed. The data was expressed in terms of rates, ratios and percentages and analyzed by chi-square test and test of proportion. A probability value ('p' value) of < 0.05 was considered as statistically significant.

RESULTS

The present one year Prospective Cross sectional study was conducted in the Department of Ophthalmology, KLES Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre, Belgaum during the period of January 2012 to December 2012.

Out of 57 cases screened, 45 patients with suspected microbial (bacterial,fungal,protozoal) corneal ulcer were deemed eligible to participate in the study.

The data obtained was tabulated on Microsoft excel spreadsheet and analysed as below.

Table 6: Sex distribution

Males	38 (84.44%)
Females	07 (15.55%)
Total	45 (100%)

Majority of patients were males 38(84.44%) and females were only 7(15.55%) out of total 45 patients enrolled in the study. Males were predominant with. M:F ratio of 5.42:1 in our study.

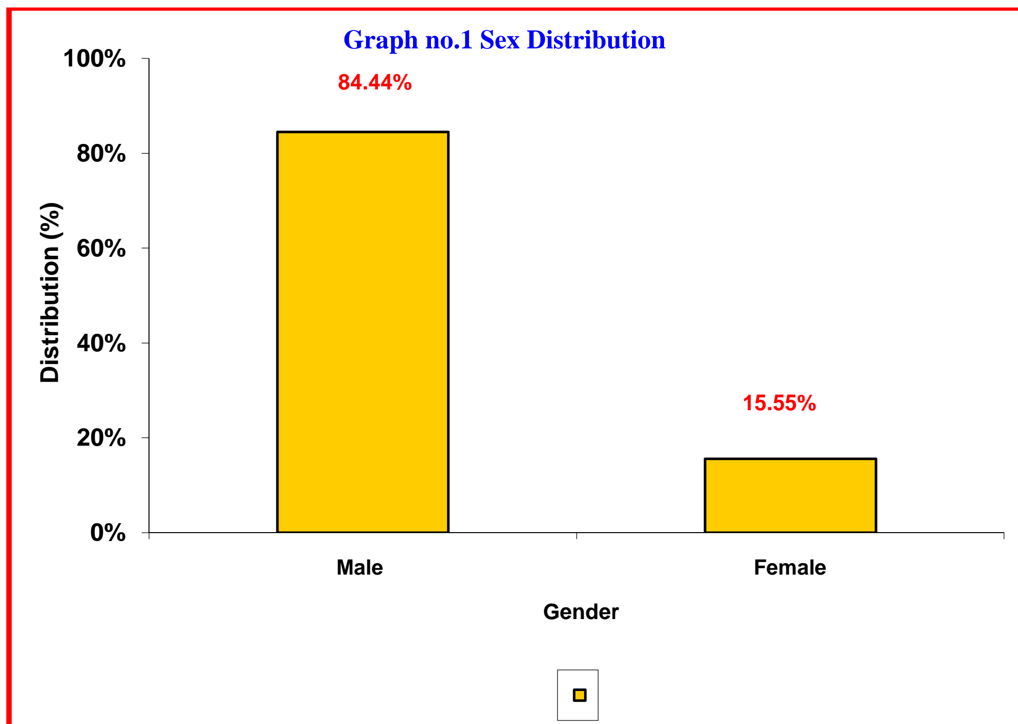
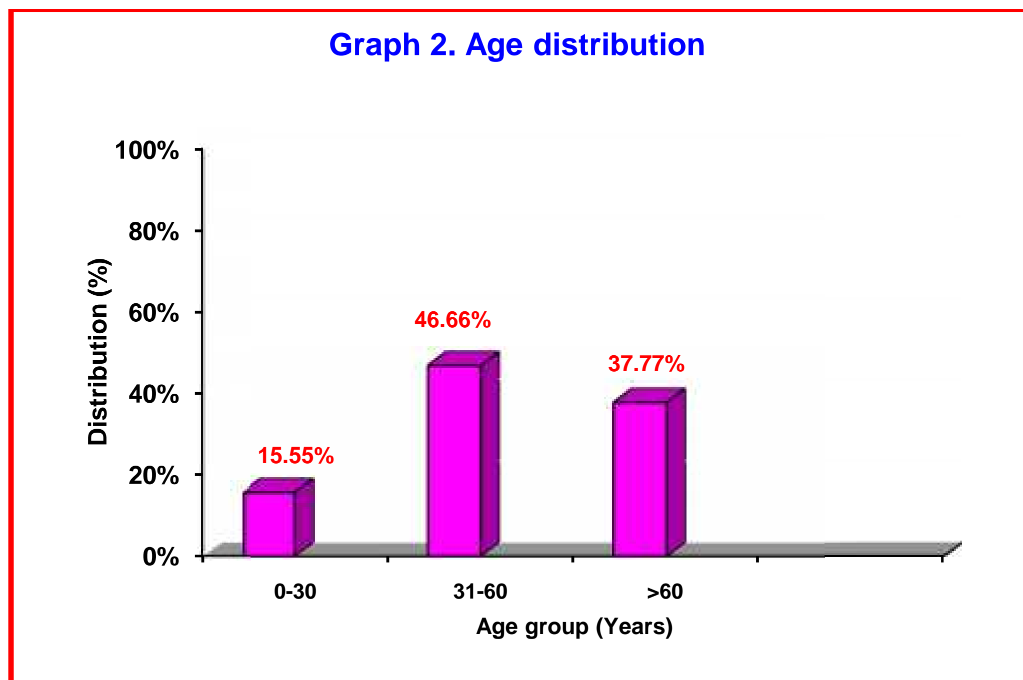


Table 7: Age distribution

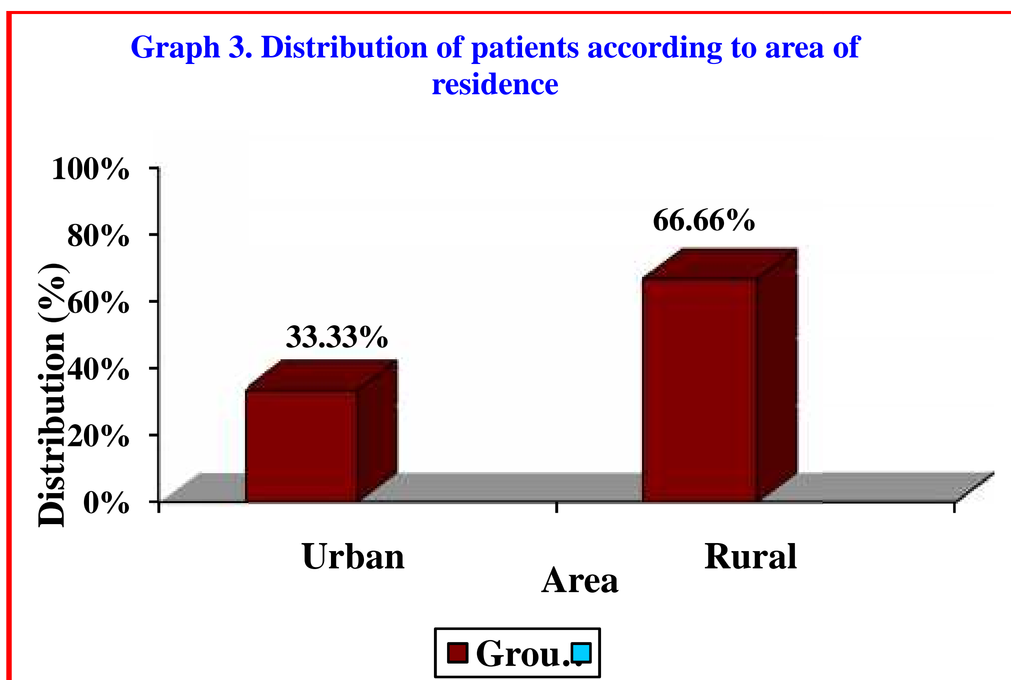
Age group	Number of individulas	Percentage
0-30	07	15.55%
31-60	21	46.66%
>60	17	37.77%
Total	45	100%



Age distribution include 15.55% patients in 0-30 age group, 46-66% patients in 31-60 years of age group and 37.77% in >60 years of age group. Mean age of presentation is 50.8 years.

Table 8. Distribution of patients according to area of residence

Urban	15	33.33%
Rural	**30**	**66.66%**
Total	**45**	**100%**

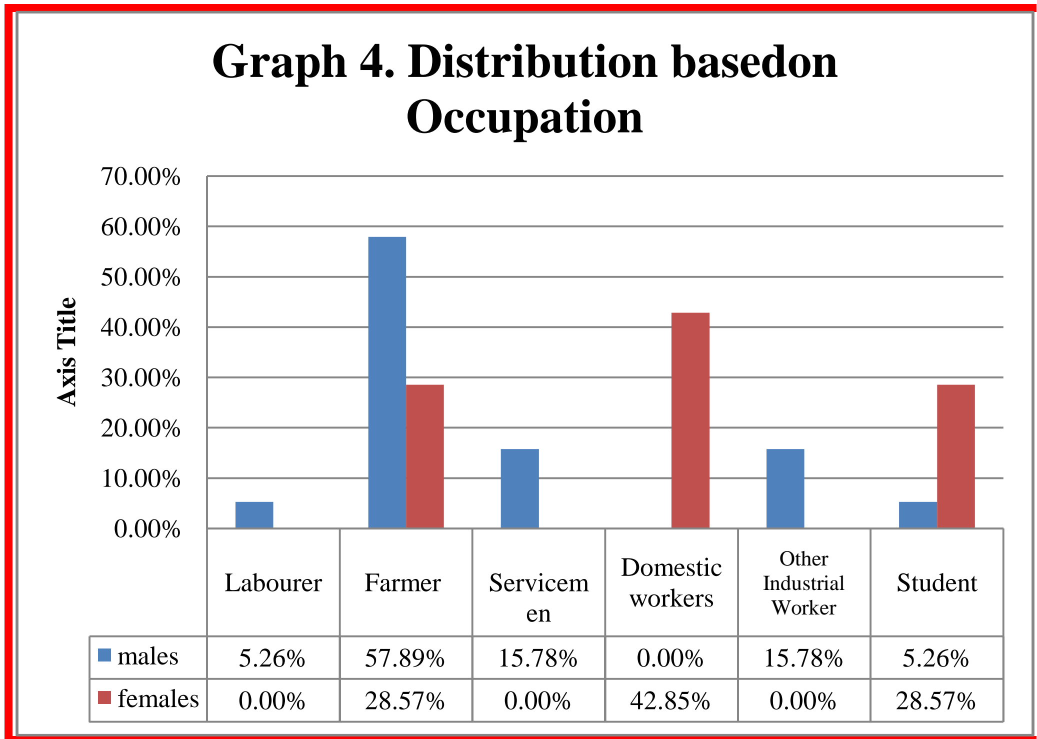


Majority of the patients were from rural population 30 (66.66%). Only 15 (33.33%) patients were from urban population.

