# 'INFECTIOUS KERATITIS IN KOLAR REGION'


**BY**

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**DISSERTATION SUBMITTED TO**

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**TAMAKA, KOLAR, KARNATAKA**

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**IN**

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*UNDER THE GUIDANCE OF*

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**APRIL 2015**

**SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH, TAMAKA, KOLAR, KARNATAKA.**

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*Date:* *Signature of the Candidate*

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| SDA | Sabourauds dextrose agar |
| PDA | Potato dextrose agar |
| MHA | Muller Hinton agar |
| CLSI | The Clinical and Laboratory Standards Institute |
| LPCB | Lacto phenol cotton blue mount |
| BOD | Bio Chemical Oxygen Demand |
| SCD | Slide calibration divisions |
| EMD | Eye micrometer divisions |
| NFCCI | National Fungal culture collection of India |
| KOH | Potassium hydroxide |
| PCR | Polymerase chain reaction |

# ABSTRACT

**INTRODUCTION:**

Corneal blindness is a major public health problem worldwide. Annually 1.2 to 2 million cases of corneal blindness are estimated to occur globally with 90% of them occurring in developing countries. Corneal ulcer has been estimated to be responsible for 9% cases of blindness in India. In a population based study from south India, the incidence of corneal ulcer was reported to be as high as 1130 per million. Among the causes of corneal ulcer, infectious keratitis occupies the predominant position. Infectious keratitis from bacterial, fungal or parasitic infection exists in all geographic regions of the world. Proper identification of the infectious agents causing keratitis and specific treatment directed at them would reduce the consequences of corneal ulceration such as corneal perforation and endopthalmitis and eventual loss of the eye. The causative agents in infectious keratitis vary significantly from country to country and even from region to region within the same country. So there is a need to determine the etiology within a given region. As there is no data available on the spectrum of infectious agents causing keratitis and the relative proportion of the diseases due to them in Kolar region, this study is being conducted.

This study was undertaken to isolate and identify the bacterial, fungal and protozoal agents causing keratitis in Kolar region.

**MATERIALS AND METHODS:**

During the period from January 2013 to June 2014, corneal scrapings were collected from 75 patients with suspected infectious keratitis attending both outpatients and in patients departments of Ophthalmology at R.L.Jalappa Hospital, and Sri Narasimha Raja (SNR) Hospital Kolar. Typical viral ulcers, healing ulcers, Mooren's ulcers,

marginal ulcers, interstitial keratitis, sterile neurotropic ulcers, and ulcers associated with autoimmune conditions were excluded from the study.

Sociodemographic features, occupation, predisposing factors, history of corneal trauma, traumatic agents, associated ocular conditions, other systemic diseases, therapy received prior to presentation, and all clinical findings were recorded. After a detailed history and thorough clinical examination the patients were examined under slit-lamp bio microscope by an ophthalmologist and subjected to investigations which included microscopy and, culture for bacteria, fungi and protozoa causing infections keratitis. For this purpose corneal scrapings were collected by applying multiple, moderately firm, unidirectional strokes, under slit lamp illumination by using a 26 guage sterile needle under aseptic conditions from the base and margin of each ulcer after instillation of 4% lignocaine (lidocaine) without preservative. The corneal scraping were obtained by an ophthalmologist. The corneal scraping was spread onto 3 clean sterile labelled glass slide for Gram stain, KOH mount and Saline mount. The corneal scraping material was inoculated directly on to the solid media and liquid media such as Blood agar, Chocolate agar, MacConkey agar, Sabourads dextrose agar and liquid media such as Thioglycolate and Brain heart infusion broth. Care was taken in the collection of material and in inoculating aseptically to the appropriate culture media. Informed consent was taken from the patients.

**RESULTS:**

Of the 75 patients studied 63(84%) belong to 21-70 years, 48(64%) were males and 27(36%) were females. There were no patients in the age group between 0-10 years. Farmers and manual laborers accounted for 31.2% whereas house wives constituted 21.33% .Corneal ulcers were encountered in all seasons but 69% of the cases were found during November and February. Corneal trauma was the major predisposing

factor among the cases studied which accounted for 82.67%. Fall of vegetable matter in eye was complained by 29 patients (38.67%). Paracentral ulcers predominated in 55(73.33%) cases and Hypopyon was present in 13(17.33%) patients. Out of 75 cases, Gram stain was positive in 37 (49.33%) cases. KOH mount was positive in 15 cases (20%) which showed fungal elements and pigmented fungi. Saline wet mount did not show any cysts or trophozoites. The positivity rate of total microbial isolates in our study is 25.33%. Among the bacterial isolations made in the study, 5 were Streptococcus pneumoniae sensitive to Penicillin, Erythromycin, Cotrimoxozole Gentamicin, Chloramphenicol, Tetracycline, Ciprofloxacin, Linezolid, and Vancomycin and 1 was AmpC β lactamase producing Klebsiella pneumoniae and was only sensitive to ciprofloxacin and carbapenems. 13 (%) fungi were isolated : Fusarium species(7:53.85%) Aspergillus species (3:23%) Colletotrichum species (2:15.38%) and Curvularia species. (1:7.69%) Among the 7 species of Fusarium isolated 3 could be speciated as Fusarium semitectum and 1 as Fusarium solani morphologically. Empirically Natamycin (5%), ceftriaxone tobramycin eye drops were advised to be instilled as eye drops. 13(64.42%) patients could be followed up for first week and patients had shown signs of improvement in symptoms and visual acuity.

**CONCLUSION:**

1. Corneal ulcers in Kolar region are more common in the age group of 21-70 years among the population.
2. No cases of corneal ulcer in children below 10 years was recorded in our study.
3. Males were more commonly affected with corneal ulcer than females with a sex ratio of 1.8:1.
4. Patients with corneal ulcer seen in our study, most often were farmers, manual laborers or housewives.

5. Corneal trauma with vegetable matter was the most common predisposing factor.
6. We could isolate bacterial and fungal etiological agents in 25.33% of patients with corneal ulcer.
7. Fungal infections predominated accounting for 17.33%.of isolates.
8. The fungal isolates included *Fusarium, Aspergillus, Colletotrichum* and *Curvularia* species.
9. *Fusarium* species dominated among the isolates 53.85%.
10. 4 out of 7 *Fusarium* species, 3 were *Fusarium semitectum* and 1 was *Fusarium solani.*
11. Among the 6 bacterial isolates, pencillium sensitive *Streptococcus pneumoniae* in 5 cases and 1 AmpC β lactamase producing *Klebsiella pneumoniae* could be isolated.
12. We did not find: mixed infection, protozoa, or yeasts in the study.
13. Among patients who received Natamycin, Tobramycin, Ceftriaxone and Voriconozole eye drops improvement in symptoms and visual acuity was observed even by the first week of follow up.

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# 1. INTRODUCTION

Corneal blindness is a major public health problem worldwide[1]. It is estimated 1.2 – 2 million cases of corneal blindness occur globally and 90% of them are from developing countries[2]. A recent survey by the Government of India[3] estimates that corneal lesions are responsible for blindness. Among the causes of corneal lesions, ulcers due to infectious agents predominate. Infectious keratitis could be of bacterial, fungal or parasitic origin[4]. There are many studies that document fungal, bacteria and protozoa as positive agents of keratitis. However fungi top the list. There are regional variation in fungal isolates from different parts of the world[5]. In addition to *Fusarium*, *Candida* and *Aspergillus* species predominate in western countries. In India[6] *Aspergillus* is the main fungal isolate from corneal ulcer followed by *Fusarium* in studies from Northern India. However *Fusarium* species occupy the first place among the reports on fungal isolations from corneal ulcer from Southern India[6]. Though corneal ulcers are seen among agricultural population attending the outpatient and inpatient departments of Ophthalmology at Kolar. There is no information available on spectrum of infectious agents and fungal species involved in Kolar region. This study has been undertaken to contribute information on these aspects as treat options depend upon the etiological agents prevalent.

# 2. OBJECTIVES

1. To isolate bacterial pathogens causing keratitis and to find out their antibiotic sensitivity pattern.
2. To isolate fungi from ulcerative keratitis and identify them.
3. To detect protozoal pathogens causing keratitis.

# 3. REVIEW OF LITERATURE

## Keratitis in ancient Indian medical texts

Keratitis is a disease described in ancient medical texts. Sushrata samitha (around 600 BC) in Uttara tantrum gives a good description of anatomy of the eye, etiology, classification, signs and symptoms of ophthalmic diseases. The diseases of the cornea are described in chapter 5, uttara tantrum[7] .Corneal ulcers are described as Savarna sukra as Krishna (Sanskrit word: Black) agata Roga or diseases of black part of the eye. Savarana sukra(corneal ulcer) means puncture like dip in the region of the Krishna mandala with a sensation of needle prick with excruciating pain and hot exudation. The etiology of eye diseases is based on Tridosha theory.

Akispakatyaya( Keratitis)

A whitish opacity which covers whole of the cornea, owes its origin to inflammation of the eye and is extremely painful is called Akispakatyaya. If the ulcer is long standing, extensive deep seated with a depression in the center, elevated marigins with loss of vision, involving both coats (cornea and iris) it is not curable[8]. Some these clinical entities described in ancient text could represent infectious keratitis and its complications.

## Historical developments in 19th-20th century

In Modern medicine, the developments in understanding keratitis and it causative agents seem to begin in 19th century. In 1729 Micheli in Florence was the first to recognize the genus *Aspergillus*, who noted the resemblance between the sporulating head of an *Aspergillus* species and an aspergillum used to sprinkle holy water[9].In 1808

James Wardrop published his essays on the morbid anatomy of the human eye and he coined the term keratitis. Prior to this it was general practice to refer all ocular inflammations as "ophthalmia"[10].In 1809, Link was the first to describe the genus *Fusarium* as fusisporium[11] .In 1849, Sir William Bowman was first to describe the changes occurring due to cornea inflammation. He was the first to describe Mooren's ulcer, and then by McKenzie in 1854 as "chronic serpiginous ulcer of the cornea or ulcus roden[12].In 1856, Virchow published the first complete microscopic descriptions of the *Aspergillus*[8].Virchow first introduced the term mycosis for fungal infection [13] .In 1879, Theodor Leber reported the first case of fungal keratitis in a 54 year old farmer, who was working with a shredder, the oat chaff struck in the eye resulting in keratomycosis caused by *Aspergillus glaucus*[14]. Leber first established an early clinical diagnosis by mycological methods [13].In 1955 Gingrich observed first case of *Fusarium* keratitis[15].Various authors have reported clinical cases of mycotic infections from 1879 to 1959[16].A review of literature reported 148 case reports of corneal mycotic infections following Leber's observation. Only 84(57%) cases occurred between 1951 and 1962. This was due to extensive use of antibiotics and steroid therapy. The fungi most frequently isolated were members of the genus *Aspergillus*[17]

**AGENTS OF KERATITIS[18,19,20,21]**

Microbes causing keratitis fall into 4 categories: Fungal, Bacterial, Viruses and Parasitic. Fungal keratitis is far more common than other etiological agents. Fungi are primitive, eukaryotic, non-motile, plant like structures that lack chlorophyll. They live as symbiotic parasites or saprophytes. Fungi grow optimally between 20 and $30^0$C. They reproduce by fragmentation, fission, budding and sexual or asexual spore formation. Cell wall is made up of polysaccharide (80-90%), proteins and lipids. The polysaccharide is either cellulose or chitin and usually only one type is found in a given

fungus. Their cell membranes are rich in sterols. They can be distinguished from bacteria by the presence of a nucleus, mitochondria, 80 S ribosomes, centrioles and the preference for an acidic environment. Fungi have the ability to grow at body temperature, to survive at the low redox potential of tissue and to neutralize the humoral and cellular defenses of the host. Most of the fungi are environmental in nature. Frequently, fungi can be isolated from the flora of the normal eyelid and conjunctiva, especially in individuals who work outdoors. Fungi are opportunistic invaders in compromised corneas as well as following trauma with plant or vegetable matter. The increasing incidence of fungal keratitis is related to a greater recognition of the clinical features, improvement in laboratory techniques, better reporting and increasingly wide spread, indiscriminate use of corticosteroids, antibiotics and immunosuppressive drugs. More than 70 genera of filamentous fungi and yeasts have been identified in fungal keratitis. For purposes of discussing their role in ocular disease, it is easier to classify fungi into filamentous organisms (molds) and yeasts. This simple classification is helpful in discussing geographic distribution, predisposing factors, clinical features, laboratory diagnosis and medical therapy. A dimorphic fungi has both filamentous phase (25-30$^0$C) and yeast phase (37$^0$C). However these organisms rarely cause keratitis. The frequency of keratitis due to filamentous fungi and yeasts varies between tropical and temperate zones. However filamentous fungi appear to be important agents of keratitis in both regions of the world.

**Filamentous fungi**

Filamentous fungi are multicellular organisms. They produce distinctive, long branching hyphae that form a tangled feathery or powdery mass above the culture medium. The hyphae are septate or nonseptate. Fungi with nonseptate hyphae include *Mucor, Rhizopus and Absidia*

They are responsible for lethal infections of the orbit and paranasal sinuses, but are rarely responsible for exogenous keratitis. Fungal keratitis is commonly caused by fungi with septate hyphae. Fungi with septate hyphae are divided into nonpigmented fungi (Moniliaceae) and pigmented fungi (Dematiaceae). The predominant causes of fungal keratitis are *Fusarium spp, Aspergillus spp, Acremonium spp and Penicillium spp* which are all nonpigmented fungi. Important pigmented fungi include *Curvularia, Alternaria, Bipolaris and Phialophora spp.*

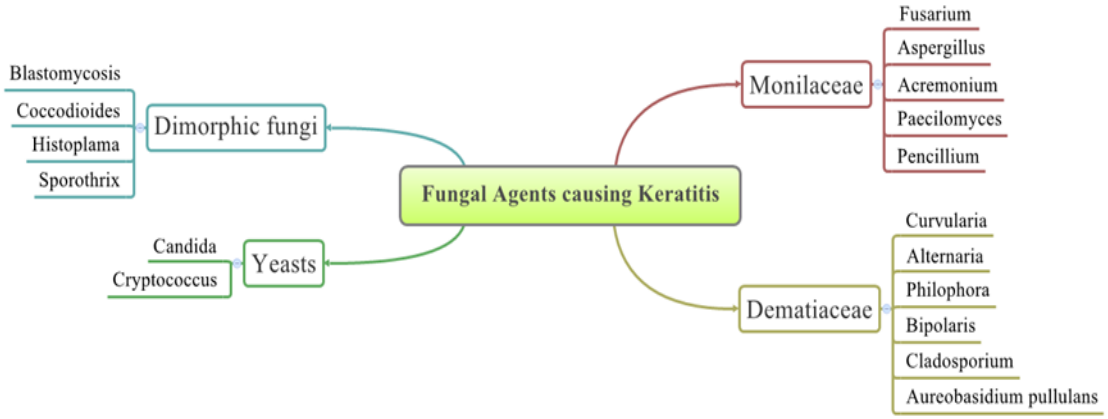



**Figure 1. Mind map of Fungal Agents causing Keratitis**

**Hyaline filamentous fungi**

Filamentous fungi are the principal causes of mycotic keratitis in most parts of the world with *Fusarium* and *Aspergillus* most commonly encountered[21]. There are many species of filamentous fungi causing keratitis. The most common causes of filamentous fungi causing keratitis are the species of *Fusarium, Aspergillus, Curuvalaria* and other *phaeohyphomycetes, Scedosporium apiosperum and Paeilomyctes*[22].Worldwide, *Aspergillus* species has been reported as the predominant isolate including Northern parts of India[6]. However, *Fusarium* species was found to be the most common cause of fungal corneal infections in Southern United States, Florida, Brazil, Ghana, Nigeria,

Paraguay, Columbia, China, Vietnam, Hongkong, Singapore[23] as well as from some parts of Southern India. A study in China reported 73.6% patients with positive fungal cultures. *Fusarium* species was 48.2%. Among *Fusarium* species, *Fusarium solani* was the commonest isolate (35.2%) followed by *Aspergillus* species (18.7%)[24] In Vitenam, National Ophthalmology Hospital reported *Fusarium* species (39.6%) was the predominant fungal isolate followed by *Aspergillus* species (25.9%)[25]. In India, *Fusarium* is predominate in Southern India (Tamil Nadu, Madurai and Bangalore) in contrast to *Aspergillus* spp. predominant in Northern India as shown in various studies[6].