Table 9. Distribution based on Occupation

Occupation	Males		Females	
Student	02	5.26%	02	28.57%
Domestic workes	00	0.00%	03	42.85%
Service men	06	15.78%	00	0.00%
Other industrial workers	06	15.78%	00	0.00%
Labourers	02	5.26%	00	0.00%
Farmers	22	57.89%	02	28.57%
Total	38		07	

Out of 45 patients enrolled in this study, maximum patients were farmers accounted for 24(53.33%) of the study group, males were 22(57.89%) and females were 2(28.57%). Maximum number of females were domestic workers active in household activities, they were 3(42.87%) out of total 7 females in study population.



Right eye	**31**	**68.88%**
Left eye	**14**	**31.11%**
Total	**45**	**100%**

Involvement of right eye was found in 31(68.88%) patients and left eye in 14(31.11%) patients.

Table 10. Risk factors - Ocular trauma

Traumatic injury present =27 (60%)			Traumatic injury absent = 18 (40%)		
Agricultural	Domestic	Industrial	No cause identified	Contact lens wearers	Foreign body
16	10	01	09	02	07
35.55%	22.22%	2.22%	20%	4.44%	15.55%

Out of 45 cases, patients with injury with a vegetative matter in form of seeds,leaf,stems and sticks were found to be 35.55% and considered as major risk factor associated with development of corneal ulcers.

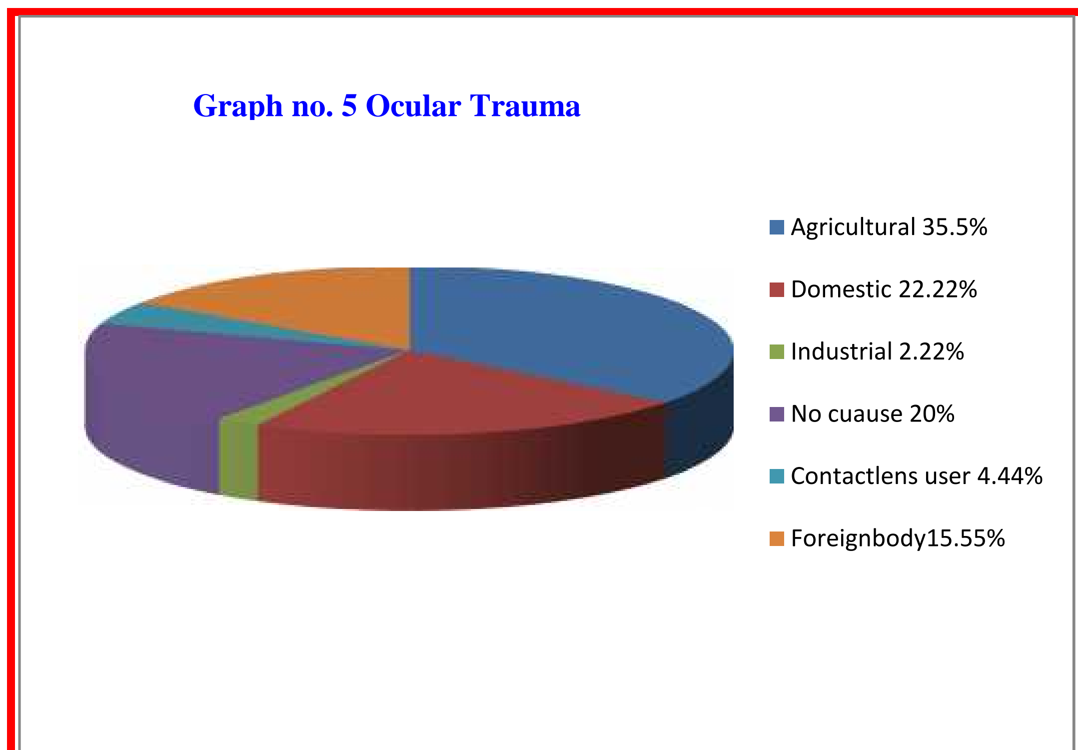


Table 11. Other risk factors

Risk factors	No.	Percentage
Lid disease	**2**	**4.44%**
Herpes zoster	**3**	**6.66%**
Retro viral disease (RVD)	**01**	**2.22%**
T2DM	**08**	**17.77%**
Dacryocystitis	**04**	**8.88%**
Leprosy	**01**	**2.22%**
No risk factor found	**26**	**57.77%**
Total	**45**	**100%**

Out of 45 cases, 17.77% cases had history of T2DM, 8.88% had concurrent Dacryocystitis who also underwent Dacryocystectomy, pasr history of Herpes zoster in 6.66%, Lid disease were found in 4.44% and 2.22% were positive for Retroviral Disease and Past history of leprosy.

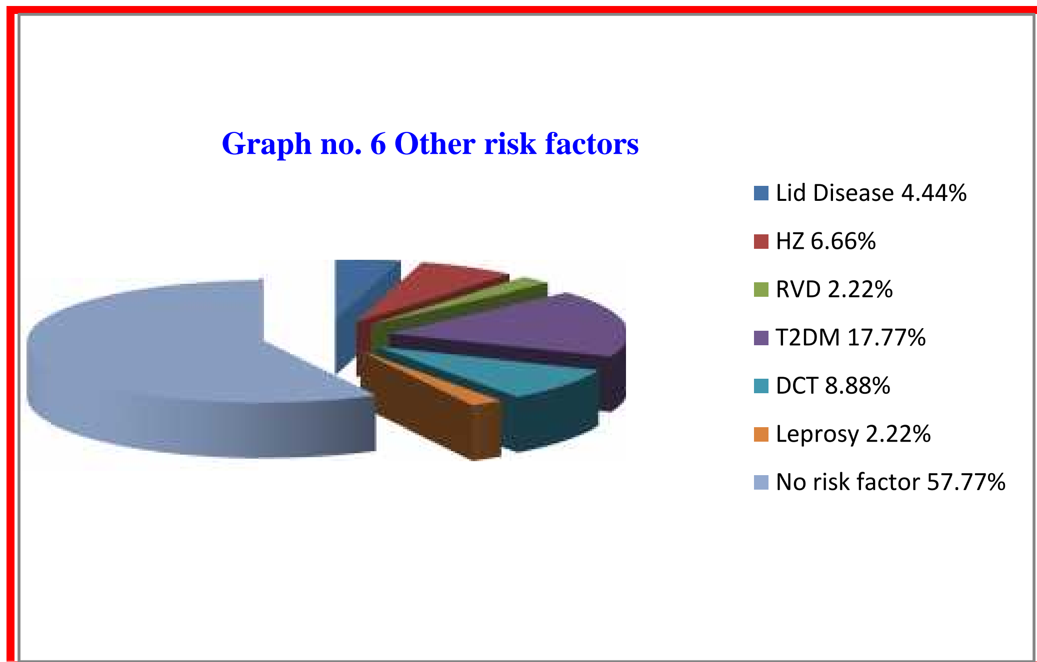


Table 12: Distribution of Symptoms at Presentation

Symptoms with which Patients were presented	No of patients	Percentage
Diminuation of vision	**40**	**88.88**
Pain	**44**	**97.77**
Photophobia	**27**	**60.00**
Watering	**18**	**40.00**
Redness	**22**	**48.88**
Discharge	**05**	**11.11**

In current study, we observed that 41/45(97.77%) patients presented with pain in and around the eye, Diminution of vision was noted in 40/45(88.88%) and Photophobia accounted for 27/45(60%) cases. History of Redness was seen in 22/45(48.88%), watering from involved eye in 18/45(40%) and discharge was noted in 5/45(11.11%) cases.

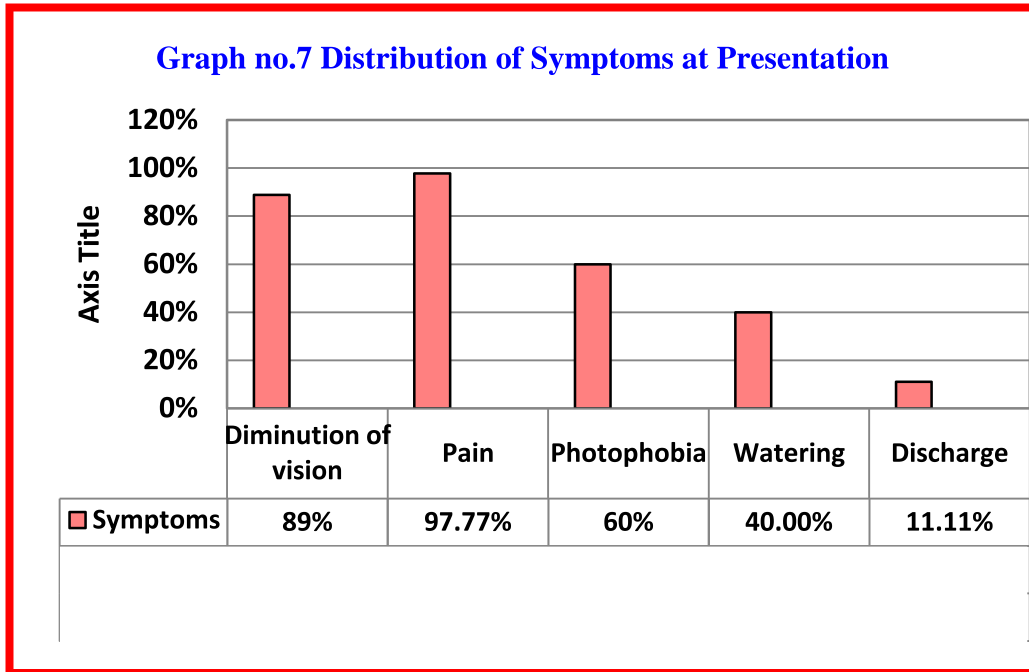
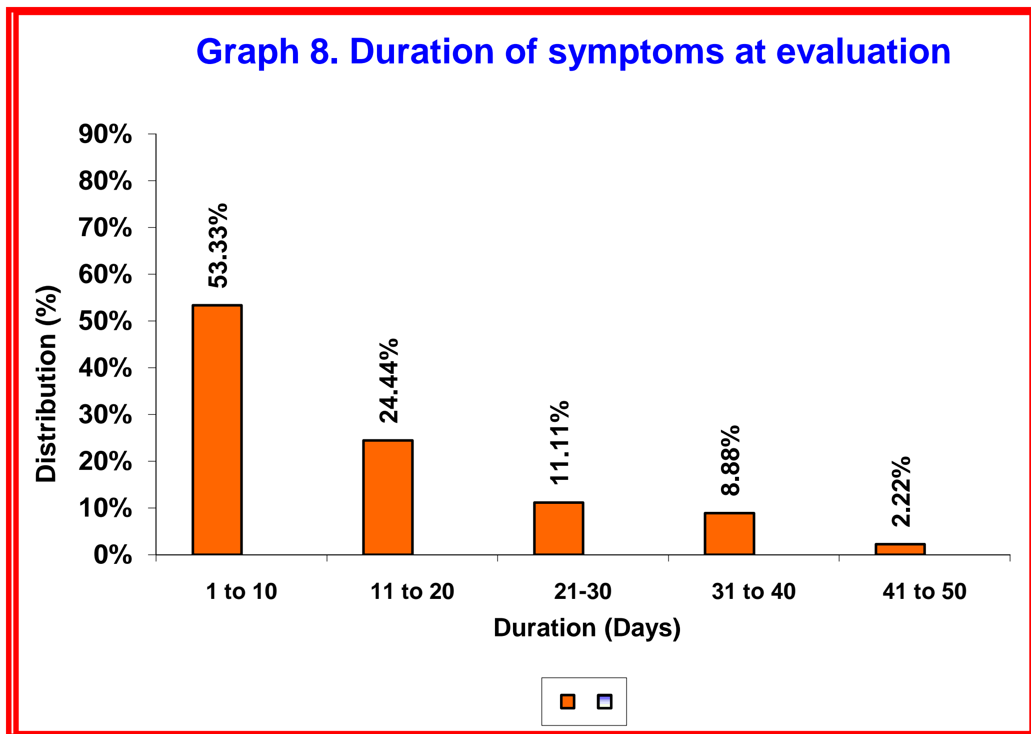