**Fusarium:** [11,18,20,21]

The genus *Fusarium* was introduced by Link in 1809. The genus classified is under the order Hypocreales, class hyphomycetes (Ascomycetes). Formerly *Fusarium* belongs to imperfect fungi of the class Deuteromycetes,

**Primary characteristics of Genus Fusarium**

The *Fusarium* classification system is developed mainly based on the morphology of the conidia produced by the representatives of the genus. Macroscopic and microscopic features, such as color of the colony; length and shape of the macroconidia; the number, shape, and arrangement of microconidia; and the presence or absence of chlamydospores are the key features for the differentiation of *Fusarium* species.

*Fusarium* species produce three types of spores i.e. microconidia, macroconidia, and chlamydospores. However, the presence of macroconidia is the most important characteristic feature that differentiates *Fusarium* species from other genus. Macroconidia are formed in sporodochium and has a shape of a moon crest or a boat or banana with multiseptum. Basically, there are three shapes of macroconidia i.e. straight or needle-like, dorsiventral curvature, and dorsal curvature. The shapes of the end,

apical and basal cells are important characteristics to determine species. Generally, the apical cell has four shapes i.e. blunt, papillate, hooked and tapering, while the basal cell also has four shapes i.e. foot-shaped, elongated foot shape, distinctly notched and barely notched. Macroconidia may be produced on condiogenous cells in the aerial mycelium. There are two types of conidiogenous cells i.e. monophialides and polyphialides. A monophialide is a condiophore with only one opening or pore through which endoconidia are extruded, while a polyphialide has two or more such openings or pores per cell. Microconidia are produced only at the aerial mycelium from conidiogenous cells and not from sporodochia. The arrangement of microconidia on the conidiogenous cells is important in identification. They may be arranged in single, false heads, or in chains. Moreover, the presence and absence of microconidial chain is very important to identify species in sections. Furthermore, the shapes of microconidia are oval, reniform, obovoid, pyriform, napiform, globose, and fusiform.

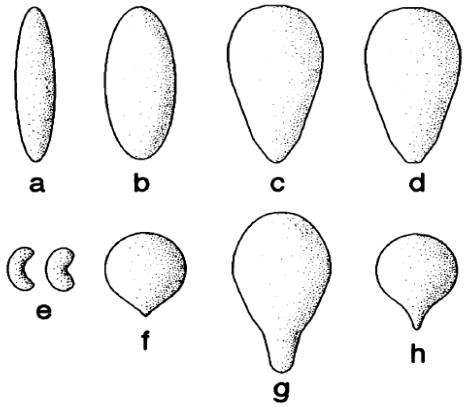



**Figure 2. Shapes of microconidia of various Fusarium species:**

a, fusiform; b, oval; c, obovoid; d, obovoid with a truncate base; e, allantoid; f, napiform; g, pyriform; h, turbinate.

Another type of spore are chlamydospores. They have a thick wall with a lipid substance inside that give the fungus the ability to survive in an extreme condition even outside the host. *Fusarium* species that produce chlamydospores is an important characteristic feature for identification. The formation of chlamydospores could be single, double, clumps, and in chains. In the laboratory, the formation of chlamydospores takes a long time, sometime up to six weeks. The chlamydospores

could be formed in the aerial mycelium or embedded on the agar. The other important morphological characteristic feature is mesoconidia. Mesoconidia are the fusoid conidia that are longer than microconidia with 3-4 septa but shorter than macroconidia with lack of foot-shaped and notched basal cell. These conidia are produced in the aerial mycelium on the polyphialides that appear as "rabbit ears" when viewed in-situ. Furthermore, this type of conidia is the most important feature to distinguish F. semitectum.

**Secondary characteristics**

In the process of species identification and delimitation, secondary characteristics such as pigmentations, growth rates, and secondary metabolites are considerably important. The most widely used by researchers for secondary characteristics is pigmentations. Under fixed condition, the colors of pigmentation are taken after a week of incubation. Another commonly used secondary characteristic is the growth rates. A growth rate of an isolate is measured after three days of dark incubation on PDA at either 25°C or 30°C. Besides pigmentation and growth rates, secondary metabolite profiles are considerably useful to distinguish some species. However, there is still lack of information on the profiles because most of the studies done were on temperate isolates.

**Differentiating features of Fusarium from other genus[27].**

The septate hyphae and their conidial stages (imperfect stages) of Fusarium are very similar to conidial stages of Ascomycotina. This suggests that most imperfect fungi are Ascomycotina in which sexual stages have either been lost in the course of evolution or the observations have not been recorded. Teleomorph(sexual reproductive stage) stages are not known for all *Fusarium* fungi, but they are all related to the sections *Gibberella*, *Nectria* of the order Hypocreales. *Gibberella* produce perithecia. These are

flask shaped structures opening by a pore or ostiole (short papilla opening by a circular pore) through which the ascospores escape. Macroscopically they may appear as black, but their dark blue color can be seen when viewed under a microscope. The eight ascospores per ascus are smooth-walled, fusiform, slightly curved, with blunted ends, hyaline and usually three-septate.

*Nectria perithecia* are usually red to reddish brown. Their asci usually contain eight ascospores that are hyaline to tan, ellipsoidal to obovate, not curved, usually one-septated and striated. *Calonectria* species have bright yellow to orange colored perithecia and possess asci containing 4-8 ascospores, whose shape and septation resemble that of *Gibberella*. *Fusarium* can be distinguished from *Acremonium* by its curved, multicellular macroconidia, while *Cylindrocarpon* is distinguished from *Fusarium* by its straight to curved macroconidia which lack foot cells. In *Micronectriella* the light brown-colored perithecia remain immersed within the plant tissue or substrate with only a papillate ostiole protruding. Ascospores, usually eight per ascus, are hyaline and, like Nectria, ellipsoidal with a central septum [26,27].

*Fusarium* is a common soil saprophyte and important plant pathogen. *Fusarium spp*. are ubiquitous filamentous fungi in all major geographic regions of the world and are routinely isolated from environmental sources such as soil, plant roots, plant debris, and water systems. As a plant pathogen, *Fusarium* has been reported as the one of the agent of major devastating diseases in most economically important plants, e.g., banana, wheat, and barley. It can various diseases in plants such as crown rot, head blight and scab on cereal grains .It cause a broad spectrum of human disease, including mycotoxicosis, and infections, which can be superficial, locally invasive or disseminated. It is likely that *Fusarium spp*. colonize patients prior to hospitalization and the subsequent immunosuppression and neutropenia could then result in a variety

of infections. Although skin, sinus and lung, infections are most common, other body organ systems can also be affected.

*Fusarium* species cause four patterns of invasive infections predominantly in immunocompromised patients: refractory fever of unknown origin, sinopulmonary infection, disseminated infection, and a variety of focal single-organ infections. *Fusarium* species have been documented as etiological agents in localized tissue infections, including keratitis, endophthalmitis, breast abscess, brain abscess,cystitis, peritonitis and septic arthritis. The mortality rate is greater than 70% in systemic infections. In addition, secondary metabolites produced by *Fusarium spp*. are associated with cancer and other developmental defects in humans and animals. There are over 70 *Fusarium spp*., known to cause human infections. Among them, almost half (50%) of the disease cases include *F. solani*, followed by *F. oxysporum* (14%), *F. verticillioides* (11%), *F. moniliforme* (10%), and *F. proliferatum* (5%). Further common human pathogens are *F. dimerum, F. chlamydosporum, F. nygamai, F. napiforme, F. semitectum, F. equiseti. F. anthophilum, F. sacchari.*

**Species of Fusarium causing Keratitis:**

1. ***Fusarium solani*** (Martius) Appel & Wollenweber emend. Snyder & Hansen

Cultures of *Fusarium solani* usually are white to cream with sparse mycelium. Sporodochia is a small, compact stroma (mass of hyphae) usually formed on host plants parasitised by mitosporic fungi. This stroma bears the conidiophores on which the asexual spores or conidia are formed. Sporodochia is often are produced in abundance. They may be cream, blue or green in color. Many isolates do not produce pigments in the agar although some violet or brown pigments may be observed. Macroconidia they are relatively wide, straight, stout and robust. Apical cell is blunt and rounded. Basal

cell may have a distinct foot shape or it may be developed poorly. They may be straight to almost cylindrical, usually with a notched or a rounded end. They are 5- to 7 septa. Usually abundant in sporodochia. Microconidia: They are oval, ellipsoid, reniform and fusiform in shape, with 1or occasionally 2 septa. Aerial mycelium presents with false heads. Monophilades are often quite long. Microconidia are abundant in aerial mycelia. Chlamydospores: Commonly formed abundantly and rapidly, usually within 2-4 weeks on carnation leaf agar. They may be intercalary in the hyphae or formed terminally on short lateral branches usually singly or in pairs, but occasionally in short chains. Chalmydospores may be globose to oval in shape and smooth or rough walled.(Table 1)

**2. *Fusarium semitectum* (Berkeley & Ravenel)**

Cultures usually grow rapidly and produce abundant dense aerial mycelia that initially is off white and becomes beige or brown with age. Brown pigments also may be produced in the agar. Light orange sporodochia may be produced by some strains. Macroconidia Relatively slender with a curved dorsal surface and a straighter ventral surface. Apical cell are curved and tapering to a point. Basal Cell is foot shaped. Septa may be 3-5. Macroconida may be difficult to find in some cultures. Microconidia are pyriform to obovate in shape. They are usually 1-septate and most common in older cultures. Mesoconidia are fusoid and 3 to 5 septate. Aerial mycelium presents individual spores per phialide, but often two spores per polyphialide to give a "rabbit ear" appearance. Conidiogenous cells may be Monophialides and polyphialides. Mesoconidia are abundant in the aerial mycelia. Microconidia are scarce and often are difficult to find. Chalmydospores are present, but not common. The absence of chlamydospores is not a reliable diagnostic character. Found in the hyphae both singly

and in chains, and singly within conidia. They appear globose and smooth. Initially hyaline, but may become a light yellow color with age.(Table 1)

3. ***Fusarium oxysporum*** (Schlechtendahl emend. Snyder & Hansen)

Colony morphology on Potato Dextrose Agar(PDA) varies widely. Mycelia may be floccose, sparse or abundant and range in color from white to pale violet. Abundant pale orange or pale violet macroconidia are produced in a central spore mass in some isolates. *F. oxysporum* usually produces a pale to dark violet or dark magenta pigment in the agar but some isolates produce no pigment at all. Macroconidia are short to medium length, straight to slightly curved, relatively slender and thin walled. Apical Cell is tapered and curved, sometimes with a slight hook. Basal Cell is foot shaped to pointed. The number of septa: is usually 3-septate. They are sparse in some strains, but usually abundant in sporodochia and occasionally from hyphae growing on the agar surface. Microconidia are Oval, elliptical or kidney shaped and usually 0-septate. Aerial mycelium presents with false heads. Conidiogenous cells present with short monophialides. They are abundant in the aerial mycelia. Chlamydospores are formed abundantly and quickly (2-4 weeks on CLA) by most isolates, but some isolates form chlamydospores slowly if they form them at all. Usually formed singly or in pairs, but also may be found in clusters or in short chains. May be either terminal or intercalary in aerial, submerged, or surface hyphae. Appearance is smooth or rough walled.

4. ***Fusarium verticillioides*** **(Saccardo) Nirenberg**

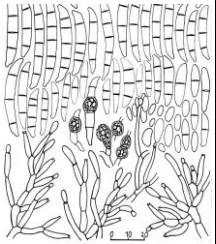
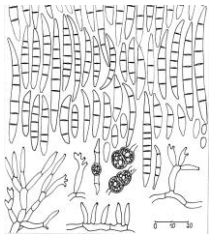
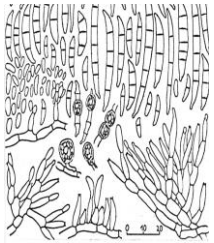
Initially cultures have white mycelia but may develop violet pigments with age. Pigmentation in the agar varies, ranging from no pigmentation or grayish orange to violet grey, dark violet or dark magenta (almost black) in others. Macroconidia. Generally they are relatively long and slender, slightly falcate or straight, and thin

walled. Typical of macroconidia produced by species in the *Gibberella fujikuroi* species complex. Apical Cell is curved and often tapered to a point. Basal Cell is notched or foot shaped.Number of septa is 3- to 5-septate. Macroconida vries by strain, but may be difficult to find. Microconidia are oval to club shaped with a flattened base and usually 0-septate.Aerial mycelium presents with long chains are common, but small aggregates of a few spores occur occasionally. Conidiogenous cells mainly monophialides, which are occasionally produced in pairs to give a "rabbit ear" appearance. Abundant in the aerial mycelia. Chlamydospores are not produced,although some isolates may produce swollen cells in the hyphae that can easily be mistaken for chlamydospores or pseudochlamydospores.

5. ***Fusarium proliferatum*** **(Matsushima) Nirenberg**

Characters on PDA. The abundant aerial mycelium initially is white but may become purple-violet with age. Sporodochia may be present as discrete entities or nearly confluent over portions of the colony. Violet pigments usually are produced in the agar, but with overall pigmentation varying in intensity from nearly colorless to almost black. Macroconidia are slender, thin-walled, relatively straight and typical of those produced by species in the G. fujikuroi species complex. Apical Cell is curved. Basal Cell is poorly developed. Number of septa is usually 3- to 5-septa. The abundance of macroconidia varies since this character can be lost in this species following repeated subcultures. Fresh cultures usually produce large numbers of macroconidia in sporodochia. Microconidia are club shaped with a flattened base and 0-septate. Pyriform microconidia also may occur but generally are rare. Aerial mycelium may be found in chains of varying, but usually moderate, length, false heads, or aggregates of a few microconidia. Conidiogenous cells are monophialides and polyphialides. Microconida are abundant in aerial mycelia. Chlamydospores are absent.