Table 13. Duration of symptoms at Presentation

Duration of symptoms before evaluation	No of patients	Percentage
0-10	24	53.33%
11-20	11	24.44%
21-30	05	11.11%
31-40	04	8.88%
41-50	01	2.22%
	Total=45	Total =100%



Majority of patients in presented during first 10 days of the onset of symptoms 24(53.33%), while 11 (24.44%) subjects presented during 11-20 days and remaining 10 (22.22%) presented after 20 days of duration.

Table 14. Visual acuity at initial presentation

Visual acuity	Total cases (n=60)	
	Number	Percent
6/5 to 6/9	1	2.22
6/12 to 6/24	7	15.55
6/36 to 6/60	10	22.22
CF to HMCF, PL +	27	60
No PL	0	0
Total	**45**	**100.00**

In the present study majority of the patients 27/45 (60%) presented with an visual acuity of CF to HMCF, PL +. While 10/45 (22.22%) presented with 6/36 to 6/60, another 7 patients (15.55%) presented with vision between 6/12 to 6/24 and 1 (2.22%) had vision ranging from 6/5 to 6/9.

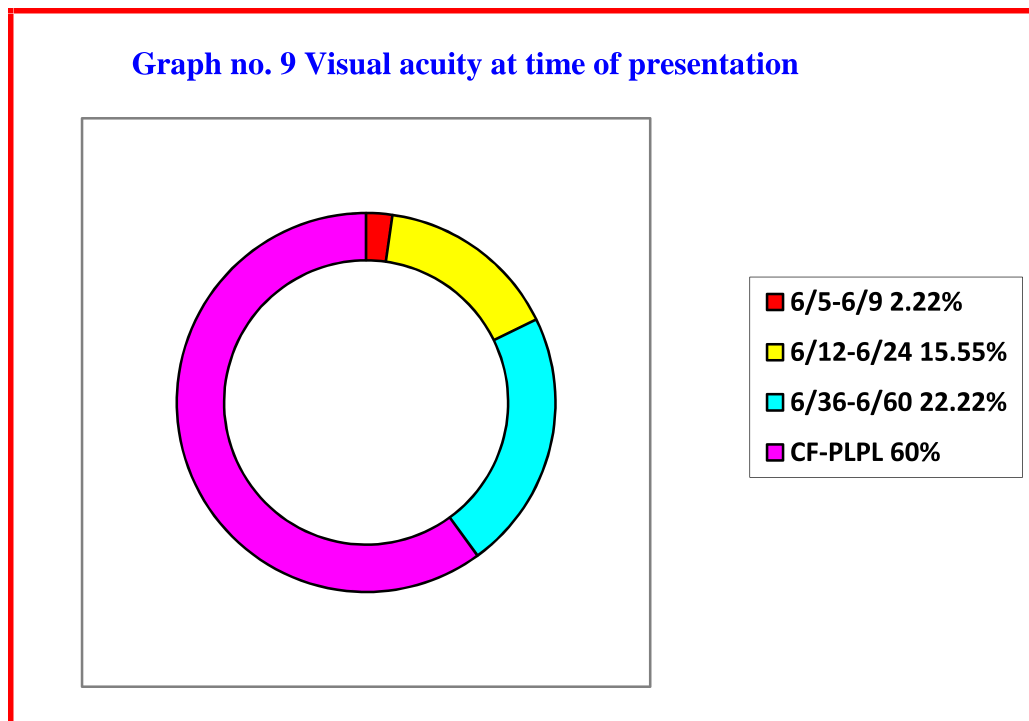


Table 15. Ulcer characteristics at time of presentation

Characteristics	(n=45)	
	No	%
Location		
Central	29	**64.44**
Peripheral	15	**33.33**
Total	1	**2.22**
Severity		
Non Severe	26	**57.77**
Severe	19	**42.22**
Vascularization	22	**48.88**
Hypopyon	21	**46.66**
Descematocele	09	**20**
Corneal thinning	**22**	**48.88**

In this study majority of ulcers were centrally located 29(64.44%) whereas 15 (33.3%) showed peripherally located ulcer. Of the 45 cases, 26(57.77%) had non severe and 19(42.22%) had severe ulceration at the time of presentation. Hypopyon was present in 21 (46.66%) cases. Vascularization was present in 22 (48.88%). Descmatocele was seen in 9(20%) cases and Conreal thinning occurred in 22(48.88%) cases.

Table 16. Gram stain

	Final diagnosis with Bacterial isolated positive	Final diagnosis with Negative Bacterial isolates
Gram test positive	**18(TP)**	**02(FP)**
Gram test negative	**4(FN)**	**21(TN)**

Final Diagnosis was considered gold standard and based on above chart sensitivity of Gram's stain was 81.81% and specificity was found to be 91.30%. Positive predictive value was 90% and Negative predictive value was 84%.

Table 17. Bacterial isolation on Culture

Characteristics	Total (n=17)	
	No	%
Gram positive organism	***13***	*76.47*
Staph epidermidis	2	**11.76**
Staph aureus	5	**29.41**
Strept. Pneumoniae	6	**35.29**
Gram negative organism	***4***	*23.52*
Pseudomonas	**4**	**23.52**

In the present study, 17/45(37.77%) of the patients were found to have positive bacterial cultures on blood agar, chocolate agar and Maconkey'agar. 13/17(76.47%) were found to be Gram positive and 4/17 (23.52%) were Gram

negative. Among Gram positive most commonly isolated organism was Streptococcus Pneumoniae 6/17 (35.29%) and among Gram negative most commonly isolated organism was Pseudomonas species 4/17(23.52%).

Table 18. KOH preparation

	Culture isolated with positive Fungi	Culture isolate with Negative fungal growth
KOH positive	**30**	**1**
KOH negative	**4**	**10**

Out of 45 cases 31 patients had fungal species isolated on culture hence sensitivity of KOH preparation was 88.25% from above chart. Specificity of KOH preparation was 90.90%. Posisitve predictive value was 96.77% and negative predictive value was 71.42%.

Table.19 Fungal Species isolated on Culture

Fungal species	No.(n=30)	Percentage
Aspergillus Sp	12	40
Fusarium Sp	7	23.33
Aureobassidium sp	1	3.33
Cladosporium sp	1	3.33
Alternaria sp	1	3.33
Curvularia sp	2	6.66
Bipolaris sp	1	3.33
Candida sp	3	10
Penicillium sp	1	3.33
Zygomycetes sp	1	3.33

Table.20 KOH and Fungal growth

	BA,CA	BA,SDA	CA,SDA	N	SDA	Total
KOH N	0	0	0	15	0	15
KOH Y	1	2	1	0	26	30
Total	1	2	1	15	26	

Kappa stastics = 1

Out of 45 cases 30 patients had fungal isolates on chocolate agar, Blood agar and Sabroud's dextrose agar and accounted for 30/45(66.66%) cases of microbial corneal ulcers. Majority of patients had Aspergillus sp positivity with 12/30(40%) followed by Fusarium 7/30(23.33%). Kappa stastics 1 denotes that there is total agreement between KOH preparation and fungal isolation by culture.

Table.21 Clinical diagnosis and Final diagnosis

	Final bacterial	Final fungal	Final mixed	Total
Clinical Bacterial	**6**	**4**	**4**	**14**
Clinical fungal	**1**	**19**	**3**	**23**
Clinical mixed	**4**	**2**	**2**	**8**
Total	**11**	**25**	**9**	**45**

Kappa statistics = 0.338 **p=0.002**

Final diagnosis was made on microbiological investigations and response to the treatment and found that there is a positive correlation between clinical and final diagnosis which is statistically significant P= 0.002

Table.22 Antibiotic sensitivity

	Staph.aureus	**Staph.epidermidis**	**Strepto pneumoniae**	**Pseudomonas**
Ciprofloxacin	S	S	S	S
Ampicillin	R	R	S	R
Chloremphenicol	S	S	S	R
Gentamycin	R	R	R	S
Tetracycline	R	R	S	S
Imipenam	-	-	S	S
Vancomycin	S	S	R	-
Cefazolin	S	S	S	S
Piperacillin	-	-	S	S
Erythromycin	S	S	S	R

Isolated bacterial organisms were tested for antibiotic sensitivity and results concluded that all organisms were sensitive to Ciprofloxacin group of drugs.

DISCUSSION

Microbial keratitis is a serious sight threatening disease and an important cause of corneal blindness in India[83].

In our study, majority of patients were males 38(84.44%) and females were only 7(15.55%) out of total 45 patients enrolled in one year period from January 2012 to December 2012. Males were predominant with M:F ratio of 5.42:1 in our study.