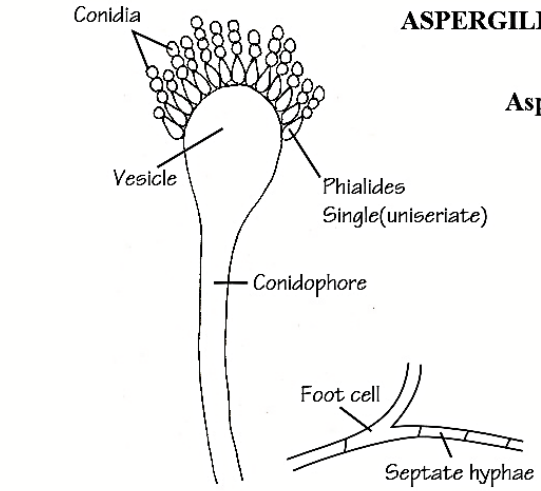
**Table 1. Macroscopic and Microscopic features of Fusarium species**

| Fusarium species | Figure | Colony characteristics | Macroconidia | | | Microconidia | | | Chalmydospores |
|---|---|---|---|---|---|---|---|---|---|
| | | | Apical cell | Basal cell | No. of septa | Shape | No. of septa | Conidogenous cell | |
| Fusarium solani |  | white to cream with sparse mycelium Sporodochia abundant. They may be cream, blue or green in color. | Blunt and rounded | distinct foot shape, straight to almost cylindrical, usually with a notched or a rounded end | 5-7 septae | oval, ellipsoid, reniform and fusiform | 1 or 2 septa | Aerial mycelium presents with false heads. Monophilades are often quite long. | Intercalary,singly or in pairs, but occasionally in short chains. Chalmydospores may be globose to oval in shape and smooth or rough walled. |
| Fusarium semitectum | .  | off white and becomes beige or brown with age | Relatively slender with a curved dorsal surface and a straighter ventral surface | Foot shaped | 3-5 septae | pyriform to obovate | 1 septa | Monophialides & polyphialides | globose and smooth singly and in chains |
| Fusarium oxysporum |  | Floccose, sparse or abundant and range in color from white to pale violet. | Short to medium length, straight to slightly curved, slender & thin walled sometimes with a slight hook. | Foot shape and pointed | 3 septae | Oval, elliptical or kidney shaped | No septa | Short monophialides. | Single or in pairs, clusters or in short chains. May be either terminal or intercalary in. Appearance is smooth or rough walled. |

***Aspergillus***

*Aspergillus* is a large genus, containing over 200 species. Only a small number of these species, has been associated with disease. Of these, over 95% of all infections are caused by *A. fumigatus, A. flavus, and A. niger*. Several additional species of clinical importance include *A. nidulans, A. terreus, A. oryzae, A. ustus and A. versicolor. Aspergillus* species are ubiquitous moulds that grow on organic matter. They are commonly isolated from soil, decaying plants and vegetables, and from indoor air environment.

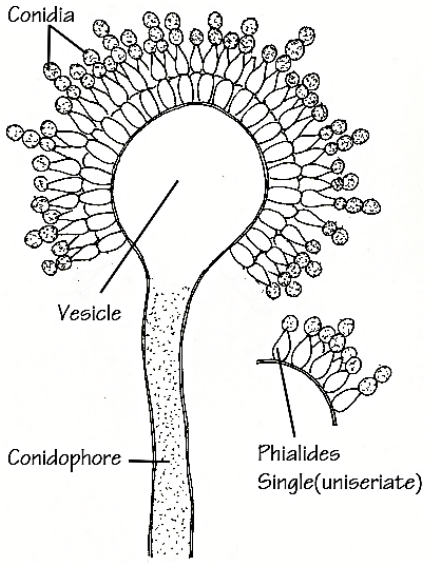
Colonies of *Aspergillius* may be powdery, granular, or cottony with a variety of colors: black, brown, yellow, red, white, green, or other colors depending on the species and the growth conditions. The growth rate of *aspergillus* is rapid to moderately rapid (1.9 cm in diameter after 7 days of culture at $25^0$C), with the exception of the slow growing species *A. nidulans* and *A. glaucus*. Only *A. fumigatus* is thermo tolerant with the ability to grow up to $50^0$C, a feature that is helpful to separate this species from the others species. On microscopic examination the characteristic features for this genus include hyphae that are hyaline and septate with dichotomous branching. Conidiophores arise from a basal foot cell and germinate into a vesicle at the apex. Conidiogenic phialides arise directly from the vesicle (uniseriate) or from intersected metulae (biseriate) depending on the species. Conidia are round (2.5 µm) and formed into radial basipetal chains (Figure 3). Species-specific features include the presence or absence of sclerotia, cleistothecia, Hulle cells, aleuriconidia, chlamydoconidia, and morphology of the vesicles and arrangements of phialides (Figure 3 ).




**ASPERGILLUS SPECIES**

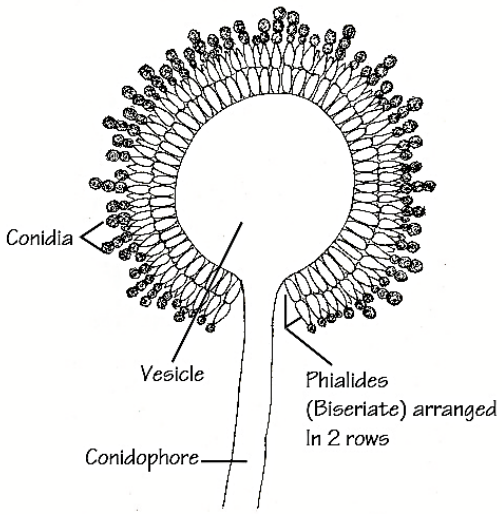
**Aspergillus fumigatus**

- **Macroscopy:**
  - *Obverse:* Colonies are velvety, powdery at first, turning to smoky green.
  - *Reverse:* white to tan
- **Microscopy:** Condiophore is smooth, phialides are single(uniseriate) usually cover upper half vesicle parallel to the axis of stalk.




**Aspergillus flavus**

- **Macroscopy:**
  - *Obverse:* Colonies are velvety, yellow to green or brown.
  - *Reverse:* is golden to red brown.
- **Microscopy:** Condiophore of varying length, rough pitted and spiny: phialides are single and double cover entire vesicle




**Aspergillus niger**

- **Macroscopy:**
  - *Obverse:* Colonies are woolly at first,white to yellow, then turning brown to black.
  - *Reverse:* white to yellow.
- **Microscopy:** Condiophore of varying length, phialides are biseriate, i.e arranged in two rows, covering the entire vesicle, form radiate head.

**Figure 3. Colony morphology of Aspergillus species**

Worldwide, *Aspergillus* species is predominant isolate in mycotic keratitis. *Aspergillus* species may cause disease due to inhalation of conidia or ingestion of mycotoxins resulting in allergic or toxic disease, chronic infection, and/ or acute infections. Allergic aspergillosis occurs largely in patients with asthma, atopy, or cystic fibrosis (CF) while invasive pulmonary disease usually only occurs in immunocompromised patients with inhalation being the primary route of infection. Invasive aspergillosis (IA) has increased due largely to advances in the treatment of malignant diseases with an increasing number of patients undergoing severe immunosuppression, as part of intensive chemotherapeutical regimes, hematopoietic stem cell or organ transplantation. *A. fumigatus* is the most common species involved in infections (85%–90%), but A. *flavus, A. niger, A. terreus, and A. nidulans* are also regularly recovered, with varying species differentiation.

***Acremonium***

The genus *Acremonium* is classified within the mitosporic Hypocreales group, order Hypocreales, class Sordariomycetes, subphylum Pezizomycotina, phylum Ascomycota, and kingdom Fungi. It is characterized by the formation of narrow hyphae with solitary, slender (2 μm), unbranched, awl (needle)-shaped phialides (or weakly branched conidiophores) arising from vegetative hyphae and producing clusters (slimy messes) or chains of small, one-celled conidia mostly aggregated at the apex of each phialide. In tissue sections, *Acremonium* often shows hyaline, septate hyphae and characteristic reproductive structures known as phialides and phialoconidia.The genus *Acremonium* consists of 33 recognized species. Most important species causing human infections are *Acremonium falciforme, Acremonium kiliense, Acremonium strictum,*

and *Acremonium recifei*. *Acremonium* is a ubiquitous, saprophytic fungus. Commonly isolated from soil, plant debris, rotting mushrooms, etc. in Europe, Asia, Egypt, and North and Central America. *Acremonium* shows a high degree of morphological similarity to *Fusarium, Verticillium Lecythophora, Phialemonium, Gliomastix, and Cylindrocarpon* as well as *Candida.* Strains of Fusarium, which do not produce macroconidia, is differentiated from those of *Acremonium* by their faster growth and production of deeply woolly colonies. In comparison with *Acremonium, Lecythophora* and *Phialemonium* phialides are not separated from hyphae by a septum; while Gliomastix generates olive-green to greenishblack colonies and chains or balls of dark conidia. *Acremonium* is differentiated from hyaline isolates of Phialophora by the absence or very limited development of a collarette on the phialide and the predominant formation of awl-shaped phialides with a basal septum. Compared to *Acremonium*, microconidial Fusarium isolates usually grow faster and have colonies with a characteristic fluffy appearance. Potato dextrose agar and cornmeal agar are the most suitable media for their identification, and exposure to daylight maximizes their culture color characteristics. *Acremonium* has been recognized as an etiologic agent of nail and corneal infection, mycetoma, peritonitis and dialysis fistulae infection, osteomyelitis, meningitis following spinal anesthesia in a normal person, cerebritis in an intravenous drug abuser, endocarditis in a prosthetic valve operation, and a pulmonary infection in a child.

***Paecilomyces*[20]**

*Paecilomyces* are hyaline species of filamentous fungi whose sexual stage belongs to the division Ascomycota. *Paecilomyces* species are saprophytic filamentous fungi that are found worldwide in soil and decaying vegetable matter. Often associated with decay of food products and cosmetics. Colonies are fast growing, flat spreading, powdery or

velvety, and are usually white, brownish, or in bright colors, reverse off-white to brown. *P. variotii* shows yellowish brownish greenish colony and *P. lilacinus* with characteristic vinaceous to violet colonies. Conidiophores occur solitarily, in pairs, as verticils, and in penicillate heads. Conidiogenous cells are phialidic, swollen at the base, and gradually narrowed into a long beak; they bend away from the axis of the conidiophore. Microscopically resembles *Penicillium spp*. but the phialides are more elongated. *Paecilomyces* lilacinus[28] and *Paecilomyces variotii*[29] are the species most frequently involved in human infection.

***Penicillium*.**

The genus *Penicillium* comprises anamorphic (asexual) species with connections to the ascomycete family Trichocomaceae. Closely related to *Aspergillus*. It is a dimorphic fungus. The only true pathogen is *Penicillium marneffei*, a member of the subgenus Biverticillium. Colonies are initially white, change to a brownish red color and later to green or bluish green color. The colony surface appears flat and powdery [20].*Penicillium marneffei* should be incubated at 30°C for 2 weeks to display dimorphism. The yeast phase (37°C) displays colonies that are white to tan, soft, and dry. Microscopically, the organism grows as a single yeast-like cell and reproduces by fission rather than budding. The round or oval or sometimes elongate cells (approximate diameter 3 μm) are septate. Elongated and septate allantoid forms (length 8–13 μm) and short filaments may also be present. The most distinguishing characteristic of the mould phase (at 25°C) is the early presence of a red pigment that diffuses into the agar. The colonies start as pinkish-yellow and evolve into a bluish-green color in the center with a white periphery. *P. marneffei* displays the characteristic brush-like conidia with terminal conidiophores that bear groups of 4–5 metulae supporting groups of 4–6 phialides. *Penicillium* is differentiated from *Scopulariopsis* which forms annelides having

annelloconidia with truncate bases and *Paecilomyces* which forms phialides having long, tapering apices. *Penicillium marneffei* is the only *Penicillium* species (among more than 200) to cause significant human disease in healthy individuals. Patients with human immunodeficiency virus are susceptible particularly to *P. marneffei.* It is restricted to Asia (Southeast and Far East) where it is considered an indicator for AIDS. It is also known to cause keratitis. *Penicillium,* a relatively infrequent cause of fungal keratitis contributing to less than 10% of cases.[30].Penicillium spores have been identified in normal ocular flora. In 1972, 5 cases of *penicillium* keratitis were reported in a study at Hyderabad[15].Incidentally from last decade incidence of *Penicillium* keratitis is being frequently reported from India. A study at Delhi reported *Penicillium* keratitis in vernal keratoconjunctivitis [30]. Study from West Bengal, eastern, India on epidemiology and microbiological diagnosis of suppurative keratitis isolated 10.1% cases due to *Penicillium*. Similarly a study of mycotic corneal ulcer in upper Assam, India was found to have 15.2% cases due to Penicillium spp[31]. A case report from Maharashtra[32] reported *Penicillium marneffei* in an immunocompetent 15 year old agricultural worker, trauma to the eye by working fan during the farm work seems to be the main contributing factor leading to injury and inoculation of fungus.

**DEMATIACEOUS FUNGI**

The dematiaceous fungi are common soil and plant saprophytes categorized on the basis of their dark pigment. Dematiaceous fungi are reported to be responsible for 10–15% of all fungal keratitis and are the third most frequently encountered fungi following *Aspergillus* and *Fusarium*[21] .Members of the dematiaceous fungi that have been recovered from infected corneas include species of *Curvularia, Exophiala, Exserohilum, Fonsecaea, Lecythophora, Phialophora, Scedosporium and Lasiodiplodia. Lasiodiplodia,* a cause of rot in fruit and vegetables, causes an especially

severe form of keratitis. In their review of 32 patients with culture-proven Curvularia keratitis over a 30-year period, authors have reported that dematiaceous fungi accounted for 22% of all fungal corneal isolates, one-third of the isolates were comprised of Curvularia spp.[33]

Curvularia was the fourth most common fungal isolate identified from cases of keratitis, following *Candida, Fusarium, and Aspergillus*, at a large hospital in Texas[33]. Curvularia spp. isolated from the Texas study included *C. senegalensis*, *C. lunata, C. pallescens,* and *C. prasadii.* Many of the largest case series of keratitis involving dematiaceous fungi originate from around India[34,35,36]. In these series, Curvularia caused 3.3%–7.4% of all mycotic keratitis cases [37,] 8.2% of fungal corneal ulcers in North India, and 6% of all cases of suppurative keratitis in Bangladesh[38]. In a large series of mycotic keratitis reported from India, *Curvularia spp*. was the most common cause of dematiaceous fungal keratitis (approximately 23% of all dematiaceous keratitis cases) [39]. In South India, among 1352 cases of fungal keratitis reviewed, *Curvularia* represented 2.8% of all fungal etiologies and was the most common dematiaceous fungus isolated [40]

Similarly, among a hospital-based study in New Delhi of 191 patients with mycotic keratitis presenting with corneal ulcers, *Curvularia spp*. was the second most common cause of ulceration, second only to *Aspergillus spp*[41].*Curvularia* has been reported as the most frequent pigmented fungal pathogen in corneal ulcers by other investigators as well [42-45].In Thailand, Curvularia was second only to *Fusarium* as a cause of severe mycotic keratitis[46].Curvularia was also a fairly common cause of fungal keratitis in far north Queensland, Australia, where a retrospective review was performed of all cases of fungal keratitis over a 10-year period [47,] A higher incidence of fungal keratitis was

demonstrated during the monsoon and winter seasons than during the drier summer months.

***Curvularia***

*Curvularia* comprises part of the Dematiaceae family of thermally monomorphic dematiaceous molds, which possess brown melanin or melanin-like pigments in their hyphal and/or conidial cell walls. The genus is comprised of approximately 40 species, including *C. lunata* (the most commonly isolated species in humans), *C. clavata, C. brachyspora, C. geniculata, C. inaequalis, C. pallescens, C. senegalensis,* and others.

Colonies of *Curvularia spp*. appear brownish-black to dark olive-green surface and a dark colored to black reverse. The colonies are filamentous. Colonies grow rapidly on plates, generally reaching maturity within 5 days. The optimal temperature for growth is 28°C, although growth may occur from 25°C to 35°C. On microscopic examination conidiophores are brown, erect, and multicellular, producing conidia sympodially (bent at points of conidium formation). Conidia are dark, thin-walled, ellipsoidal, and large (up to 14 μm in width and 35 μm in length), with a variable number of true transverse septae that differ according to the species. As the central cell of the conidium is larger and darker than the others, the conidia show a characteristic curve, or boomerang-like bend, which becomes more pronounced with age.

*Curvularia* causes a wide range of human diseases. First reported from a mycetoma by Baylet in 1959. *Curvularia spp*. have been commonly associated with certain diseases: ocular infections such as keratitis and endopthalmitis, sinuses, as well as allergic fungal sinusitis (AFS).In addition other diseases associated with *Curvularia* include cutaneous and subcutaneous lesions (such as phaeo hyphomycosis and chromo blastomycosis),peritonitis, onychomycosis, and other less common clinical entities.

Increasingly recognized as an emerging cause of human infection, particularly in immune compromised persons, but can it can affect immune competent hosts as well.

***Alternaria***

Growth is rapid, mature within 5 days. The **s**urface of the colony at first appear grayish white and woolly and later becomes greenish black or brown with a light border, may eventually become covered by short, grayish, aerial hyphae. Reverse is black. Microscopically it has a dark septate hyphae. Conidiophores are septate, sometimes have a zig-zag appearance .Conidia are large and brown, have longitudinal septations, sometimes produce germ tubes and found singly or in chains , they are usually rather round at the end nearest the conidiophores while narrowing at the apex, producing a club like shape.

***Bipolaris species***

Growth is rapid mature within 5 days. The surface of the colony at first grayish brown, becoming black with a malted centre and raised greyish periphery. Reverse is dark brown to black. Microscopically dark septate hyphae is seen .Conidiophores elongate and bend at a point where each conidium is oblong to cylindrical, appear thick walled and have 3-5 septations and a slightly protruding hilum.

***Cladosporium species***

These are saprophytic contaminants. They have only occasionally been implicated in infections. Mature within 7 days at 25°c. The **s**urface of the colony is greenish brown or black with velvety appearance, becoming heaped or slightly folded. Reverse is black. Microscopically dark septate hyphae is seen. Conidiophores are dark and branched and usually produce 2 or more conidial chains. Conidia are brown, round to oval and usually smooth, they form branching tree like chains and are easily dislodged, showing dark

spots hila at the point where they are attached to the conidiophores or other conidia. The cells bearing the conidial chains are large and sometimes septate resemble shields and mistaken for macroconidia when seen alone.

***Colletotrichum species*** [48]**:**

They belong to the order Melanconiales under the class Coelomycetes. The members of the genus *Colletotrichum* are primarily plant pathogens which cause anthracnoses (fungal infection in plants). *Colletotrichum* is a ubiquitous fungus and is most frequently isolated from soil and plant vegetation. They have been increasingly reported in causing keratomycosis, subcutaneous and systemic infections. Coelomycetes are asexual fungi that produce their hyphae in specialized structures called conidiomata, which are often of two types, namely pycnidia and acervuli. The acervular conidiomata covered with setae, producing elongated slimy conidia, and the presence of appressoria, are the key morphological features of the genus. Macroscopically they appear grayish brown with conidia forming forming in orange, red slimy masses with reverse is brown. The falcate conidia can be confused with *Fusarium spp.* The falcate conidia are present only in *C. dematium* and *C. graminicola*. The characteristic presence of 4-6 μm-wide conidia and irregular margins of appressoria seen in *C. graminicola* helps in easy identification from C.dematium, which has a 3-4 μm-wide (narrower) conidia and smooth margins of appressoria. Five species of *Colletotrichum* have been reported to cause infections in humans, namely *C. coccoides, C. crassipes,C. dematium, C. gloeosporioides and C. graminicola. C. dematium and C. gleosporoides* are the principal causative agents in keratitis.

**YEASTS**

The Yeasts consists primarily of *Candida spp.* These unicellular oval organisms reproduce by budding and form pseudohyphae under reduced oxygen tension or in tissue. The pseudohyphal phase is the most invasive and virulent phase. As with filamentous fungi, the large size of the pseudohyphae prevents complete ingestion by neutrophils. World over yeasts are the major pathogens isolated from Europe and North America. In contrast, studies from tropical and subtropical parts of the world have reported predominance of filamentous fungi: *Aspergillus* and *Fusarium species*. A study conducted in Philadelphia, United States, reviewed the records of 24 culture positive fungal keratitis and observed that most common isolated organism was C*andida albicans*(45.8%) followed by *Fusarium* species(25%)[49].10 year study done at University of Minnesota United states, reported 19 cases of culture proven fungal keratits. *Aspergillus* and *Candida* species were the largest number of fungal isolates[50].Various other studies at California, Atlanta and Northern United states, *Candida* species was the most common isolate with 4%, 94%,and 42% respectively[51].In the United Kingdom, *Candida* species (57.5%) tops the list of fungal isolates, followed by *Aspergillus* species (17.5%)[51].In Melborne, Australia, *Candida albicans* (37.2%) was the common fungal isolate followed by *Aspergillus species* (17.1%) and *Fusarium* species (14.3%)[52].In Midde east, Asia including India, *Candida* is less and designated to second place among fungi causing keratitis[23].In Chennai India, candida was only 4% of the total isolates. Among the Candida isolates, *Candida albicans* was the most common. *Candida krusei* and *Candida gullermondi* were common isolates in different studies conducted in Pondicherry and Chandigarh[53].

*Candida* is ubiquitous yeast. Infection with It is the most common ocular fungal pathogen. *C. albicans,* usually occur in immunosuppressed patients, those with ocular

surface disease or lid margin defects, or those receiving long-term topical corticosteroids. *Candida* is not linked to environmental factors as seen with infection due to filamentous fungi. *Candida* is recovered from various places such as soil, inanimate objects, and food and community environments. The presence of budding yeasts in corneal scrapings is diagnostic of Candida infection. They can produce true as well as pseudo hyphae. Both yeast and hyphal forms can be seen in corneal scrapings in Candida infections. Growth is rapid, mature within 3 days. Colony morphology is white, tan and opaque with cream colored, smooth pasty consistency with fruity yeast odour. Microscopically candida form round to oval budding yeast cells, blastoconidia 3-6 μm singly, in chains or in small loose clusters. Pseudohyphae are chains of blastoconidia that have elongated, not separated from one another and have distinct constrictions at the septa. True hyphae have no constrictions at the septa, and also no septation at the initiation of a branch.

***Cryptococcus Keratitis*** [54]: *Cryptococcus neoformans* the causative agent of torulosis does not produce mycelia or spores. In artificial media the colonies are mucoid and appear whitish to tan. Individual cells measure 5 to 20 μm. are round to ovoid and are surrounded by wide, gelatinous, polysacharide capsules. Budding is evident. A case of Keratomycosis due to *Cryptococcus* was reported [54]. The author has described the corneal involvement as being deep and very extensive which resulted in thin opacity rather transparent.

**BACTERIAL KERATITIS[55]**

Any bacteria has a potential to cause keratitis. Classification of Bacterial keratitis is shown in Figure 4. The majority of all bacterial keratitis is caused by five major groups of organisms: *Staphylococcus spp.; Streptococcus spp. (Streptococcus pneumoniae,*

*groups A to G streptococci)*; other gram-positive organisms (*Bacillus spp. and Propionibacterium spp.)*; gram-negative organisms, such as *Pseudomonas, Haemophilus, Moraxella*; and the members of Enterobacteriaceae (*Proteus, Serratia, Klebsiella, Enterobacter, Citrobacter).*

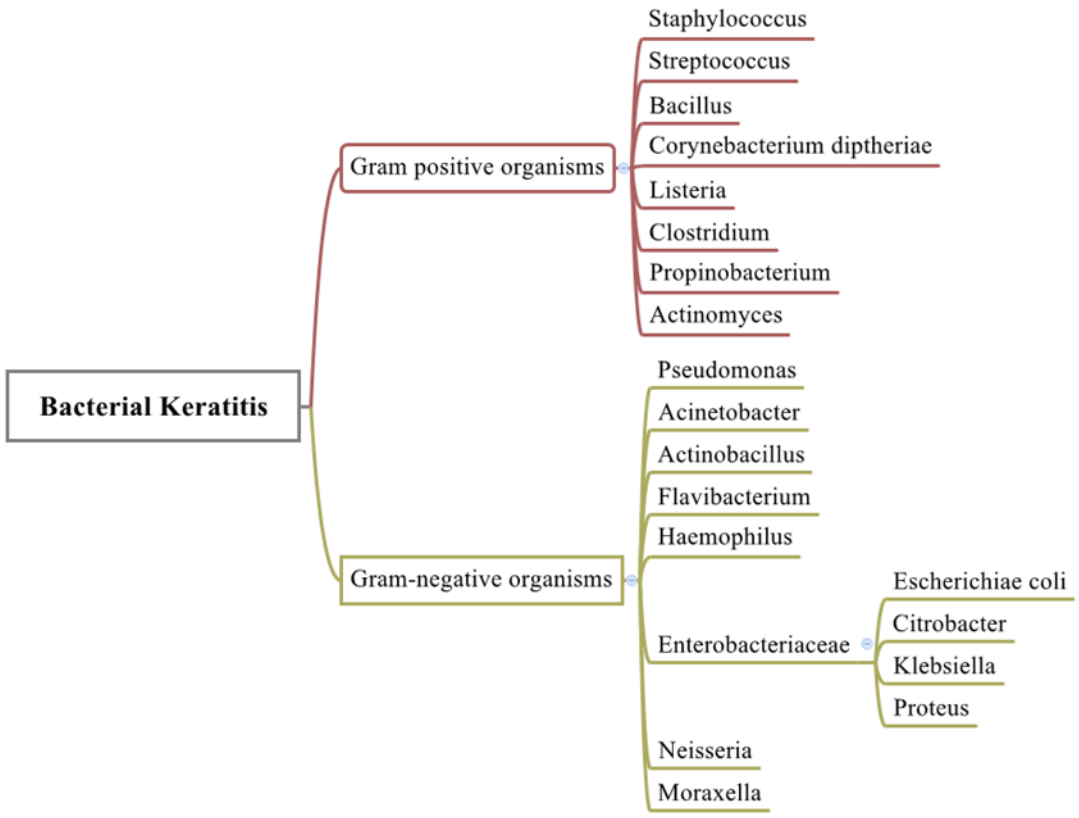



**Figure 4. Classification of Bacterial Keratitis**

**Distribution of bacterial keratitis[55]**

The published data shows that 65% to 90% of all cases of microbial keratitis are bacterial pathogens[56,57]. The relative frequency of different bacteria as causative agents in keratitis may vary geographically. In one large survey, a microbial organism were isolated in 49% of 5845 cases of suspected infectious keratitis; 82% were bacterial, 16% fungal, and 2% parasitic. A report from a small survey of organisms causing bacterial keratitis, 83% were gram-positive, 17% gram-negative, and 2%

polymicrobial. *Staphylococcus* species continue to be the predominant cause of bacterial keratitis. In several reports *Staphylococcus epidermidis* or coagulase negative *staphylococci* (CONS) are the leading causes. In series of studies from the southern part of the United States, *Pseudomonas* species is reported to be the most commonly isolated organism especially with daily use of contact lenses. *Pseudomonas* is widely distributed in nature and can easily contaminate ophthalmic preparations, cosmetics, and other materials. In developed countries the incidence of pneumococcal keratitis, which is commonly associated with chronic dacryocystitis, has decreased as a result of modern antibiotics and finer techniques for dacryocytorhinostomy. This shows that there is change in the spectrum of bacteria causing keratitis with time, especially in the United States. The prominence of certain organisms responsible for bacterial keratitis has been changing over many years. *Streptococcus pneumoniae* was the most common responsible agent of keratitus in the past, but gram-positives organisms, opportunistic commensals, Pseudomonas, anaerobes, and protozoa are now increasingly being reported. With the advent of refractive surgery, especially laser-assisted in situ keratomileusis (LASIK), more unusual organisms, such as *Nocardia* and *Mycobacterium spp*., are causing keratitis. These changes could be attributed to various factors: increased use of topical corticosteroids (i.e., refractive and cataract surgery), increased population of immune deficient patients, the use of soft contact lenses, especially extended-wear and cosmetic lenses improved isolation techniques. A 11 year study from Toronto [58] reported that there was significant decrease in the percentage of gram-positive microorganisms and also showed increased resistance to the antibiotics when compared to Gram negative isolates. Methicillin resistant *Staphylococcus aureus* was most common 29.1% of all Gram-positive cultures. As a result of the use of vancomycin in the setting of severe suspected bacterial keratitis may be justified the

shifting trends. A study from Tamil Nadu[59] showed that among 3183 corneal ulcers evaluated, 1043(32.77%) were found to be of bacterial aetiology. The commonest bacterial species isolated was Streptococcus pneumonia (37.5%). LV Prasad Eye institute Hyderabad[60] reported that majority of the bacterial infections were caused by Staphylococcus epidermidis (42.3%). The Moraxella group of organisms have been reported to cause keratitis in malnourished individuals with diabetes, alcoholism or other conditions, however, they have also been reported in healthy individuals. Less frequently reported organisms causing bacterial keratitis include: members of *Enterobacteriaceae, Corynebacterium* species, *Propionibacterium acnes*, and *Neisseria gonorrhoeae*. After the development of vaccine corneal involvement with *Corynebacterium diphtheriae* is rare, though the organism is known to penetrate intact corneal epithelium. Atypical mycobacteria causing keratitis include *Mycobacterium fortuitum, Mycobacterium chelonae, Mycobacterium gordonae and Mycobacterium avium-intracellulare*. Nontuberculous mycobacteria is being reported with increasing frequency as a cause of infectious keratitis after laser in situ keratomileusis[61,62] *Mycobacterium leprae* can occasionally be a causative agent of keratitis and invade along peripheral corneal nerves. Rare organisms causing bacterial keratitis include *Bacillus species, Nocardia asteriodes, Listeria monocytogenes*, and primary tuberculous keratitis

**Viral Keratitis[55]**

The leading causes of viral keratitis in humans include HSV, varicella zoster virus (VZV) and adenovirus. Less common virus causing viral keratitis include DNA viruses from the *Poxviridae, Herpesviridae* (excluding herpes simplex and herpes zoster), and *Papovaviridae* families. The RNA viruses affecting the eye are members of the *Picornaviridae, Togaviridae, Paramyxoviridae, and Orthomyxoviridae* families

**Herpes simplex virus (HSV)** [55]

Herpes simplex virus (HSV) is a large double-stranded DNA virus. It has an icosahedral capsid surrounded by a poorly defined tegument enclosed in a host cell membrane-derived envelope. The envelope has viral-derived glycoprotein projections. The global incidence of herpes simplex virus (HSV) keratitis has roughly been estimated to be 1.5 million, including 40,000 new cases of severe monocular visual impairment or blindness each year. Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2) have an affinity for the sensory ganglion cells and, therefore, are called neurotrophic viruses. Ocular herpes may be classified into three general groups: congenital and neonatal, primary, and recurrent. Vast majority of all ocular herpes is caused by HSV-1 infection as it is acquired by passage through an infected birth canal where as 80% of neonatal cases are caused by HSV-2.[63] Keratitis is rare in primary herpes simplex infection. It occurs in only 3–5% of cases, though severe bilateral disease can occur in atopic or immunocompromised patients. Multiple recurrences are far more common with genital and oral herpes than with ocular herpes. Based on pathophysiology HSV keratitis can be classified into epithelial and stromal/endothelial keratitis.