Similar results were seen in a study conducted by Srinivasan et al[20] in which 61.52% were males and 38.47% females, with male to female ratio 1.6:1 Earlier studies also show a male : female ratio ranging from 1:1 to 3:2.[72-74] . Male predominance was seen presumably because they are more physically active and undertake more outdoor activities and hence predisposed for external injury and so were at a higher risk of developing corneal ulcers. SK Basak[49] et al also reported a study of 1198 patients concluded that 846 (70.6%) patients were males and 352 (29.4%) were females ($p< 0.0001$) which is in association with our study. Of the 5897 cases of infectious keratitis, 4087 (69.3%) were males and 1810 (30.7%) were females, the overall male to female ratio of patients being 2.25:1 in a study conducted by Gopinathan[47] et al.

In our study age distribution include 15.55% patients in 0-30 age group, 46.66% patients in 31-60 years of age group and 37.77% in >60 years of age group and mean age of presentation is 50.8 years. Similar findings have been reported in a study conducted by Upadhyay MP [57]et al with 50 % of the patients presented with more than 50 years of age.

In our study majority of the patients were from rural population 30 (66.66%) an only 15 (33.33%) patients were from urban population. Other studies are also in support with this observation. SK Basak[49] et al also reports similar results in which most of the patients (941; 78.5%) were from rural areas (P< 0.0001) in the study.

Out of 45 patients enrolled in this study, maximum patients were farmers accounted for 24(53.33%) in the study group, males were 22(57.89%) and females were 2(28.57%). Maximum number of females were domestic workers active in household activities accounted for 3/7(42.87%). Labourers were 5.26% , servicemen and other industrial workers were 15.78%. Majority 32 (53.3%) of the patients were agricultural workers and daily wage earners, an occupation profile similar to South Indian study (66.8%)[20] as well as a study by Upadhyay (53.1%) [57] and SK Basak [49]et al (691; 57.6%). Bharathi[33] et al also reported similar results, with majority of the patients (709; 64.75%) were farmers (P<0.0001) in their study.

Involvement of right eye was found in 31(68.88%) patients and left eye in 14(31.11%) patients in our study.

Traumatic injury was found as a risk factor in 27/45(60%) cases and non-traumatic cases were 18/45(40%) out of which; agricultural injury with seeds, stone, mud, sand, dust, leaves, stem and root of a plant, seeds were accounted for 16/45(35.55%) , domestic injury with a broom, household objects like paper, metal accounted for 10/45(22.22%) and only 1 case was found to have an industrial trauma with an iron dusts in our study. History of foreign body entering into the eye was positive in 7/45(15.55%) and 2(4.44%) patient has history of extended wear contact lens use. 9/45(20%) patient did not correlate the disease with any traumatic or non traumatic event. SK Basak[49] et al reported , recent corneal injury was accounted in

994 (82.9%) patients out of which 715 (59.6%) patients had injury with vegetative matter; mostly (526; 43.9%) paddy or paddy stalk (P< 0.0001), followed by jute plant (128; 10.6%) and other agents were twig of a tree, flying insect, dirt, mud, sand, etc. Other studies by Upadhyaya[79] and Srinivasan[20] had also shown ocular trauma as the most common risk factor with 65.4% and 52.8% respectively; and chronic dacryocystitis to be 5% and 2.2% respectively. Corneal trauma (1009; 92.15%) was identified as the predominant predisposing factor in study by Bharathi[33]et al and the correlation between trauma and fungal keratitis was highly significant (P<0.0001) in a study of 3183 patients at Tamil nadu .They also showed 671 (61.28%) patients had corneal injury with vegetative matter and this correlation was also highly significant (P<0.0001).

In our study 8/45(17.77%) patient had used a home remedy or an ayurvedic medicine by a local quack , topical antibiotic/steroid was used in 4/45(8.88%) , both antibiotic and antifungal eye drops were used in 3/45(6.66%) patients and 29/45(64.44%) patients did not use any topical medication at the time of their presentation.

Srinivasan[20] in his study on 162 patients, found 32 (19.8%) patients were using herbal medicines and had put some kind of oil into the eye.

Contact lens usage has been accused to be the most common cause of bacterial keratitis in most studies, as seen in 50% of cases in the study by Bourcier et al[74] and in 22% of cases seen in the study by Green M et al.[73] In our study history of contact lens was found in 2/45(4.44%) cases. The difference in our study as compared to others is probably due to the poor socio economic class of people that usually are seen at our hospital.

Out of 45 cases, 17.77% cases had history of T2DM, 8.88% had concurrent Dacryocystitis who also underwent Dacryocystectomy, pasr history of Herpes zoster in 6.66%, Lid disease were found in 4.44% and 2.22% were positive for Retroviral Disease and Past history of leprosy in our study. SKBasak et al concluded that other risk factors in his study were chronic dacryocystitis 29 (2.4%) , dry eyes 18 (1.5%). T2DM 92 (7.6%) and 6 patients had past history of leprosy. The rate of dacryocystitis was a little higher in our study as compared to a study in Madurai,[20] with 5% prevalence of chronic dacryocystitis . Corticosteroids 86 (7.85%) and T2DM 172 (15.71%) are associated with development of fungal keratitis, concluded by Bharathi[33] et al in her 3 year retrospective review in a study conducted at a hospital in South India.

Majority of patients in our study presented during first 10 days of the onset of symptoms 24(53.33%), while 11 (24.44%) subjects presented during 11-20 days and remaining 10 (22.22%) presented after 20 days duration since onset of symptoms. These findings were in correlation with the study Srinivasan M et al[20] where 60.8% presented during first week, 18.7% in second week and 19.7% in more than two week duration. Whereas Basak[49] et al concluded (621; 51.8%) patients were seen between 2-3 weeks of their illness and 156 (13%) patients reported after 4 weeks. Bharathi[33] et al study also had similar results, 511 (46.66%) patients reported within 7 days, 352 (32.15%) between 1 to 2 weeks, 106 (9.68%) between 3 to 4 weeks, 66 (6.03%) between 1 to 2 months and the remaining 60 patients (5.48%) more than 2 months after onset of illness.

In the present study majority of the patients 27/45 (60%) presented with an visual acuity of CF to HMCF, PL +. While 10/45 (22.22%) presented with 6/36 to

6/60, another 7 patients (15.55%) presented with vision between 6/12 to 6/24 and 1 (2.22%) had vision ranging from 6/5 to 6/9. Gangopadhyay[85] in his study found 47.8% patients with visual acuity ranging from 3/60 to HMCF.

Common microbial keratitis usually affect central two-thirds of the cornea. Centrally located ulcers were more commonly seen in 29 (64.44%) and 15 (33.33%) showed peripherally located ulcer. Of the 45 cases, 26 (57.77%) had non severe corneal ulcers and 19(42.22%) had severe ulceration at the time of presentation. Hypopyon was present in 21 (46.66%) cases. Vascularization was present in 22 (48.88%). Descematocele was seen in 9(20%) cases and Corneal thinning occurred in 22(48.88%) cases in our study.

In a study by Gangopadhyay,[85] 64.5% ulcers were central in location. There were 47.8% non severe and 52.2% severe ulcers.

In current study, we observed that 41/45(97.77%) patients presented with pain in and around the eye, Diminution of vision was noted in 40/45(88.88%) and Photophobia accounted for 27/45(60%) cases. History of Redness was seen in 22/45(48.88%), watering from involved eye in 18/45(40%) and discharge was noted in 5/45(11.11%) cases.

In the present study, 20/45(44.44%) organisms could be detected on Gram staining. Final Diagnosis was considered gold standard and based on that sensitivity of Gram's stain was 81.81% and specificity was found to be 91.30%. Positive predictive value was 90% and Negative predictive value was 84%. The sensitivity of Gram-stained smear was (88.73%) ($P<0.0001$) in study by Bharathi[33] et al.

Our findings correlated with study by McLeod SD[87]wherein Gram positivity was seen in 26.8% of cases. Constantinou[86] et al on 229 patients found Gram stain positivity in 36% of the cases.

In the present study, 17/45(37.77%) of the patients were found to have positive bacterial cultures on blood agar, chocolate agar and MacConkey's agar. 13/17(76.47%) were found to be Gram positive and 4/17 (23.52%) were Gram negative. Among Gram positive most commonly isolated organism was Streptococcus Pneumoniae 6/17 (35.29%) and among Gram negative most commonly isolated organism was Pseudomonas species 4/17(23.52%).

Culture positivity seen in a study conducted by Basak[49] et al on 1198 patients with culture positivity in 67.7% of patients, most common bacterial isolate was Staphylococcus Aureus, representing 127 (42.6%) of all the bacterial culture ($P < 0.0001$) followed by Pseudomonas species 63 (21.1%). Other studies in Ghana[35] and South India,[20,33] had shown 57.3%, 68.4% and 70.6% culture positivity respectively. All isolated organisms were tested for antibiotic sensitivity.

In our study 16/45(35.55%) patient were already on topical medications in form of topical steroids, antibiotics, antifungals and home remedies and only 3/16(18.75%) did not show any growth of bacteria and/or fungi on culture media. These patients received antibiotic and/or antifungal topical medications and 13 patients received home remedies in form of oil, butter and ayurvedic powders. McDonnel[88]*et al* and Kowal[89] *et al* reported that patients who were already on treatment prior to culture showed delayed healing of the ulcer, probably due to the toxic effect of ineffective and prolonged antibiotic/antifungal therapy. Sub-optimal concentration of antibiotics

does allow bacterial and fungal growth to occur in culture media and also delays recovery of organisms on media.

Out of 45 cases in present study , 30 patients had fungal isolates on chocolate agar, Blood agar and Sabouraud's dextrose agar and accounted for 30/45(66.66%) cases of microbial corneal ulcers. The incidence of fungal keratitis in a study conducted by Bharathi[33] et al was 34.4% Similar reported incidence in other regions of India are 7.3% in North India, 32% in East India, 38.9% in West India and 32% - 39.8% in South India. Majority of patients in our study belong to rural population and from agricultural background hence incidence of fungal etiology is higher in our study.

Majority of patients in present study had Aspergillus species positivity with 12/30(40%) followed by Fusarium sp 7/30(23.33%), Aureobassidium sp 1(3.33%). Cladosporium sp 1(3.33%), Alternaria sp 1(3.33%), Curvuleria sp 2(6.66%) , Penicillium 1(3.33%) , Candida sp 3(10%) Zygomyecetes sp 1(3.33%) and Bipolaris sp 1(3.33%). Kappa statistics of 1 denotes a total agreement between KOH preparation and fungal isolation.