**Herpes zoster:** Similar to herpes simplex disease, corneal manifestations of herpes zoster (varicella zoster) infection have been described as epithelial keratitis, stromal keratitis,intersititial keratitis, keratouveitis, endotheliitis and sclerokeratitis. Corneal complications (keratitis) may be due to inflammatory and/or immune reaction to the virus, vasculopathy, and neuropathy.

**Parasitic Keratitis** [55]

*Acanthamoeba* is the most important pathogens among parasitic infections of the cornea, which can lead to painful, sight-threatening and difficult-to-treat ocular complication. *Acanthamoeba* is a free-living ubiquitous protozoa that is mostly found in water and soil. It lives in two forms: either a trophozoite or cyst. Trophozoites are the proliferative and active forms with an irregular shape and pseudopodia. This form can move by gliding and feeds on bacteria such as E. coli. When the trophozoite is exposed to unfavorable conditions, like desiccation, lack of food, contact with a toxic substance, exposure to antimicrobial agents, freezing, and the chlorine levels routinely used in water supplies, immediate encystment is achieved. *Acanthamoeba* cysts are the resistant and dormant stage, which are characterized by a double-walled envelope. Therefore, eradication is difficult and can survive in cornea for many years even after several aggressive treatments are performed. This may also be a major factor accounting for the severity and reentry of *Acanthamoeba* infection. The reported incidence of *Acanthamoeba* keratitis all over the world increased dramatically through 1989 and then it reached a plateau, especially in the United States. While the incidence continues to increase in developing countries it is reported to have declined in the U.K. *Acanthamoeba* keratitis occurs in immunocompetent, healthy young individuals.Several important risk factors have been identified which are associated with *Acanthamoeba* keratitis. A history of contact lens wear and particularly in developing countries, exposure to contaminated water or soil is the principal risk factors. The incidence of *Acanthamoeba* keratitis has increased continuously with occasional major outbreaks in various parts of the world. In a series of 189 cases of Acanthamoeba keratitis from the U.S. 85% of cases were contact lens related. In contrast, the commonly identified risk factor in patients of *Acanthamoeba* keratitis seen

in developing countries is history of corneal trauma or exposure to contaminated water. A study from South India [64] reported only 8(0.49%) patients were found to be culture positive for *Acanthamoeba* species out of 1618 corneal ulcers.

**Microsporidia**

*Microsporidia* are ubiquitous obligate intracellular parasites closely related to fungi. These organisms exist in three developmental stages within infected cells: infective, proliferative (binary or multiple fission and termed merogony) and sporogony. Sporogony results in spore production, ranging from 1–20 microns, with the coiled polar filament or tubule characteristic of *Microsporidia*. Only six genera of *Microsporidia* are known to cause ocular disease [*Nosema (renamed Vittaforma), Encephalitozoon, Entercytozoon, Trachipleistophora, Pleistophora and Septada*] [65]

It is a rare disease, but the diagnosis of microsporidial keratitis is increasing especially in Asia, where the disease may correspond with the monsoon season. Bilateral ocular infections have been described from swimming in ponds and lakes, presumably caused by insect larvae parasitized by *Microsporidia.*

**TRANSMISSION AND PREDISPOSING FACTORS**

**1. Trauma:** Injury to the cornea is the leading cause of microbial keratitis, particularly fungal keratitis. A history of corneal trauma with vegetable matter or organic matter is reported in 55-65% of fungal keratitis. Ocular trauma was accounted for about 37.1% of patients with fungal keratitis in a study done at Royal Victorian Eye and Ear hospital, Melbourne[66], Australia from 1996 to 2004. A retrospective study at L.V Prasad Eye hospital, Southern India[67] reported 54.4% of fungal kaeratitis were due to ocular trauma. Ocular trauma (1009; 92.15%) was a highly significant risk factor and vegetative injuries (671; 61.28%) were identified as a significant cause for fungal

keratitis in a retrospective analysis from tertiary care hospital in South India[68] for a over a 3-year period, 1999 to August 2002. A study from Tirunelveli[69] reported corneal injury predisposing to fungal keratitis was significantly higher, compared to other predisposing factors. Corneal injury was responsible for 71.5 per cent cases of corneal ulcer as a risk factor. This figure is supported by a higher incidence of microbial keratitis following corneal injury in developing countries (82.9% in East India, 65.4% in South India 55 per cent in North India, 52.8% in Nepal, 39.2 per cent in Ghana and 23.8 in Taiwan) in contrast to developed countries (8.33 per cent in Phailadelphia 23.5 per cent in New Zealand, 15% in Perth, and 3.7 % in Sydney. These differences are due to differences in occupation. In developing countries, agriculture is the primary occupation. History of corneal injury was noted in 92 per cent cases of fungal keratitis, the most common agent being vegetative matter. This is in consistent with similar studies[69] of corneal injury reported in Calcutta (72%), Hyderabad (54%), Sri Lanka (55%), Singapore (55%) and Florida (44%).In all 33 culture proven cases of *Acanthamoeba* keratitis, history of corneal trauma with mud (injury with wet sand) or vegetative matter was reported. In contrast, corneal injury was a predisposing factor in 28 per cent of all bacterial keratitis, consistent with studies in Paris (15%, third most common factor), Switzerland and Hong Kong. A study from West Bengal[70], trauma with vegetative matter was the most common cause followed by paddy, jute plant, tree twig, flying insect, dirt, mud, sand.

**2. Contact lenses:** Patients wearing any type of contact lens are more prone for fungal keratitis. A retrospective analysis from Brisbane[71], Australia reported significant risk factors were contact lens wear (53; 22%), followed by ocular surface disease (45; 18%), ocular trauma (41; 16%). Contact lenses predisposed to 1 per cent of all corneal ulcers evaluated in study from Tamil Nadu[69] and all cases had bacterial keratitis. However,

in this study contact lens did not prove to be significant predisposing factor for *Acanthamoeba* and *microsporidial* keratitis. This is in contrast to the predisposition to *Acanthamoeba* keratitis in contact lens wearers in developed countries. In developing countries predisposition to bacterial keratitis may be attributed to bacterial contamination and formation of slimy bio-film on the posterior surface of the contact lens leading to spoilage of Contact Lens and risk of subsequent bacterial infection[69]. This difference can be attributed to the difference in prevalence of contact lens wear between developed and developing countries, and the care of Contact lens and environmental factors.

**3. Topical steroid use:**

Topical steroids is principal risk factor in enhancing fungal growth in the cornea. Topical steroids predisposed to microbial keratitis in 26 cases, of whom 50 per cent had fungal etiology[69]. Other studies reported 10-25 per cent cases of mycotic keratitis due to topical steroid usage[69] .600 cases of corneal ulcer patients attending the Sarojini Devi eye Hospital and Institute of Ophthalmology, Hyderabad. 36 cases out of 600 corneal ulcers were found be of fungal aetiology. In this series 36% patients gave history of steady and frequent application of steroids and antibiotics to the eyes, before the development of fungal keratitis[14] .Prolonged use of broad-spectrum antibiotics and indiscriminate use of steroids such as corticosteroids has generally been described to enhance the problem of mycotic keratitis. This may be attributed to disturbance in the microbial flora of the eye and local immunosuppression that probably helps the saprophytic fungi to become pathogenic[72]. A review study during 1971 to 1981, at the University of Minnesota[73] Hospital showed that all patients received systemic/topical corticosteroids prior to the treatment of keratomycosis.

**4. Co-existing ocular diseases:** These include chronic dacryocystitis, spheroidal degeneration of the cornea, blepharitis, conjunctivitis, pre-existing viral keratitis, bullous keratopathy, dry eyes and exposure keratitis were all associated with microbial keratitis. Co-existing ocular diseases (listed above) predisposing to bacterial keratitis accounted for 69% patients, and 78.3%were frequently documented among patients older than 50 years in a study from south India4. Systemic diseases – Diabetes mellitus, Alcoholism, malnutrition, vitamin A, deficiency, Immunosuppression, Burns, Coma. Diabetes mellitus was identified as the predominant systemic disease associated with fungal and bacterial keratitis similar to a study from Hyderabad[67]. However, several other large series of mycotic keratitis reported from tropical countries do not support diabetes as a risk factor for the development of mycotic keratitis[74] .*Microsporidial* keratitis is seen with immune compromise, especially in persons with HIV.

**5. Age/Sex:** Males are commonly affected as compared to females due to their higher outdoor exposure. Fungal keratitis commonly seen in persons aged between 20 to 45years of age.

A study in Assam, India showed that 67.6% were males. The most commonly affected age group was 41-50 years (25.5%) [31]. The incidence of fungal keratitis was more in males (75.21%) compared to females (24.78%) and the most common age group was between 31 to 40 years[75] in a study from western part of India. A study from Tamil Nadu[69], reported fungal and *Acanthamoeba* keratitis was found to be higher in the younger age group while bacterial keratitis was frequent among elderly patients. The authors have explained that there are more chances of corneal injury in the young as an occupational hazard and more chances of co-existing ocular diseases in the elder population.

**6. Occupation:** Fungal keratitis commonly seen in persons engaged in agricultural activities[70]. A fungal corneal ulcer in people involved in onion harvesting was reported in Southern Taiwan[76]. Fungal and *Acanthamoeba keratits* were more common in agricultural workers and rural population as against bacterial keratitis among non-agricultural workers. This can also be explained by the greater incidence of vegetative corneal injury among the young rural population engaged in agriculture thus predisposing to fungal ulceration and *Acanthamoeba* keratits[69].

## PATHOGENESIS [77,78]

### Normal mycotic flora

Fungi are not part of the normal flora of the lids or conjunctiva of normal eyes. They are only transient colonizers. When specimens are taken from the conjunctiva or lids, the same fungus is rarely isolated sequentially in an individual. Most cultures grow only one or two fungal colonies, suggesting a very low burden of organisms. Fungal species are found in soil and vegetable matter and are associated with corneal infections. Fungi can be cultured from 2.5% to 52% of normal eyes, depending on climate and occupation.[79]

Innate immune mechanisms play an important role in host defenses by[80,81]:

1. Blinking: Blinking is a very effective cleansing mechanism. The lashes are able to trap microbes, preventing access onto the globe.
2. Eyelid contains sebaceous glands that secrete lactic acid and fatty acids in a low pH environment, which has a direct inhibition on bacterial replication.
3. The tear film at neutral pH and constant blinking act as a mechanical barrier against infection.

4. Lactoferrin, lysozyme, β-lysin, secretory immunoglobulin, IgG and complement are all present within the tear film protecting against bacterial invasion.

5. The normal ocular flora provides a balance to help prevent overgrowth of exogenous organisms

6. The conjunctiva contains subepithelial mucosal associated lymphoid tissue with a collection of lymphoid cells with specific defense mechanisms.

7. Smooth corneal surface with intact corneal epithelium.

Barrier functions of cornea against infections is compromised by the following factors Mechanical or chemical damage to endothelium, status of the disease, calcium free solutions, oxidation of intracellular glutathione, PH and preservative of ophthalmic solutions.

**Fungal keratitis[54]**

The intact corneal epithelium is generally resistant to fungal penetration and infection. The pathogenicity of fungi is dependent on the characteristics of the invading organism and the status of the normal defense of the host. Fungi gain entry into the eye in the following manner.

a) Exogenous by direct invasion of the external eye as in fungal conjunctivitis, fungal keratitis, fungal infection of the lacrimal passages. Infection may extend to deeper tissues, e.g., various strains of A*spergillus, Candida, Nocardia, Cephalosporium, Actinomyces* etc.,
b) After instillation of herbal drops as a part of native treatment, or through indiscriminate use of antibiotics and cortico-steroids.
c) Extension from infected neighbouring structures as in fungal dermatitis, nasopharyngitis, sinusitis.

d) May gain entry into the inner eye by perforating wounds or during operation or postoperatively causing fungus endophthalmitis.

e) Haemotogenous spread often missed especially when they are from an occult source.

f) Lack of personal hygiene especially in women suffering from fungal vaginitis and allied fungal disorders.

Fungi gain entry into the corneal stroma through the defect in the epithelial barrier by various mechanisms listed above. Fungi adheres by a number of surface molecule adhesions, some of which will inhibit attachment of neutrophils and helps in protection of the organism within the stroma. The virulent fungi can penetrate deep into the stroma and through an intact Descemet's membrane. Once fungi gains access into the anterior chamber, or to the iris, lens, eradication of the fungi becomes extremely difficult. Also the fungi extending from the cornea to the sclera is also difficult to control. Avascular tissues of the eye such as cornea, anterior chamber and sclera are not accessible for growth inhibiting factors and treatment via blood. So the fungi grows rampantly despite the treatment. Once in the stroma, fungi multiply and can cause tissue necrosis and a host inflammatory reaction[77.]

The Fungi produce proteases and toxins such as trichothenes, ochratoxins, cytotoxins, aflatoxin and gliotoxins. Gliotoxins are produced by *Aspergillus fumigatus* and *Penicillium* .They have antibacterial, antiviral, antitumor, antiphagocytic properties and are known to suppress the immune function. Several factors contribute to C. albicans pathogenicity, such as surface adhesins, protease secretions, and morphological transformations from yeast to the hyphal form[82.] Hyphal and pseudohyphal mannoproteins inhibit attachment and digestion by neutrophils. Hyphal forms have

ability to invade epithelial cells and leucocytes. The large size of the hyphal and pseudohyphal forms also may inhibit phagocytosis [78,83].

**Bacterial keratitis[77]**

The surface of the cornea is protected by innate immune mechanisms as described above. The protective epithelial barrier has to be breached for the microbes to enter the cornea and establish the disease. However there are number of organisms that can penetrate the intact epithelium of the cornea or conjunctiva. These include *Neisseria gonorrhoeae, Neisseria meningitidis, S. pneumoniae, Listeria monocytogenes,* and *Corynebacterium diphtheriae*. There are a number of mechanisms [2] that are involved in bacterial keratitis: adherence, invasion and inflammation and tissue damage.

Once corneal trauma has occurred, the pathogen will adhere to the damaged epithelial cells of the cornea, basement membrane or to the stroma near to the edge of the wound. This is initiated by the bacterial adhesins and bacteria will interact with the glycoprotein receptors of the damaged corneal epithelium. Pili (fimbriae) will facilitate the adherence as seen with *Pseudomonas* and *Neiserria* species. The frequent occurrence of keratitis due to *Staphylococcus aureus, Streptococcus pneumoniae* and *Pseudomonas* is attributed to this adherence mechanism to an epithelial defect. Bacteria starts to invade within hours of exogenous contamination of a corneal wound or after the application of a heavily contaminated contact lens. Sometimes colonization of the corneal surface precedes stromal invasion. If there is no intervention with antibiotics at this stage, the bacteria continue to invade and replicate within the stroma resulting in progressive enlargement or extension of infectious foci into the surrounding cornea.

The bacterial capsule and other surface components are important in corneal invasion. For example, some bacteria avoid activation of the alternate complement pathway

because of their capsular polysaccharide. Lipopolysaccharides, the subcapsular constituents of bacteria, are the major mediators of corneal inflammation. Bacterial invasion into surface epithelial cells is partially mediated by the interactions between the bacterial cell-surface proteins, integrins, epithelial cell-surface proteins, and the release of proteases by bacteria. Microorganisms in the anterior stromal lamellae produce proteolytic enzymes, which destroy stromal matrices and collagen fibrils forming an ulcer. The bacteria that are viable are found near the peripheral margins of the infiltrate or deep within a crater of central ulcer. Acute inflammatory cells invade within a few hours after bacteria gain entry. As neutrophils accumulate at the infected site, more cytokines such as leukotrienes, tumor necrosis factor (TNF)-alpha and interleukin-1, and complement components are released to attract additional leukocytes. Macrophages subsequently begin to migrate to the cornea to ingest invading bacteria and degenerating neutrophils. There is extensive stromal inflammation which eventually leads to proteolytic stromal degradation and liquefactive tissue necrosis.

**Viral Keratitis[55]:**

**Herpes simplex virus (HSV):** Initial HSV infection occurs by direct contact of mucus membranes with infected secretions. On contact the virus enters epithelial cells, replicates, enters the sensory nerve endings and travels in a retrograde fashion to the trigeminal ganglion where it remains latent. The cornea may also be a site of HSV latency and replication. After an initial round of replication in the trigeminal ganglion, the virus travels back down the nerve in an antegrade fashion, causing primary infection in 1–6% of patients. It then remains latent until certain triggers cause it to reactivate, replicate, and travel back down the nerve to cause recurrent infection. There are many factors involved in the activation of recurrent HSV ocular disease. Factors include

trauma (including surgical trauma) abnormal body temperature, other infectious diseases and emotional stress.

**Herpes Zoster Keratitis:** Herpes zoster ophthalmicus (HZO) caused by Varicella zoster. It occurs by two mechanisms. The most common mechanism is reactivation of latent trigeminal nucleus infection occurs following previous episode of varicella (chicken pox) or herpes zoster ophthalmicus. The other mechanism is by direct inoculation of the exogenous virus by contact in patients having varicella or herpes zoster. The HZO virus reactivates in 10–25% of the population. The ophthalmic division of the trigeminal nerve is affected 20 times more frequently than the maxillary or mandibular divisions. Ocular involvement occurs in more than 70% of patients with zoster of the first (ophthalmic) division of the trigeminal nerve. Once virus gain entry it attaches to host cells by binding to heparan sulfate proteoglycans and mannose-6-phosphate receptors. After attachment, the glycoproteins within the viral envelope fuse with the host cell membrane, and the virus uncoats and travels to the nucleus where it initiates transcription of viral genomes.

Corneal pathology results from three pathophysiologic mechanisms: (1) active viral infection; (2) immune-mediated inflammation; and (3) chronic neurotrophic keratopathy. Active viral infections tend to affect the epithelium, leading to punctate epithelial keratitis and pseudodendrites. These lesions likely contain live virus. An immune-mediated stromal keratitis can take multiple forms. Nummular keratitis is the earliest finding of corneal stromal involvement and presents during the second week of the disease in 25–30% of patients. Active zoster infections travel through branches of the ophthalmic division of cranial nerve. Each reactivation damages the corneal nerves causing progressive neurotrophic cornea.

**Parasitic Keratitis[55]:**

Parasitic keratitis can be due to:

1. Direct inoculation: Ex: *Acanthamoebae, Microsporidiosis*
2. Endogenous origin due result of a systemic infection. Ex: Onchocerciasis, Leishmaniasis, Trypanosomiasis

**Acanthamoebae:**

*Acanthamoebae,* found ubiquitously in water, soil, and air. They are free living protozoans that exist in an active trophozoite form and a dormant cyst form. The trophozoite feeds on microorganisms and reproduces by binary fission, but if deprived of a food source will encyst. Keratitis caused by *Acanthamoeba* is less common than caused by bacteria or fungi. *A. castellanii* is the most common species associated with keratitis. The risk factors associated with *Acanthamoeba* are: contact lens users and exposure to fresh water sources. *Acanthamoebae* binds to corneal epithelial cell by expression of mannose binding glycoprotein. This expression increases due to corneal injury such as contact lens users. Once bound, *Aanthamoebae* then express proteases such as MIP-133 which degrade both corneal epithelium and corneal stroma, promoting invasion and ulceration. In the corneal stroma, the organism can proliferate, and engulfs the resident bacterial or, possibly, resident keratocytes. This is followed by systemic immune sensitization expressed as serum anti-Acanthamoeba IgG, probably from other exposures. Once *Acanthamoebae* are established in the stroma, this systemic immune sensitization appears ineffective in clearing infection. The exact mechanism of contact lens-induced risk for *Acanthamoeba* infection is not clear, however it is known that they cause a significant immune deviation of the ocular surface, permit binding of microorganisms, and promote biofilm formation on the lens. This provides rich food

supply to encourage proliferation and extend contact time of the amoeba with corneal epithelium, increasing chances of successful binding and ulceration

**Microsporidia**

*Microsporidia* is a group of obligate intracellular parasites closely related to fungi. They are transmitted through air or water by highly efficient spores which utilize an integrated filament to pierce and attach to a target cell's wall. Once attached by this tubule, the infective sporoplasm enters the host cell's cytoplasm, and replicates either freely in the cytoplasm or within a cytoplasmic vacuole with the formation of new spores. These spores reach maturity and burst opens the host cell. The major risk factor is exposure to water or mud, contact lens wear and immune compromised states like HIV and topical use of corticosteroids.

**Parasitic Keratitis of Primarily Endogenous Origin**

These organisms all utilize an insect vector to penetrate systemic defenses. Once established, spread occurs hematologenously to the limbal end vessels, most commonly resulting in invasion of the corneal stroma to produce a centripetal interstitial keratitis. Ex: Onchocerciasis (river blindness), Leishmaniasis and Trypanosomiasis.

## CLINICAL FEATURES [77,78]

1. **Fungal keratitis.**

Onset is insidious but slowly progressive. In most infections, symptoms and signs occur within 1-2 days of ocular trauma with vegetable matter. The patient experiences the symptoms of ulceration namely foreign body sensation, watering, photophobia, pain and redness. Keratitis due to candida may present with gradual onset of pain, grittiness, photophobia, blurred vision and watery or mucopurulent discharge.

Slit lamp examination shows specific and non-specific signs as shown in Figure. 5

Signs of candida keratitis shows yellow-white densely suppurative infiltrate and a collar-stud morphology[84] Signs like elevated areas, branching ulcers, irregular feathery margins, a dry rough texture, and satellite lesions suggests diagnosis of keratitis caused by filamentous fungi [85,86].

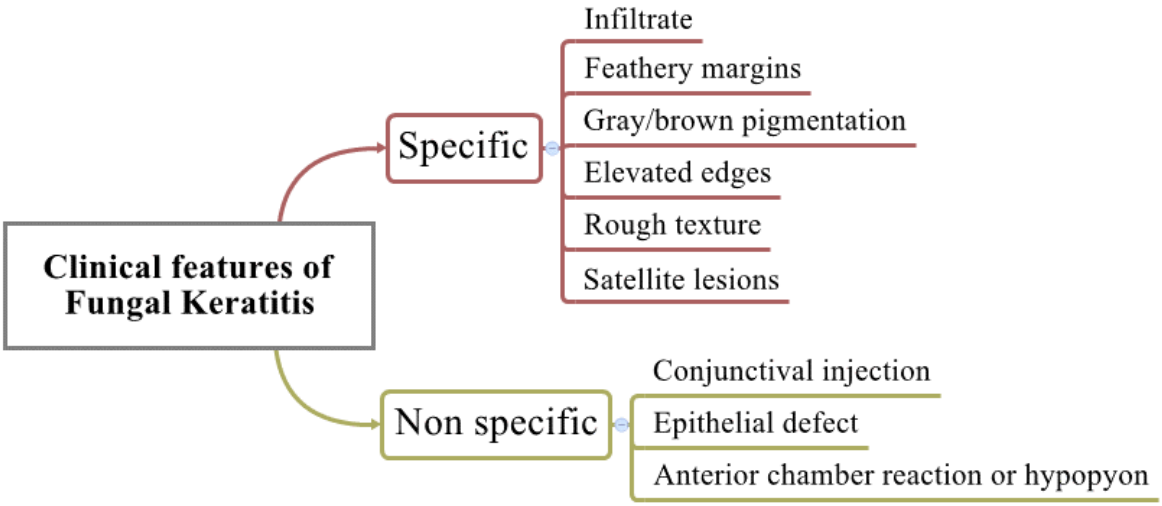



**Figure 5. Classification of Fungal Keratitis**

*Aspergillus* may be more likely presents with a ring infiltrate, whereas dematiaceous fungi (Curvularia lunata) shows brown pigmentation or raised infiltrate [84].

Confocal microscopy[87] is a novel investigation procedure in the early diagnosis of fungal keratitis. It can differentiate fungal keratitis from other forms of microbial keratitis .It also differentiates filamentous keratitis from yeast keratitis. Filamentous fungi grow in a plane horizontal to the stromal lamellae, whereas budding yeast cells grows in a plane perpendicular to the stromal lamellae [88]

**2. Bacterial keratitis**

Patients present with pain, photophobia, blurred vision and mucopurulent or purulent discharge. The signs of bacterial keratitis appear in a chronological sequence.An

epithelial defect associated with a larger infiltrate. There is enlargement of the infiltrate and the epithelial defect. The infiltrate may be well defined cream color and presents with more severity and necrosis as in Staphylococcus aureus infection. In case of nocardia keratitis, infiltrate is superficial, gray white with appearance like a wreath. There is stromal oedema, folds in Descemet membrane and anterior uveitis. Chemosis and eyelid swelling is seen with severe cases especially with bacillus spp. This is followed with rapid progression of infiltration with an enlarging hypopyon. Pneumococci infection presents with severe anterior chamber reaction with hypopyon. Severe ulceration may lead to descemetocele formation and perforation, particularly in Pseudomonas infection. Endophthalmitis is rare in the absence of perforation. Finally there is scarring, vascularization and opacification. Improvement is usually by reduction of eyelid oedema and chemosis, as well as shrinking of the epithelial defect and decreasing infiltrate density.

### 3. Viral keratitis[77,78]

Herpes simplex virus keratitis (HSVK) is broadly classified into epithelial and stromal/endothelial keratitis. Epithelial keratitis: presents with mild discomfort, redness, photophobia, watering and blurred vision. The epithelial cells are swollen and arranged in punctate or stellate pattern. Desquamation occurs frequently in the center resulting in ulcer with a linear branching pattern.(dendritic ulcer).The ends of the ulcer have characteristic terminal buds and the bed of the ulcer stains well with fluorescein. The margin of the ulcer has virus and can be stained with rose Bengal. There is reduced sensation of the cornea. Indiscriminate use of topical steroids will increase the size of the ulcer in a progressive manner. (geopgraphical ulcer). Healing occurs with mild subepithelial scarring. Stromal/Endothelial Keratitis: Presents with a gradual onset of blurred vision often with haloes around the light. Mild discomfort and redness. Based

on the site and type of involved area, they are classified as endotheliitis, localized endotheliitis, diffuse and linear endotheliitis, Necrotizing keratitis, Immune stromal keratitis and keratouveitis.

4. **Parasitic keratitis**[77,78]

*Acanthamoeba:* The most characteristic signs and symptoms include severe, incapacitating pain, the presence of a ring infiltrate, and radial keratoneuritis.

*Microsporidial* keratitis Patients complain of redness, pain, photophobia and variably decreased vision. This form is most commonly associated with *Encephalitozoon* spp., but other members of *Microsporidium* have been reported. Signs include bilateral chronic diffuse punctate epithelial keratitis. Unilateral slowly progressive deep stromal keratitis may rarely affect immunocompetent patients. Sclerokeratitis and endophthalmitis are rare.

## LABORATORY DIAGNOSIS [18, 19]

**Specimen collection and transport**

1. Corneal scrapings

2. Corneal biopsy

3. Anterior chamber aspirate

**Materials required for specimen collection**

1. Proparacaine hydrochloride 0.5%

2. Kimura platinum spatula

3. Alcohol lamp

4. Glass slides (frosted ends, etched circles)

5. Methyl alcohol 90%; Coplin jar

6. Calcium alginate swabs (plastic shaft)[18,19]

**Corneal scraping[89].**

The corneal scrapings will be collected by an ophthalmologist by using standard techniques under all aseptic precautions.

1. Corneal scraping is indicated whenever microbial keratitis is suspected. It provides material for a microbiological diagnosis, debrides necrotic tissue and enhances antibiotic penetration

2. Scraping is collected after anaesthetizing the cornea with 0.5% Proparacaine drops. With the help of sterile Kimura spatula or Bard-Parker blade No.15 or Iris repositor, scraping is done by applying moderately firm, multiple, unidirectional strokes, under slit lamp illumination

4. Material is collected both from the base as well as from the edge of the ulcer, after retracting the lids properly and after cleaning any discharge or debris from the vicinity of the ulcer.

5. Collection of a corneal swab is not recommended. Use of a calcium alginate swab is sometimes advised for better yield of fungus.

**Corneal biopsy[78]**

1. It is a relatively invasive (trephining) procedure and requires minor OT.

2. The indications of biopsy are

(a)Strong clinical suspicion of fungal keratitis

(b)At least twice negative smear and culture report

(c) No clinical improvement on empiric antibiotic therapy

3. The biopsied material is preferably removed enbloc.

4. It is bisected, half being sent to microbiology laboratory for culture, smear examination, and the remaining half put in 10% buffered formalin for histopathological examination

**Anterior chamber aspirate**

Anterior chamber (AC) paracentesis is done when there is

1. Strong clinical suspicion of intra ocular infection

2. Progressive corneal damage and persistent hypopyon

Procedure:

1. The AC is tapped via the limbus using sterile tuberculin syringe or 22 gauge needle

2. The needle should be removed before the specimen is submitted in order to decrease the danger to laboratory personnel

3. 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.

**Processing of samples**

The scraped out corneal tissue or the biopsied material after homogenization is divided into four portions, one for Gram staining, one for 10% KOH wet mount, one for fluorescent microscopy and the last portion for culture.

Stains: Different strains are used for the detection of various organisms. The Gram and Giemsa stains are the most common initial stains used for the rapid identification of fungi. Other methods of examining smears in patients with suspected fungal keratitis

are potassium hydroxide (10–20%) wet mounts, acridine orange staining, Grocott's methenaminesilver technique,lectins, and calcofluor white preparations

**Interpretation of smear and culture:**

**Smear:**

**a. Gram stain:**

Stains both bacteria and fungi. It classifies the bacteria into two major groups based on the cell wall of the bacteria. Gram- positive bacteria retain the crystal violet-iodine complex and appears purple, whereas the gram- negative bacteria lose their crystal violet –iodine complex with decolorization step and appear pink when counterstained with safranin. Fungal filaments exhibit variability in their staining pattern, with cell wall and septae remaining unstained and only the protoplasm being stained. Yeast on the other hand stain typically blue. It has been reported to yield an accuracy of 66-75%.