SK Basak[49] et al also concluded similar results with most common fungal pathogen being Aspergillus sp (623 ,59.8%) ,21.2% were Fusarium sp, 10.1% were Penicillium and Candida spp (7; 1.12%).

Out of 45 cases in present study, 31 patients had fungal species isolated on culture hence sensitivity of KOH preparation was 88.25% and specificity was 90.90%. Positive predictive value was 96.77% and negative predictive value was 71.42%. Bharathi[33] et al also reported sensitivity of 10% KOH wet mount preparation (99.23%) in their study.

In our study final diagnosis was arrived based on **microbiological investigations** and response to the treatment. We found that there is a positive correlation between clinical and final diagnosis, which is statistically significant P= 0.002 ($p<0.05$).

In our study, Staphylococcus Aureus and Staphylococcus Epidermidis are sensitive to Cephazolin, Gentamycin, Ciprofloxacin, Vancomycin and Chloremphenicol. Pseudomonas species were found to be sensitive to Piperacilin, Ciprofloxacin, Tetracycline, Cephazolin and Imipenem. Streptococcus Pneumoniae is sensitive to Ampicillin, Ciprofloxacin, Erythromycin, Gentamycin and Tetracycline. Nikhil Gokhale [90] reported that Aminoglycoside antibiotics like Gentamicin and Tobramycin give good coverage against Gram-negative organisms and are also active against *Staphylococci* and some *Streptococci* but not against *Pneumococci* . He also concluded that 4th generation Fluroquinolones offer good coverage against Staphylococcus Aureus, Staphylococcus Epidermidis, Streptococcus Pneumoniae and Pseudomonas species.

Overall, the findings of the present study showed males are predominantly affected with dominance of right eye involvement. Majority of patients belong to rural population and are agriculturists. Recent corneal injury was found to be a major risk factor in this study with majority cases are of fungal in etiology.

A small sample size of only 45 patients was an important limitation of this study another limitation was transportation of culture media and probable risk of contamination. However, to minimize this, special care was taken while inoculating the culture media. To get better yield of organisms 12-24 hours of abstinence was given to patients who were already on treatment.

CONCLUSION

The present study was undertaken to find out the clinical and microbiological profile of corneal ulcers. It successfully concludes that recent corneal injury with a vegetative matter is a most common risk factor for development of microbial corneal ulcers. Rural population and male gender is at increased risk of developing keratitis. Most common bacterial organism is Streptococcus Pneumoniae and fungal pathogen is Aspergillus sp in our study. No case of Acanthamoeba keratitis was observed in our study.

Microbiological tests offer a great help in identification of organisms whenever there is a clinical dilemma. Sensitivity of Gram's and KOH test is 81.81% and 88.25% respectively, denotes that KOH has higher sensitivity over Gram's test. Specificity of Gram's test and KOH test is 91.30% and 90.90% confirms higher specificity of Gram's test over KOH preparation. Blood agar is the most common media identified for bacterial growth and Sabouroud's dextrose agar is most common for isolation of fungal pathogen.

Microbiological investigations offer early and reliable diagnosis of corneal ulcers and treatment can be started early to prevent the sight threatening complications.

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SUMMARY

The present one year Prospective Cross sectional study was conducted in the Department of Ophthalmology, KLES Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre, Belgaum during the period of January 2012 to December 2012.

Males accounted for 38(84.44%) and females 7(15.55%) out of total 45 patients enrolled in the study. Males were predominant with. M:F ratio of 5.42:1 in our study. Majority of the patients were from rural population 30 (66.66%) and only 15 (33.33%) patients were from urban population. Farmers accounted for 24(53.33%) of the study group, males were 22(57.89%) and females were 2(28.57%) among farmers. Maximum number of females were domestic workers active in household activities, they were 3(42.87%) out of total 7 females in study population. Involvement of right eye was found in 31(68.88%) patients and left eye in 14(31.11%) patients. Out of 45 cases, patients with injury with a vegetative matter in form of seeds, leaf, stems and sticks were found to be 35.55% and considered as major risk factor associated with development of corneal ulcers. Out of 45 cases, 17.77% cases had history of T2DM, 8.88% had concurrent Dacryocystitis who also underwent Dacryocystectomy, past history of Herpes zoster in 6.66%, Lid disease were found in 4.44% and 2.22% were positive for Retroviral Disease and leprosy.

Majority of patients in presented during first 10 days of the onset of symptoms 24(53.33%), while 11 (24.44%) subjects presented during 11-20 days and remaining 10 (22.22%) presented after 20 days of duration.

In current study, we observed that 41/45(97.77%) patients presented with pain in and around the eye, Diminution of vision was noted in 40/45(88.88%) and Photophobia accounted for 27/45(60%) cases. History of Redness was seen in 22/45(48.88%), watering from involved eye in 18/45(40%) and discharge was noted in 5/45(11.11%) cases.

In the present study majority of the patients 27/45 (60%) presented with an visual acuity of CF to HMCF, PL +. While 10/45 (22.22%) presented with 6/36 to 6/60, another 7 patients (15.55%) presented with vision between 6/12 to 6/24 and 1 (2.22%) had vision ranging from 6/5 to 6/9.

Morphological features that clicked the clinical diagnosis were noted which include site, size, depth, infiltration, vascularisation, corneal thinning and hypopyon. Central ulcers were 29(64.44%) whereas 15 (33.3%) showed peripherally located ulcer. Of the 45 cases, 26(57.77%) had non severe and 19(42.22%) had severe ulceration at the time of presentation. Hypopyon was present in 21 (46.66%) cases. Vascularisation was present in 22 (48.88%). Descematocele was seen in 9(20%) cases and Corneal thinning occurred in 22(48.88%) cases.

Final Diagnosis was considered gold standard and based on that sensitivity of Gram’s stain was 81.81% and specificity was found to be 91.30%. Positive predictive value was 90% and Negative predictive value was 84%.

In the present study, 17/45(37.77%) of the patients were found to have positive bacterial cultures on blood agar, chocolate agar and MacConkey’agar. 13/17(76.47%) were found to be Gram positive and 4/17 (23.52%) were Gram negative. Among Gram positive most commonly isolated organism was Streptococcus

Pneumoniae 6/17 (35.29%) and among Gram negative most commonly isolated organism was Pseudomonas species 4/17(23.52%).

Out of 45 cases 31 patients had fungal species isolated on culture hence sensitivity of KOH preparation was 88.25% from above chart. Specificity of KOH preparation was 90.90%. Positive predictive value was 96.77% and negative predictive value was 71.42%.

Out of 45 cases 30 patients had fungal isolates on chocolate agar, Blood agar and Sabouraud's dextrose agar and accounted for 30/45(66.66%) cases of microbial corneal ulcers. Majority of patients had Aspergillus species positivity with 12/30(40%) followed by Fusarium species 7/30(23.33%). Kappa statistics 1 denotes that there is total agreement between KOH preparation and fungal isolation by culture.

Final diagnosis was made on **microbiological investigations** and response to the treatment and found that there is a positive correlation between **clinical and final diagnosis which is statistically significant P= 0.002**

ANNEXURE I

CONSENT FORM

Mr/Mrs/Ms ____ You are invited to participate in our research study titled **"**A one year prospective cross sectional study of clinical and microbiological spectrum of corneal ulcers at tertiary care hospital, Belgaum.**"** conducted by Post Graduate student in M.S. Ophthalmology,

Respected Sir/Madam, we request you to enroll yourself in our study as you are eligible for participation. Your participation in research is voluntary. If you decide to participate you are free to withdraw at any time.

<u>Purpose of the Study</u>

The purpose of research is to determine the clinical efficacy and safety of moxifloxacin in treatment of bacterial keratitis

<u>Procedure Involved</u>

If you agree to enroll yourself in this study, you will be asked your present, past and family history. You will be clinically examined and relevant investigations will be done. Then you will be asked to undergo either of the two treatment procedures based entirely on computer generated randomization. You will be asked to follow up on specified dates when your progress would be monitored, documented and if necessary photographed.

Risks and Benefits

There are no major risks involved, however some complications may occur primarily due to the disease process itself, but not related to the medications used. Your participation may benefit you and others with the same condition in future, by helping us learn more about the disease process. No financial incentives are promised for being a part of the study.

Alternatives

If you are not willing to participate you will be treated according to the existing protocol & it will not affect your relationship with this hospital.

Costs for participating in this research

There will not be any extra cost incurred by you. You will, however, have to pay for the investigations and medications which are part of the existing management protocol for the condition. There is no commitment for any reimbursement or any other compensation.

Privacy and Confidentiality

Your privacy is guaranteed. However, your medical records can be directly accessed and reviewed by authorized individuals or by the ethics committee. Records, which could reveal your identity, will be kept confidential. Personal data will remain anonymous if data is being published or written as a dissertation.

Authorization to Publish Results

When the results of the research are published or discussed, in a conference, no information will be displayed that would disclose your identity.

Compensation

In the event of injury related to the study, treatment will be made available through KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum.

There is no compensation or payment for such medical treatment by law. The doctors and the staff will provide facilities and medical attention to you.

Questions

If you have any questions about the research you may please contact:

1. Investigator, ________________________ Post Graduate student, Department of Ophthalmology, JNMC, Belgaum.
2. Guide, ________________ Professor, Department of Ophthalmology, JNMC, Belgaum
3. ________________ Principal, JNMC, Belgaum and Chairman, Institutional Ethics Committee.

CONSENT FOR PARTICIPATION IN RESEARCH TRIAL

I, Mr./Ms./Mrs ________________________________ voluntarily agree for the participation as a subject of this study. By signing this consent form, I am not giving up any of my legal rights. I may withdraw from the study at anytime. I am signing the consent form after having read or been read for me in my own vernacular language, including the risks and the benefits and having all my questions answered.