**b. Giemsa staining:**

It is used to determine the type of inflammatory cells present. It differentiates bacteria from fungi, and also identifies trophozoites of Acanthamoeba cells. With Giemsa technique the bacteria appear dark blue in color. The yeast cells and fungal hyphae absorb the stain and appear purple or blue while the cell walls and the septations do not stain.

**c. Ziehl-Neilsen Acid-fast stain**: Used for identification of *Mycobacteria*, *Actinomyces* or *Nocardia.*

**d. KOH wet preparation:**

A 10-20% solution of KOH has been used to visualize fungal elements in corneal scrapings. Owing to chitin in their cell wall; fungal elements are clearly delineated in a

homogenous background of corneal tissue digested by KOH. Yeast cells are oval or round and colorless and may sometimes produce psuedohyphae.

An ink- potassium hydroxide preparation (9 parts 10% KOH and 1 part ink) is effective in distinguishing fungal elements [20,89]

**e. Calcofluor white:**

Calcofluor white binds to chitin and cellulose of the cell walls of yeast and filamentous fungi. These organisms stain bright green with Calcofluor white under epiflorescent microscope. The cysts of Acanthamoeba also stain bright green, while the trophozoites stain reddish-orange.

**f. Acridine orange:**

It is a chemoflorescent dye, which stains fungi and bacteria yellow-orange against a green background when pH is acidic and an epiflourescent microscope is used to visualize these organisms. It identifies gram-positive and gram-negative bacteria, yeast and hyphal forms of fungi and both the trophozoite and cyst form of Acanthamoeba.

**g. Lacto phenol cotton blue stain (LPCB)** is used for microscopic examination of fungal elements.

**h. Modified Grocott-Gomori Methenamine Silver Nitrate stain:**

The specimens should be spread onto gelatin- coated slides. With the methenamine silver nitrate stain, fungus cell walls and septa stain black and can be easily seen against the background, which is fairly transparent green.

**i. Periodic Acid Schiff stain:** Hyphae as pinkish red.

A study in China[90] showed that sensitivity of potassium hydroxide wet mount was 81% and following the addition of calcoflour white it was 96.6% in diagnosing fungal keratitis, whereas sensitivity using Giemsa stain was 39.7% and following the addition of calcoflour white stain was 98.3%. A study from Tirunelveli, Tamilnadu reported the sensitivity of KOH wet mount was higher (99.3%) than that of Gram stained smears (89.2%) [91] Diagnostic sensitivity of wet preparation microscopy was found to be 84.85% by comparing its performance to yield of fungal culture, which is the 'gold standard' for laboratory diagnosis [92].Review article published in 2008 reported that sensitivity of KOH wet mount for the diagnosis of fungal keratitis varies between 33 to 92%. Gram's stain has an accuracy of 60-75% in detecting the causative organisms [93].

The sensitivity of KOH and CFW wet mount preparation [94] was 84.6% and in another study the sensitivity for KOH preparation and CFW stain respectively was 83.8% and 93.7%[95] .

**Culture:**

**a) Blood agar:** Enriched media such as blood and chocolate agar help to isolate the fastidious organisms. Blood agar is the standard medium for the isolation of aerobic bacteria at 35 degree Celsius and helps support the growth of most saprophytic fungi at room temperature. The agar is derived from the seaweed and produces optimal surface moisture, and addition of 5-10% red blood cells provides nutrients and an index of hemolysis.

**b) Sabouraud dextrose agar** is readily available universal nonselective medium for primary isolation of opportunistic fungi. Yeast extract is added to improve nutritional characteristics and an antibiotic (gentamicin or chloramphinicol) is added to inhibit bacterial contamination. This medium must not have any additives, such as

cycloheximide, that inhibit the saprophytic fungi commonly responsible for ocular infections. SDA plates are preferred over slants because of ease of inoculation, observation of colony growth, transfer to secondary media and dilution of inhibitory substances for the fungi. The agar medium must be thicker than usual to prevent desiccation during prolonged incubation and the container should be taped closed during incubation and routine examination. The majority of fungi that infect the cornea grow within 3 days and few fungi might take more than 14 days to grow. Therefore, cultures should be kept for three weeks. Once fungal growth has appeared on the primary isolation medium, colonies should be sub cultured promptly to fresh medium for isolation and identification in pure culture. For specific identification it is necessary to induce a fungus to display characteristic conidia, sporangiospores by the use of a special medium or growth conditions. Potato dextrose agar, cornmeal agar, Czapek Dox agar are used.

1. Two sets of SDA with antibiotics without actidione are inoculated and incubated at $25^0$ C and $37^0$C

2. Brain heart infusion agar and Blood agar may also be used for culture of fungal organisms.

3. Corneal scrapings are inoculated in ‘C’ or ‘S’ shaped manner to differentiate inoculum growth over laboratory contaminants

4. All cultures are checked every day during first week and twice a week during next three weeks

5. The mycelial isolates are identified by their colony characteristics and morphology in LPCB mount

6. The yeast isolates are identified by germ tube test, reduction of tetrazolium medium, chlamydospore production on corn meal agar, urease test and sugar assimilation tests

7. Filamentous fungi appear as aerial fluffy colonies on solid media and as a feathery mycelium in liquid medium

8. *Candida albicans* appear as smooth flat colonies that are pasty and milky white.

**c) Thioglycollate Broth:** It promotes the growth of aerobic bacteria, as well as obligate and facultative anaerobic organisms. It consists of the basic nutrients required to support the growth of aerobic bacteria and also has sulf-hydryl compound that act as an oxygen- reducing agent to facilitate the recovery of the anaerobic bacteria. It also supports a number of saprophytic fungi.

**d) For isolation of Acanthamoeba**: The scarped material should be directly inoculated on a confluent lawn of *Escherichia coli* (monoaxonic culture) plated on non-nutrient agar. The laboratories have recommended a temperature of 35 degrees C, possibly with a second plate at 30 or 25 degrees C. the culture plates should be sealed with adhesive tape to prevent evaporation and loss of Acanthamoeba organisms from dying. Acanthamoeba trophozoites track through the lawn of the bacteria. The bacteria do not fill in these paths as there is absence of nutrition for bacteria in the non nutrient agar. The path depicts the ingestion of bacteria by trophozoites; the bacteria are unable to reproduce fast enough to fill in the defect in the nutrient poor medium. The cultures may require more than 9 days to recover the organism and should be maintained for more than two weeks.

**Duration of isolation of organism:**

Most aerobic organisms responsible for keratitis are seen on standard culture media within 48hours. In some cases the pathogen may be recognized in 12 to 15 hours. All

plates should be examined daily with the help of dissecting microscope and liquid media should be evaluated for the presence of turbidity. Growth outside the C streak should be disregarded as it implies contamination and circled with wax pencil. Indigenous organisms in the tear film may appear on the inoculation marks but may be distinguished on the basis of their sparse growth and isolation of the same organisms from the ipsilateral lids or the conjunctiva, if these specimens have been taken. Aerobic cultures of the corneal specimens should be held for 7 days, anaerobic cultures for 7 to 14 days and mycobacterial and fungal cultures for 4 to 6 weeks before being reported as no growth. The majority of fungi causing keratitis can be detected on SDA within 48-72 hours. Initial growth occurs within 72 hours in 83% and within 1 week in 97% of culture. Culture media should be observed for at least 2 weeks before they are considered negative.

**Interpretation of culture results:**

It should be made with regard to the clinical situation, the adequacy of the sample and the possibility of contamination by organisms present on the skin, eyelids and conjunctiva.

**Positive culture:** Reported culture positive rates in presumed infectious keratitis varies from 40 to 73 percent. Criteria for a significant positive culture by some investigators include the clinical signs of keratitis plus one of the following: a) The growth of the same organism is demonstrated on two or more solid media. b) Growth of the organisms should be consistent with clinical signs. c) Smear results are consistent with cultures[64.]

**Negative cultures:** Negative cultures maybe present truly in cases of sterile or noninfectious ulcers or due to prior partial antibiotic treatment, inadequate sampling methods, and improper selection of the media and incubation conditions and false

interpretation of the data. When the culture results are negative, antibiotic treatment can be suspended temporarily for 24 hours and rescraping is done following which repeat cultures are sent and examined.

**Antimicrobial susceptibility testing:**

The preferred methods for testing the susceptibility of the antimicrobial agents are the standard disc diffusion method and the micro-dilution techniques. The limitation of the ocular antimicrobial susceptibility testing is that the results of agar disc diffusion tests relate to the levels of drug in the serum rather than the concentration of antibiotics achieved in the ocular tissues and fluids.

**Non culture methods**

Detection of circulating antigens: Eg: 1,3 beta D glucan is detected by G test. Candida specific products or antigens in clinical specimens include D-arabinitol, cell wall mannoprotein and enolase. Detection of metabolites: detection of specific fungal metabolites in body fluids is done by gas liquid chromatography[17.] Serological tests are used in establishing the diagnosis of fungal diseases in following circumstances

1. Fungal cultures have been non-productive

2. Interpreting clinical significance of positive cultures

3. Identification of new isolate

4. To monitor prognosis and outcome of therapy

The common serological tests include: ELISA, Agglutination test, Counter immunoelectrophoresis, Immunodiffusion, Complement fixation, Indirect fluorescent antibody [18]

**Molecular methods**

The DNA sequencing is generally considered as gold standard method. PCR has been used to detect segment of fungus specific DNA coding for cytochrome P-450 L1 A1 in clinical specimens, chitin synthase gene, 18S rRNA gene and aspartic protease gene in detection of *Candida*[18] The PCR is universally accepted as most popular technique as it can yield quick results, confirming the diagnosis of mycotic keratitis within a few hours. The sensitivity of PCR, taking culture as the gold standard, was quite high between 89 to 94% and specificity ranged between 50-88%7.The limitations of PCR is cost and technical expertise limits its use.[95] The most frequently used targets for identification of *Fusarium* are the β-tubulin gene, the translation elongation factor 1α gene (tef1), and the histone gene. The translation elongation factor 1-a (TEF)[96] gene, which encodes an essential part of the protein translation machinery, has high phylogenetic utility because it is (i) highly informative at the species level in *Fusarium*; (ii) non-orthologous copies of the gene have not been detected in the genus; and (iii) universal primers have been designed that work across the phylogenetic breadth of the genus. TEF is a maker of choice for single-locus identification tool for Fusarium because the gene appears consistently as single copy and shows high level of sequence polymorphism among closely related species in comparison to protein coding genes like calmodulin, beta tubulin and histone H3.

## TREATMENT [63,65,77,78]

Infectious keratitis should be considered an ocular emergency. Aggressive antimicrobial therapy is the primary approach with infectious keratitis. Topical administration is the route of choice, because it provides rapid, high levels of drug in the cornea and anterior chamber. Subconjunctival injection may be helpful in cases with

spread to sclera, or in those patients unable to instill frequent eye drops. Systemic administration results in relatively low antibiotic levels in the cornea. Therefore it is generally advised only when keratitis is complicated by scleritis or endophthalmitis or there is a risk of perforation.

**Bacterial Keratitis**

Initially, empirical topical therapy should start with a broad spectrum antibiotic regimen. The most commonly used regimens include fluoroquinolone monotherapy or combination therapy with a cephalosporin and aminoglycoside; these two regimens are thought to have similar efficacy in most cases, with approximately 90% of cases responding to therapy. Severe or central ulcers are intially treated with a loading dose (e.g., every 5–15 minutes for the first hour) followed by application of topical antibiotics for every 30–60 minutes. If a combination of two antibiotics is prescribed, the drops are given in an alternating fashion. The tapering of the dose is done based on the response to infection. If severe suppurative keratitis is seen, the Gram stain smear will guide the selection of medication. One specific medication may be selected if only one type of bacterium (gram-positive or gram-negative) can be positively identified and the patient has not started an antibiotic previously. If two or more types of bacteria are identified, if the stain and smears are equivocal, or if the patient is on any type of antimicrobial agent, then broad-spectrum therapy is started. Ciprofloxacin remains the fluoroquinolone of choice for pseudomonas aeruginosa. There is emerging resistance for several organisms causing infectious keratitis including pseudomonas aeruginosa and staphylococcus aureus, so fluoroquinolone monotherapy should be applied with great caution and with careful observation for a clinical response.

Cefazolin, a first-generation cephalosporin, is relatively nontoxic to the corneal epithelium, and the agent most commonly used for treatment of infectious keratitis. A third generation agent, ceftazidime, has efficacy against *P. aeruginosa* and is an option for resistant cases. The aminoglycosides most commonly used for the treatment of infectious keratitis are gentamicin and tobramycin. These antibiotics provide excellent Gram-negative coverage and are also active against staphylococci and some streptococci but not against pneumococci. Tobramycin may be more active than gentamicin against *Pseudomonas aeruginosa*. Amikacin is a semisynthetic aminoglycoside that is useful in the treatment of Gram-negative organisms resistant to gentamicin and tobramycin, as well as non tuberculous mycobacterial organisms. Vancomycin is a glycopeptide antibiotic with activity against meticillin-resistant staphylococci and other Gram-positive organisms. However, to minimize the development of resistance, empirical therapy should be used judiciously.

**Fungal keratitis**

There are 4 principal group of drugs for the treatment of fungal disease. These are the polyenes, the azoles (imidazoles and triazoles), the pyrimidines, and the echinocandins. The agents most commonly used for fungal keratitis include the polyenes and azoles. Among the polyenes, amphotericin B and natamycin are used extensively. Polyenes disrupt the cell by binding to fungal cell wall ergosterol and are effective against both filamentous and yeast forms. Amphotericin B is particularly effective against yeasts and is the agentof choice for keratitis caused by *Candida* species, but it is less effective against filamentous organism. Natamycin is effective against yeasts and has a broad spectrum of activity against filamentous organisms. Natamycin is the treatment of choice for *Fusarium* ulcers as it was more effective for this organism[97].

The penetration of topically applied Amphotericin B is found to be less than that of topically applied natamycin through the intact corneal epithelium. In a study from southern Florida[98], natamycin was the initial topical antifungal agent used in 91% (107/118) of patients. Another report[99] stated that 18 consecutive cases of *Fusarium solani* keratitis was treated successfully with natamycin. In cases of Candida spp., amphotericin B may be the drug of choice. Among the azoles, the most commonly used compounds have been topical voriconazole and oral ketoconazole and itraconazole. These inhibit ergosterol synthesis at low concentrations, and at higher concentrations, they appear to cause direct damage to cell walls. Oral fluconazole and ketoconazole are absorbed systematically with good levels in the anterior chamber and the cornea; therefore, they should be considered in the management of deep fungal keratitis. Azoles generally have good activity against yeasts, but more variable activity against filamentous organisms. The newer triazole voriconazole has a broader spectrum of activity but is not as effective as natamycin, especially for Fusarium ulcers.[77] Voriconazole has good corneal penetration when given topically, and good intraocular concentrations when given orally. It can also be administered as an intracameral or intrastromal injection[100]. Several clinical case reports have reported on the successful use of voriconazole on fungal keratitis which failed to respond to conventional agents.However adverse effects of oral voriconazole include visual phenomena and hepatotoxicity and liver function should be monitored. Data on the use of posaconazole in the treatment of fungal keratitis is limited; a few case reports described rapid resolution of infection after oral posaconazole was used as salvage therapy. Miconazole is the drug of choice for Paecilomyces spp. Flucytosine is converted into a thymidine analog that blocks fungal thymidine synthesis. Usually administered in combination with an azole or Amphotericin B. Echinocandins (caspofungin and micafungin) have

also been used in the treatment of fungal keratitis. They inhibit beta-glucan in cell walls. Topical caspofungin 0.5% in conjunction with intrastromal voriconazole successfully treated a patient with Alternaria keratitis. Likewise, micafungin 0.1%, used as a single agent, successfully treated three patients with Candida keratitis.