Subject Name : ____

Signature or the Left Thumb Print of Subject : _____________________________

Witness Name :____

Signature of Witness:____

Investigators Name: ____

Signature of Investigator : ____

Date:

Place:

Name of Guide: ____

Signature of guide: ____

ANNEXURE II – PROFORMA

1. PATIENT OPD NO. [ ] PATIENT IP NO. : [ ]

2. NAME:____

3. AGE: [ ] yrs SEX: [ ] (1-male, 2-female)

4. OCCUPATION:___________________________

5. DATE OF ADMISSION:_____________DATE OF DISCHARGE :___

6. ADDRESS :

____

____

7. TELEPHONE No.(s)____

8. SOCIO-ECONOMIC STATUS: [ ]

1- UPPER
2- MIDDLE
3- LOWER

9. IS THE PATIENT ELIGIBLE FOR STUDY: [ ] 1-YES 2-NO

10. HAS INFORMED CONSENT BEEN GIVEN? [ ] 1-YES 2-NO

11. PROVISIONAL DIAGNOSIS:

12. COMPLAINTS:

	RIGHT EYE	LEFT EYE	DURATION
1. PAIN 1- YES 2- NO (If 1, then, 1=mild, 2=moderate, 3=severe)	☐	☐	☐
2. PHOTOPHOBIA 1- YES 2- NO	☐	☐	☐
3. WATERING 1- YES 2- NO	☐	☐	☐
4. REDNESS 1- YES 2- NO	☐ ☐	☐ ☐	☐ ☐
5. HEADACHE 1- YES 2- NO	☐	☐	☐
6. DISCHARGE 1- YES 2- NO (If 1, then 1=serous, 2=mucopurulent, 3=purulent)	☐	☐	☐
7. DIMINUTIC VISION 1- YES 2- NO	☐	☐	☐

13. NATURE OF HISTORY:

PREVIOUS HISTORY OF APPLICATIONS OF (1- YES , 2- NO)

1. ANTIBIOTIC
2. ANTIBIOTIC + STEROID
3. CYCLOPEGIC
4. HOME REMEDY
   (INDIGENOUS MEDICATION)
5. BANDAGE CONTACT LENS

14. HISTORY OF RISK FACTORS (1- YES, 2- NO)

1. TRAUMA
2. CONTACT LENS WEAR
3. DIABETES
4. DACRYOCYSTITIS
5. ALCOHOL
6. IMMUNE SUPPRESSOR

15. PAST HISTORY :

PAST HISTORY OF (1- DIABETES, 2- HYPERTENSION, 3- BOTH, 4- ANY OTHER MEDICAL DISORDER,5- NONE)

IF 4 SPECIFY : ______________________

16. PERSONAL HISTORY:

PERSONAL HISTORY OF ☐ (1- SMOKING, 2-ALCOHOLISM, 3-BOTH ,4- NONE)

17. GENERAL PHYSICAL EXAMINATION

PALLOR ☐ (1- PRESENT, 2- ABSENT)

OEDEMA ☐ (1- PRESENT, 2- ABSENT)

LYMPHADENOPATHY ☐ (1- PRESENT, 2- ABSENT)

PULSE: ___________/ MINUTE

BLOOD PRESSURE: ____________mmHg

TEMPERATURE: ________(1- AFEBRILE, 2- FEBRILE)

18. SYSTEMIC EXAMINATION

CVS: ☐ (1-Normal, 2-Abnormal, If Abnormal, specify : __________

RS: ☐ (1-Normal, 2-Abnormal, If Abnormal, specify : __________

CNS: ☐ (1-Normal, 2-Abnormal, If Abnormal, specify : __________

PER ABDOMEN: ☐ (1-Normal, 2-Abnormal, If Abnormal, specify :

19. OCULAR EXAMAINATION:

HEAD POSTURE ☐ (1- ERECT, 2- TILTED)

FACIAL SYMMETRY ☐ (1- SYMMETRICAL, 2- ASYMMETRICAL)

VISUAL AXIS ☐ (1- PARALLEL, 2- DEVIATED)

	RIGHT EYE	LEFT EYE
• **Extraocular Movements**		
(1- NORMAL, 2- RESTRICTED)		
Uniocular	☐	☐
Binocular		
• **Visual Acuity**		
Distant Vision	☐	☐
1- 6/5 to 6/9		
2- 6/12 to 6/24		
3- 6/36 to 6/60		
4-CF to Hand movement (HM),		
-Projection of light (PL/PR)		
5-NPL (No PL)		
• **Adnexa**		
(1-Normal; 2-Abnormal, if abnormal specify : ________________________)	☐	☐
• **Sclera**	☐	☐
(1-Normal; 2-Congested)		
• **Conjunctiva**		
Normal (1=yes; 2=no)	☐	☐
Conjunctival congestion (1=yes; 2=no)	☐	☐
Circum corneal congestion (1=yes; 2=no)	☐	☐
Chemosis (1=yes; 2=no)	☐	☐
Others	☐	☐

____

• **Cornea**		
(1-Normal; 2-Abnormal)	☐	☐
Corneal ulcer		
○ Location (1=Central; 2=Peripheral)	☐	☐

- Shape
- Size
- Margin (1=well defined;
  2=feathery,3= ill defined)
- Edges (1=sloping;2=punched
  out; 3=undermined)
- Depth - (1=Superficial;2=Mid
  Stromal; 3=Deep Stromal)
- Floor (1=Fibrotic; 2=slough)
- Infiltration (1=yes; 2=no)
  If 1, then, specify
  Size
  Depth (1=Superficial;2=Mid
  Stromal; 3=Deep Stromal)
- Vascularisation – (1=yes; 2=no)
  If 1, then, (1=superficial;
  2=deep;
  3=both)
- Immune ring (1=yes; 2=no)
- Pigmentation (1=yes; 2=no)
- Satellite lesion (1=yes; 2=no) ☐ ☐
- Descemetocele (1=yes; 2=no) ☐ ☐
- Perforation (1=yes; 2=no) ☐ ☐
- Keratic precipitates (1=present; ☐ ☐
  2=absent)

- Aqueous flare/ cells (1=yes; 2=no) ☐ ☐

If 1, then specify the grade: ☐ ☐

- Scarring (1=yes; 2=No) ☐

(If 1, then, 1=nebular; 2=macular, 3=leucomatous)

- **Anterior Chamber**
  - Depth : ☐ ☐

(1-Normal depth ; 2-Shallow, 3- Deep, 4-Absent, 5- Irregular)

- Hypopyon (1=yes; 2=no): ☐ ☐

If 1, then specify,

Level (1=<2mm; 2=2-4mm; 3=>4mm) ☐ ☐

Mobility (1=mobile; 2=immobile) ☐ ☐

- Hyphema (1=yes; 2=no): ☐ ☐

- **Iris**

(1=Details visible 2=Details not visible) ☐ ☐

If 1 specify,1=Normal colour and pattern; 2=Abnormal) ☐ ☐

If 2, then, 1=synechiae; 2=staphyloma; 3=others ☐ ☐

- **Pupil**

(1-Round,regular,reacting; 2-Abnormal) ☐ ☐

If 2, then specify

- **Lens**

(1=Clear; 2=Cataractous; 3=details not made out)

If 2, then, 1=immature; 2=mature; ☐ ☐

3=hypermature

20. LACRIMAL PATENCY TEST

1. Patent
2. Blocked

21. INVESTIGATIONS: (1- Normal, 2- Abnormal)

- Blood Sugar

22. OCULAR INVESTIGATIONS:

A. Fluorescein staining

1- Staining

2- Not staining

3- Pooling

B. Corneal scraping

a. Stain (1- positive, 2- negative)

- Gram
- KOH wet mount

b. Culture (1- positive, 2- negative)

- Blood Agar
- Chocolate Agar
- Sabouraud's Slope agar

C. Antibiotic sensitivity test

23. TREATMENT GIVEN

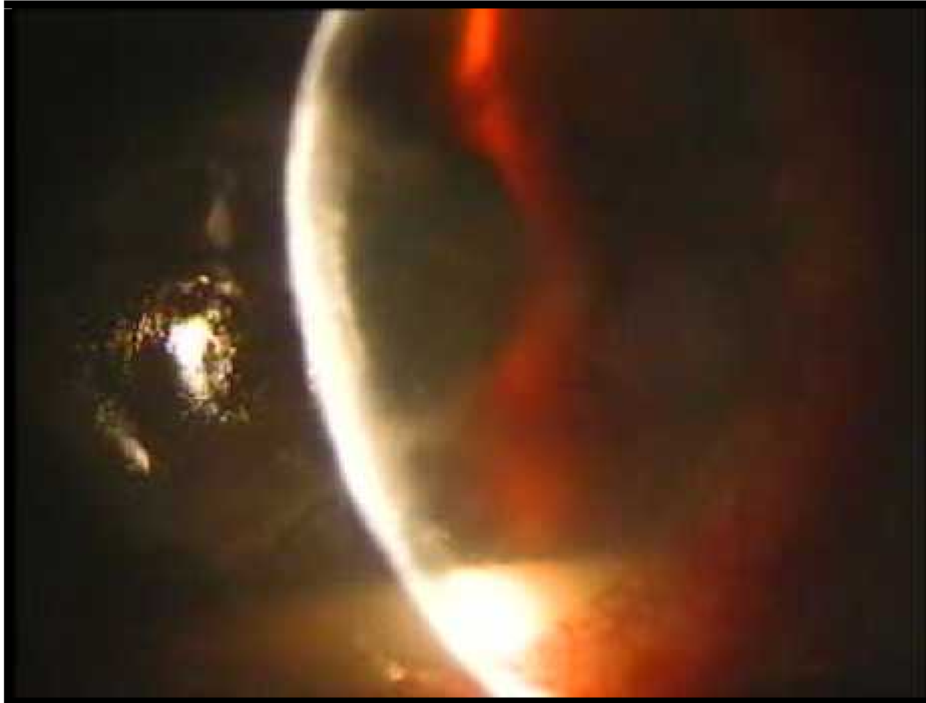
ANNEXURE III – PHOTOGRAPHS

1.Procedure of corneal scraping

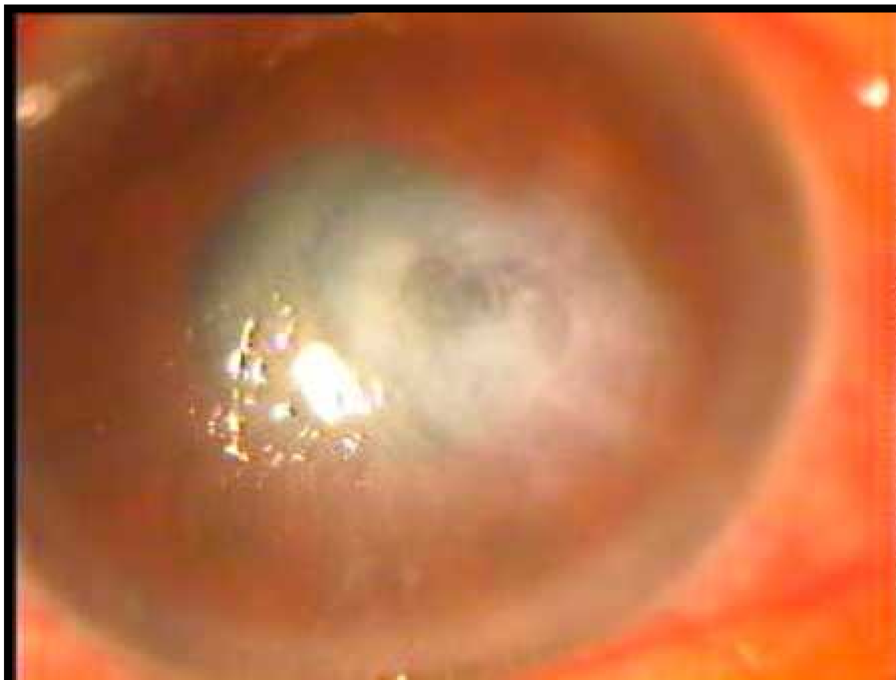
2. Laboratory armamentarium

3. Bacterial corneal ulcer with marked infiltration

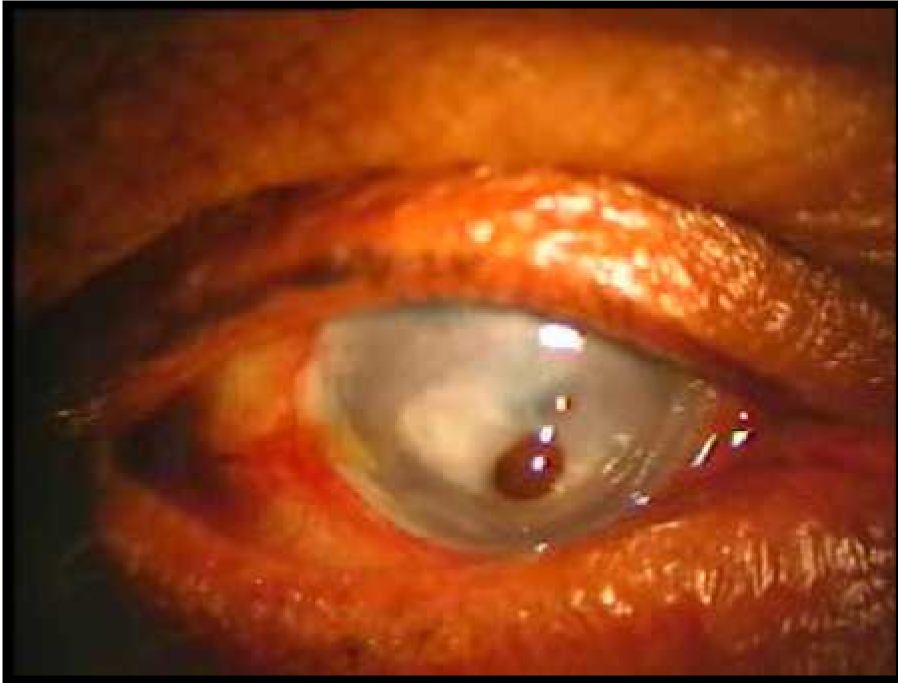
4. Hypopyon corneal ulcer with chemosis



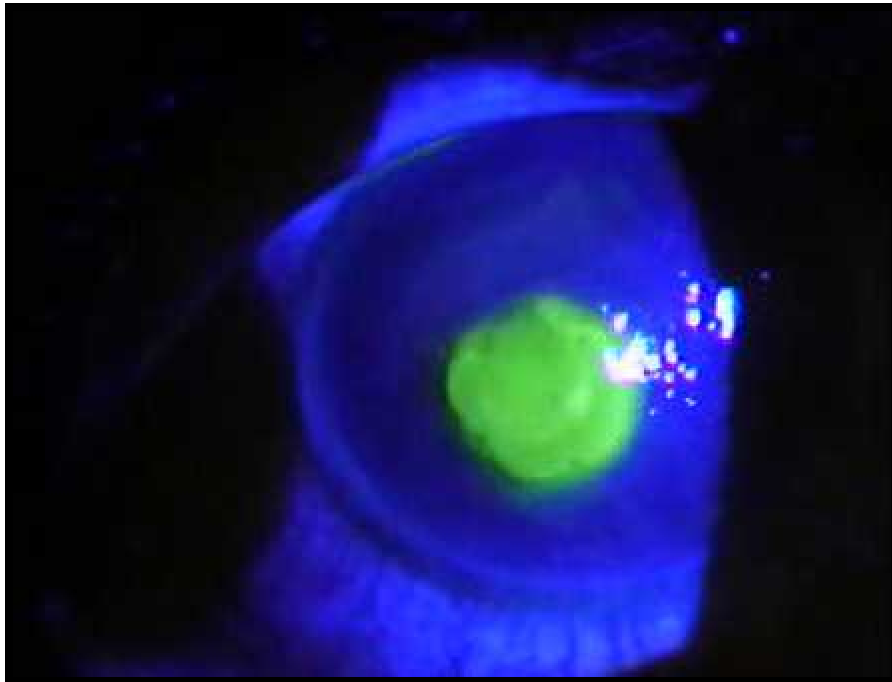
5. Fungal corneal ulcer with feathery margins



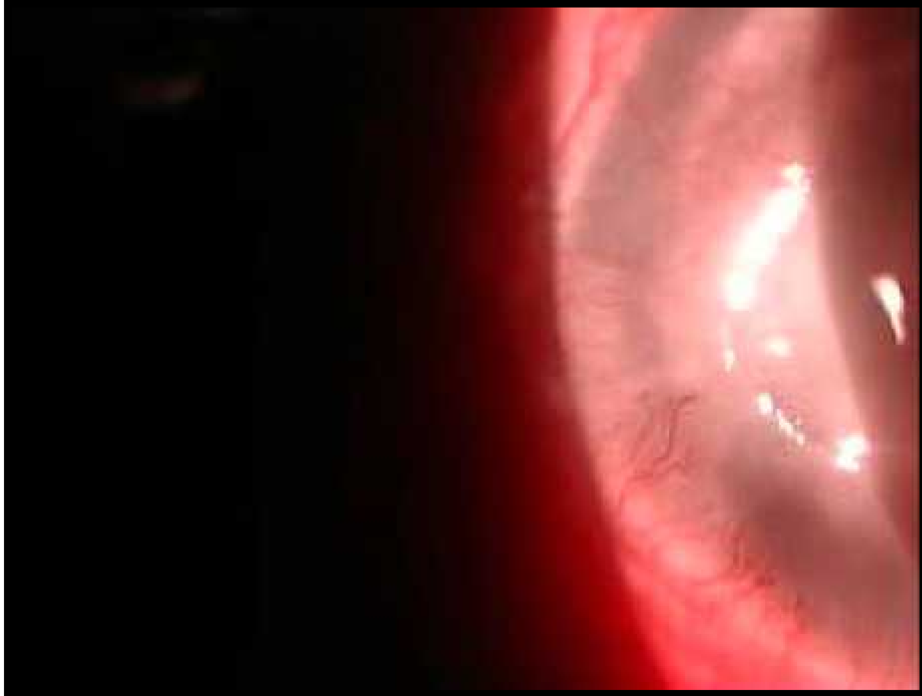
6. Perforation in a Bacterial corneal ulcer



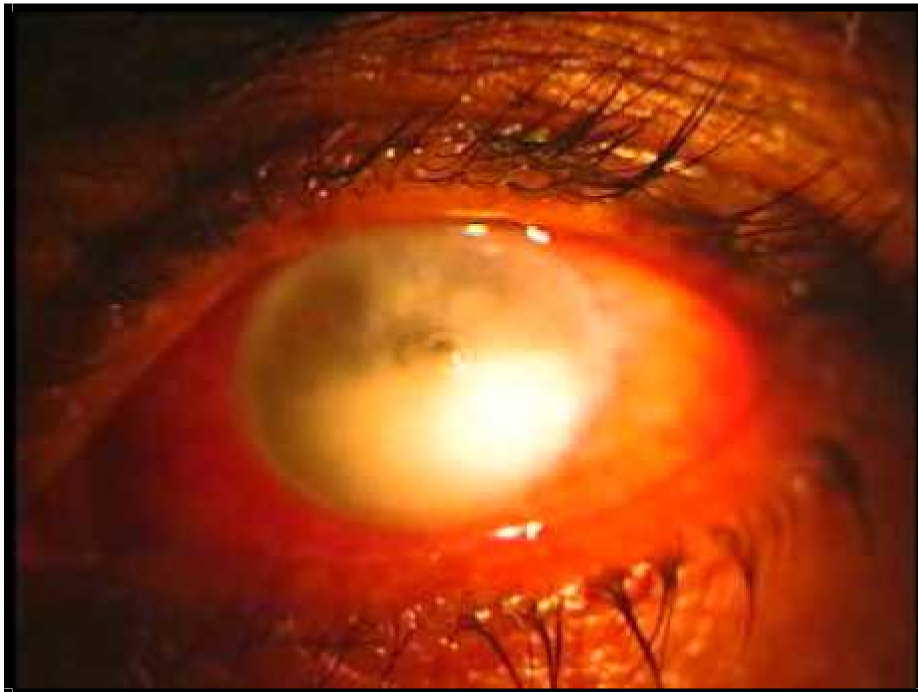
7. Epithelial defect with fluorescein staining



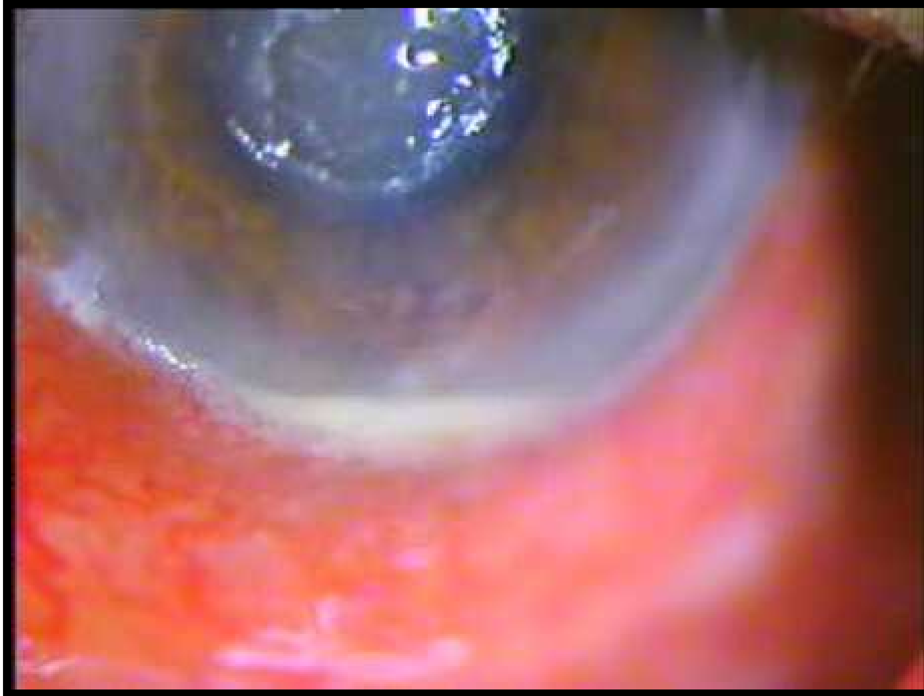
8.Corneal ulcer with both superficial and deep vascularisation



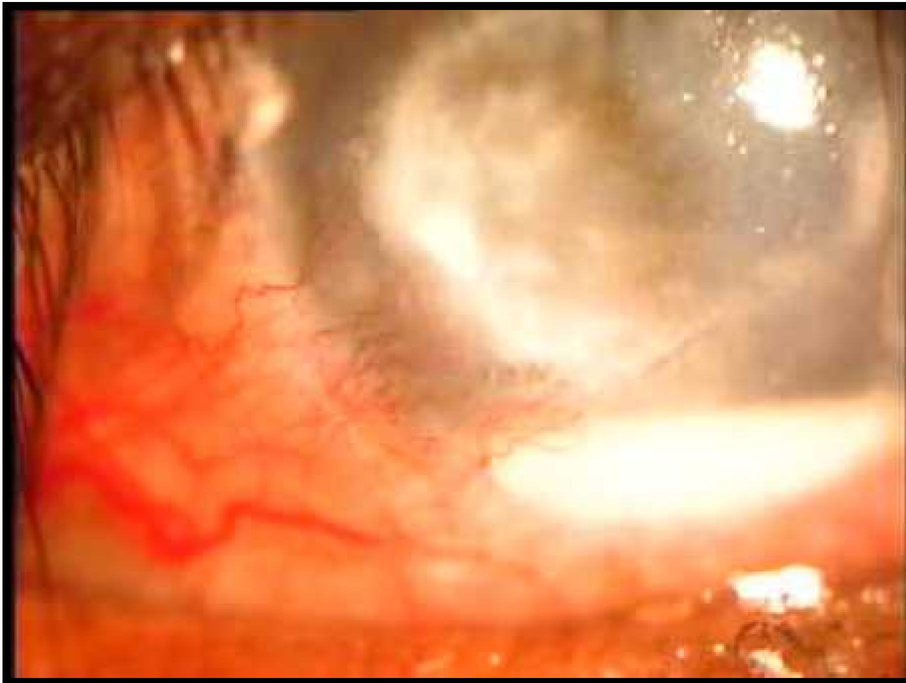
9. Endothelial plaque and hypopyon in polymicrobial keratitis



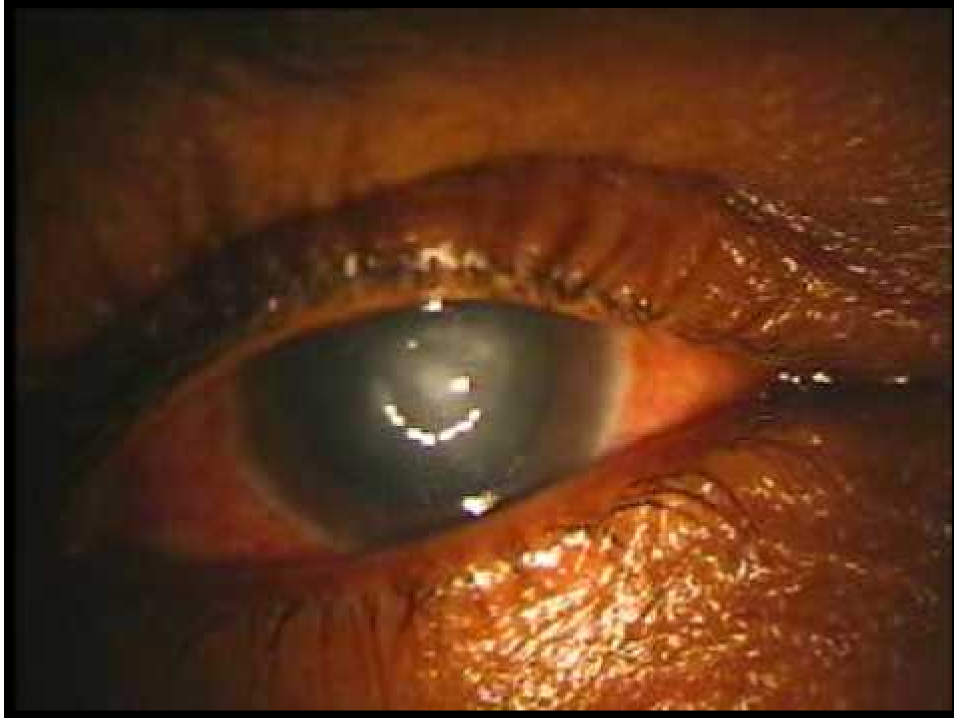
10. Pseudomonas keratitis with ring infilteration and hypopyon



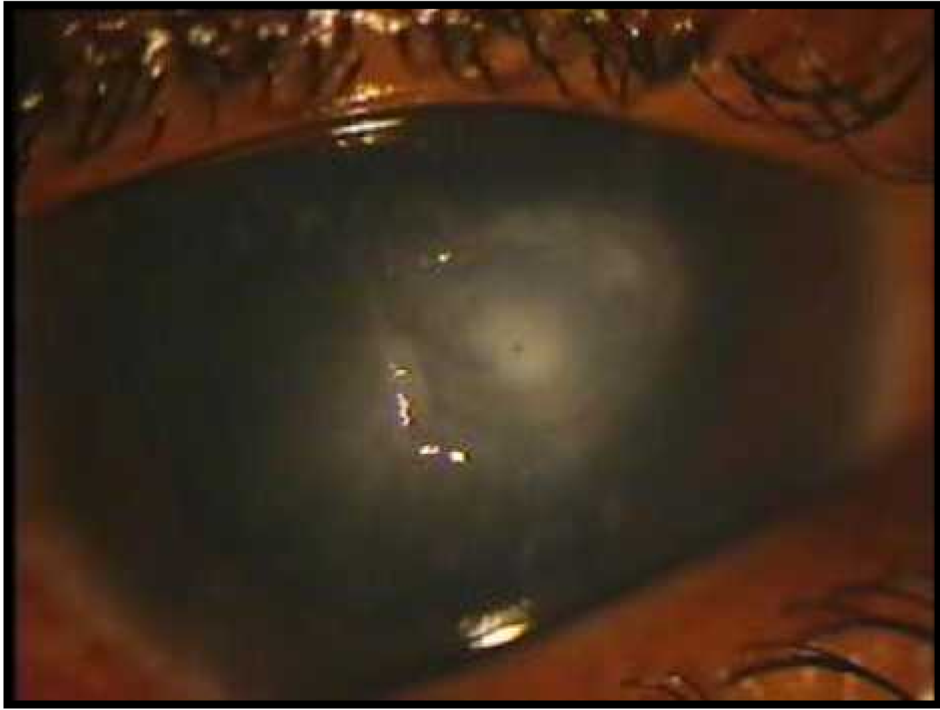
11. Superficial vascularisation in Curvuleria keratitis



12. Aspergillus Keratitis



13. Staphylococcal corneal ulcer with stromal infiltration



]

14. Aspergillus Niger growth on SDA

15. Candida sp on SDA

16. Streptococcus pneumoniae on Blood agar

17. Aureobassidium growth on Blood agar

.

ANNEXURE IV - KEY TO MASTER CHART

AB	- Antibiotic
AF	- Antifungal
ACR	- Anterior chamber reaction
Agr	-Agricultural
ASP	- Aspergillus
ALT	- Alterneria
AUR	- Aureobassidium
B	- Belgaum
BA	- Blood agar
BH	-Bhagalkot
BIP	- Bipolaris
BK	- Bullous keratopathy
C	- Central
CA	-Chocolate agar
CC	- Congestion / Chemosis
CF	- Counting fingers
CL	- Contact lens
CLAD	- Cladosporium
CS	- Cataract surgery

CUR - Curvuleria

D - Days

DCT -Dacryocystitis

DFs -Descemet folds

Dis -Discharge

Dm -Domestic

Do -Diminution of vision

DW - Domestic worker

EI - Explosive burn injury

EPK -Endothelial plaque

EXM -Exudative membrane

F -Farmer

Fac -Facet

FB -Foreign body

Fm - Female

FM -Feathery margin

FUS -Fusarium

GNB - Gram negative bacilli

GPC - Gram positive cocci

GPF -Gram positive Fungi

Gr - Greyish

HMCF - Hand movements close to face

HR - Home remedy

HW - Housewife

HU	- Healing ulcer
Hz	- Herpes zoster
IMP	- Improved
Ind	-Industrial
IW	- Other Indoor worker
IR	- Immune ring
ISA	-Intrastromal abscess
KOL	- Kolhapur
KPs	- Keratitic precipitates
L	-Left eye
Lab	-Labourer
LCO	- Leucomatous corneal opacity
LD	- Lid disorder
Lep	- Leprosy
LM	- Limbal
M	-Male
MAC	- Maconkey's agar
MCO	-Macular corneal opacity
mm	- Millimetres
mts	- Metres
MPF	-Micro perforation
N	-No
NCO	- Nebular corneal opacity
NH	- Not healed with the treatment drug

NI - Not improved

nogc - No growth on culture media

NS - Non severe

NV -Not visualised

OIW - Other industrial worker

P - Peripheral

PF -Perforation

PEN -Penicillium

PLPR - Perception of light and Projection of rays

Ph - Photophobia

Pn -Pain

PSA - Posterior stromal abscess

PSM - Pseudomonas

R - Right Eye

Rd -Redness

RU - Ring ulcer

RVD - Retroviral disease

S - Severe

Sp - Species

SDA - Saboroud's agar

SLS -Satellite lesions

SIMC -Senile immature cataract

ST - Topical steroid

STU - Student

STA - Staphylococcus aureus

STE - Staphylococcus epidermidis

STP - Streptococcus pneumoniae

STV - Streptococcus viridans

SV - Serviceman

TF - Treatment failure

T2DM - Diabetes Mellitus

TOB - Tobramycin

U - Urban

UC - Unchanged

W - Watering

WU -Worsening ulcer status

Y -Yes

ZYG - Zygomycetes