**Parasitic Keratitis:**

**Acanthoemba Keratitis:** The most effective medications are the biguanide antiseptic agents chlorhexidine and polyhexamethylene biguanide (PHMB), which act by inhibiting membrane function and are consistently cysticidal. Second-line agents include the diamidines (hexamidine, pentamidine, and propamidine), which inhibit DNA synthesis. Diamidines are also generally cysticidal, though their activity is more variable. Azole medications have activity against trophozoites, but are generally not cysticidal.

**Microsporidial keratitis:** The optimal treatment regimen for ocular microsporidiosis is not well defined. Several medications are currently used, including oral agents such as albendazole and itraconazole, and topical agents such as fumagillin, propamidine, chlorhexidine, polyhexamethylene biguanide (PHMB), voriconazole, and the fluoroquinolones. Fumagillin is one of the more frequently used medications; a 10 mg/mL suspension can be applied hourly for 24 hours and then tapered based on clinical response.

**Viral Keratitis**

Herpes simplex Keratitis (HSVK): Antivirals drugs are nucleoside analogs that competitively inhibit viral DNA polymerase. They may also interfere with host DNA synthesis and cause significant toxicity. Acyclovir and ganciclovir are the most specific for viral polymerase and thymidine kinase and are least toxic.

Topical Aciclovir 3% ointment 5 times daily or Ganciclovir 0.15% gel 5 times daily. or Trifluorothymidine 1% drops every 2 hrs. Systemic therapy - Oral acyclovir 400 mg bd in conjunction with steroid – antibiotic skin creams

**Varicella-zoster keratitis:** Oral aciclovir 800 mg five times daily for 7–10 days, started within 72 hours of onset, is the treatment of choice. Intravenous aciclovir 5–10 mg/kg t.i.d. is indicated only for encephalitis.Other oral antiviral agents such as valaciclovir 1 g t.i.d., famciclovir 500 mg t.i.d. and brivudine125 mg once daily are more expensive than but have a more convenient regimen, are better tolerated, and are as effective as aciclovir.

**Role of corticosteriods**

Some cases of bacterial keratitis may have a beneficial role with topical corticosteroid therapy however, there is no conclusive scientific evidence to show that corticosteroids can alter clinical outcome. The advantage of corticosteroids is the possible suppression of inflammation, which may reduce subsequent corneal scarring and associated visual loss. The disadvantages include recrudescence of infection, local immunosuppression, and inhibition of collagen synthesis predisposing to corneal melting, and increased intraocular pressure or cataract formation. Topical corticosteroids were contraindicated in the treatment of fungal keratitis[101] .For HSV keratitis 1% prednisolone acetate or 0.1% dexamethasone is used. The frequency should be based on the severity of inflammation and tapering must be very gradual to prevent rebound inflammation. In Herpes zoster systemic steroids (prednisolone 40–60 mg daily) should be used only in conjunction with systemic antivirals. They have a moderate effect to reduce acute pain and accelerate skin healing but have no effect on the incidence or severity of

postherpetic neuralgia. The role of corticosteroids in the treatment of Acanthamoeba infection has not been established

**Surgical intervention**

**Bacterial keratitis**

Surgical intervention is required when corneal necrosis progresses to perforation or impending perforation, and for corneal ulcers that are not responding to medical therapy. For small perforations without extensive surrounding necrosis, cyanoacrylate glue can be applied. Small perforations have also been successfully managed with fibrin glue and multilayered amniotic membrane grafts. If the perforation is large or necrosis is extensive, corneal transplantation might be necessary. Deep anterior lamellar keratoplasty is an option for corneas that have not yet perforated; this technique may reduce the risk of graft failure and appears to have similar rates of disease recurrence compared to penetrating keratoplasty. Therapeutic keratoplasty can result in favorable surgical results, although the procedure can be complicated by the difficulties involved in operating on a perforated globe, concurrent infection, and inflammation that threatens graft success. Conjunctival flaps can be used to treat infections that fail to improve with medical therapy. This is useful in peripheral infectious ulcer. The vascularized conjunctival tissue helps blood vessels that aid in healing and scarring. Conjunctival flaps should not be placed over a corneal perforation.

**Fungal keratitis**

The fungal elements has a natural tendency to invade deeply and, can penetrate Descemet's membrane. Such advanced cases requires penetrating keratoplasty to ensure complete removal of the invading fungus. Penetrating keratoplasty should be

performed sooner to maximize the probability of a graft margin free of infection and to minimize the risk of endophthalmitis or infectious scleritis.

**Viral keratitis**

Surgery is usually performed when there is limitation of vision due to corneal scarring and in patients with non-healing ulcers or impending perforations from necrotizing keratitis. Debridement is useful for dendritic ulcers. Penetrating keratoplasty (PKP) is preferably done least 6 months after an episode of HSVK. Then corneal transplantation can be performed with increased success rate in an inactive eye. Protozoal keratitis:The role of therapeutic keratoplasty for active acanthamoeba keratitis is controversial.

## PREVENTION [54]

Farmers should be educated regarding the dangers of mycotic infections and should be advised to wear protective safety glasses to avoid dust and minor trauma to the eyes. Introduction of mechanical devices in agricultural occupations may minimize the incidence of mycotic ulcers, of the cornea. Hand washing before touching eyes is essential to avoid fungal infections as seen with fungal vaginitis. Pre-operative prophylaxis with antifungal agents like sodium propionate, amphotericin B eye drops is essential. Care should be taken to prevent infections during operative procedures and post-operative infections by avoiding air-borne organisms, spores in glove powder, inadequate sterilization of instruments, by contaminated solutions and drugs. Routine antibiotics and postoperative steroid therapy should be used with great caution. Routine use of fungicidal sprays in the operating room is desirable. Cleanliness of the operating room by modern methods should be adopted to see that no dust is stirred up during cleaning. Precautions against contamination of fungi from outside environment like street dust, shoes and other uncovered clothing should be rigidly followed.

# 4. MATERIALS AND METHODS

**Source of data:**

During the period from January 2013 to June 2014, corneal scrapings were collected from 75 patients with suspected infectious keratitis attending both outpatients and in patients of the department of Ophthalmology at R.L.Jalappa Hospital, and Sri Narasimha Raja (SNR) Hospital Kolar.

**Inclusion criteria**

The patients clinically suspected to have as infectious keratitis were included in the study.

**Exclusion criteria**

Typical viral ulcers, healing ulcers, Mooren's ulcers, marginal ulcers, interstitial keratitis, sterile neurotropic ulcers, and any ulcers associated with autoimmune conditions were excluded from the study.

**Methods of collection of data:**

A standard protocol was prepared and data related to socio demographic features, occupation, predisposing factors, history of corneal trauma, traumatic agents, associated ocular conditions, other systemic diseases, therapy received prior to presentation , and all clinical findings were recorded.

**Collection of sample:** After a detailed history and thorough clinical

examination the patients were subjected to protocol of investigations which included microscopy and, culture for bacteria, fungi and protozoa causing microbial keratitis

All patients were examined under slit-lamp bio microscope by an ophthalmologist. After a detailed ocular examination using standard techniques eyelids were retracted and cleaned for any discharge or debris from the vicinity of the ulcer. Corneal scrapings were collected by applying multiple, moderately firm, unidirectional strokes, under slit lamp illumination by using a sterile needle (26 guage) under aseptic conditions from the base and margin of each ulcer after instillation of 4% lignocaine (lidocaine) without preservative. The corneal scraping procedure was performed by an ophthalmologist. The corneal scraping material was inoculated directly on to the solid media such as Blood agar, Chocolate agar, MacConkey agar, and Sabourads dextrose agar. The next scraping was done using a separate sterile needle and then inoculated into the liquid media such as Thioglycolate and Brain heart infusion broth. The next scraping was then spread onto 3 clean sterile labelled glass slide for Gram stain, KOH mount and Saline mount. For each scraping a separate sterile needle was used. Care was taken in the collection of material and in inoculating aseptically to the appropriate culture media. Informed consent was taken from the patients.

**Processing of samples**

1. **Microscopy**

Corneal scrapings were directly smeared on a clean grease free sterilized glass slide for Gram stain, KOH and saline mount for direct microscopic examination.

**A. Gram Stain:**

a) The specimen was uniformly spread over 1cm area in the center of clean grease free slide

b) The smear was heat fixed

c) Crystal violet stain was poured over the smear and allowed to stand for 1 minute

d) The stain was poured off and washed gently with water

e) Gram's iodine was poured over the smear and allowed to stand for 1 minute

f) The Gram's iodine was poured off and washed gently with water

g) The smear was decolorized with acetone and quickly washed gently with water

h) The smear was counter stained with dilute safarinin for 30 seconds and washed gently with water

i) The smear was air dried and examined microscopically under oil immersion.

Observation: The smear was observed for the presence of inflammatory cells, bacteria, fungal hyphae, budding yeast cells. The fungal elements appeared Gram positive.

**Quantitative Gram stain:** The Gram stained smears were examined for pus cells and bacteria in more than 10 oil immersion fields for each slide. The Grading was done according to Clinical Microbiology Proficiency-Testing (CMPT) program's recommended for Gram stain reporting criteria[104].(Table 2)

**Table 2: Gram stain Grading**

| | | **No. per oil immersion field (x1000)[a]** | |
|---|---|---|---|
| **Grade** | **Description** | **Cells** | **Bacteria** |
| 0 | No pus cells, No organisms | 0 | 0 |
| 1+ | Rare | <1 | <1 |
| 2+ | Few | 1–5 | 2-10 |
| 3+ | Moderate | 6–10 | 11–50 |
| 4+ | Many | >10 | >50 |

[a] Based on the reading of more than 10 fields

B. **KOH preparation:** was examined as follows: Corneal scraping was placed on clean labeled glass slide. One drop of 10% KOH solution was put over the specimen and a cover slip was placed gently over the specimen. The preparation was examined microscopically under 10x and 40 x magnifications. Undissolved specimens were placed in wet Petri dishes for some time and examined.

   Observation: The preparation was observed for fragments of septate/aseptate hyphae, yeast cells and other fungal elements.

C. **Saline wet mount** was examined as follows: A drop of saline was added to the smear of corneal scraping on glass slide and observed for the cyst and trophozoites of *acanthamoeba*. Only if cyst and trophozoites of *acanthamoeba* was observed in saline mount, then further processing culture was done.

**2. Culture of corneal scrapings**

Corneal scrapings were inoculated into solid media like blood agar, MacConkey agar, Chocolate agar, Sabourauds dextrose agar (SDA) and Potato dextrose agar (PDA) and liquid media like thioglycolate broth and Brain heart infusion broth. All inoculated media were incubated aerobically at $37^0$C and observed for growth 24, 48 and 72 hours. If any growth was observed in solid or liquid media it was sub cultured and processed further.

All methods were followed as per standard laboratory protocols and microbial cultures were considered positive only if at least one of the following criteria is met [64.]

a) The growth of the same organism is demonstrated on two or more solid media.
b) Growth of the organisms should be consistent with clinical signs
c) Smear results are consistent with cultures.

The isolated colonies on solid media were subjected to Gram stain to identify the morphology of the organism and a battery of appropriate biochemical tests were done for the identification of bacteria. Each isolate was tested for antibiotic sensitivity using Kirby Bauer disc diffusion method on Muller Hinton agar (MHA) for non-fastidious organisms and on MHA with 5 % sheep blood for Pneumococci according to The Clinical and Laboratory Standards Institute (CLSI) guidelines.

The growth of fungi on SDA and PDA plates and slopes were checked daily during first week, twice a week for subsequently for three weeks. The growths were identified by microscopy using Lacto phenol cotton blue mount and colony morphology. The media were labeled as sterile if no growth appears at the end of four weeks of incubation and discarded.

Lacto phenol cotton blue mount (LPCB) was done as follows: The fragment of fungal culture was teased on to clean glass slide with a drop of LPCB stain by using two needles. Cover slip was placed over the preparation and examined under the microscope with lower power objective (10x) and high power objective (40x).

Fungal Slide culture preparation was done in the following manner. A sterilized petridish with filter paper, bent glass rod, clean slide and two cover slips was taken. From SDA plate, 1sq cm agar block was prepared using sterile scalpel blade and carefully placed on to sterilized glass slide. Fungal culture to be identified was inoculated on four sides of the agar. Sterile cover slip was gently placed on to agar block and incubated at $25^0$ C in Bio Chemical Oxygen Demand (BOD) incubator. Sterile distilled water was added to filter paper to avoid drying of agar. If growth was observed, coverslip was carefully removed and placed on to a clean glass slide with a drop of LPCB. Agar block was removed carefully and a drop of LPCB was added and covered with a cover slip for microscopic examination under low power objective.

For quick identification of fungi, cellophane tape preparation was made. A piece of cellophane tape was carefully placed on to the fungal colony and slowly lifted and placed on to a glass slide with a drop of LPCB. Care was taken to avoid any air bubbles and both the ends of tape was firmly struck to glass slide and examined under microscope for colony characteristics [18,20]

**Micrometric measurements** [102] of Fusarium was done using eye piece micrometer and calibration slide.

The eye piece micrometer is a small glass disc which has a line of 100 divisions on its surface. Calibration slide has a line of 1mm in length and is divided into 100 divisions. Each slide calibration division is equivalent to 10 micrometers (µm).

The calibration slide was focused using low power objective (10x). The eye piece micrometer was then focused and the slide was moved until the first line on the slide was under the first line of the micrometer. Then next line which coincided again was noted. The number of divisions on the slide (Slide calibration divisions, SCD) and number of divisions on the eye micrometer divisions (EMD) was noted and calculations for low power 10x and 40x was calculated as follows.

Calculation for high power 40x: If 50 EMD corresponds to 10 SCD (Note: 1 SCD=10µm) So 50 EMD= 100µm. For 1 EMD= 100/50= 2 µm.

**Speciation of Fusarium**[27]**:**

The identification of *Fusarium* was done by observing colony morphology, growth rates and pigmentation. The micromorphological characteristics was observed by looking at three types of spores (macroconidia, microconidia, and chlamydospores).The absence or presence of one type of them was noted. The Macrconidia was observed for the size, shape, number of septa, shape of apical and basal cell and for microconidia the size, shape, number of cells, formation, nature of conidiogenous cells, and

conidiophores was observed and noted. The chlamydospores were observed for the presence or absence, if present then size, shape, position and formation All of these characteristics was noted and referred to standard manual[27,103] for tentative identification

All isolates of *Fusarium* was sent to National Fungal culture collection of India(NFCCI)- A National Facility, Agharkar Research Institute Pune, an autonomous Grant in Aid Institute under the Department of Science and Techonology, Government of India for final identification.

**Method of statistical analysis:** Statistical analysis will be done by descriptive statistical methods like mean, and proportions.

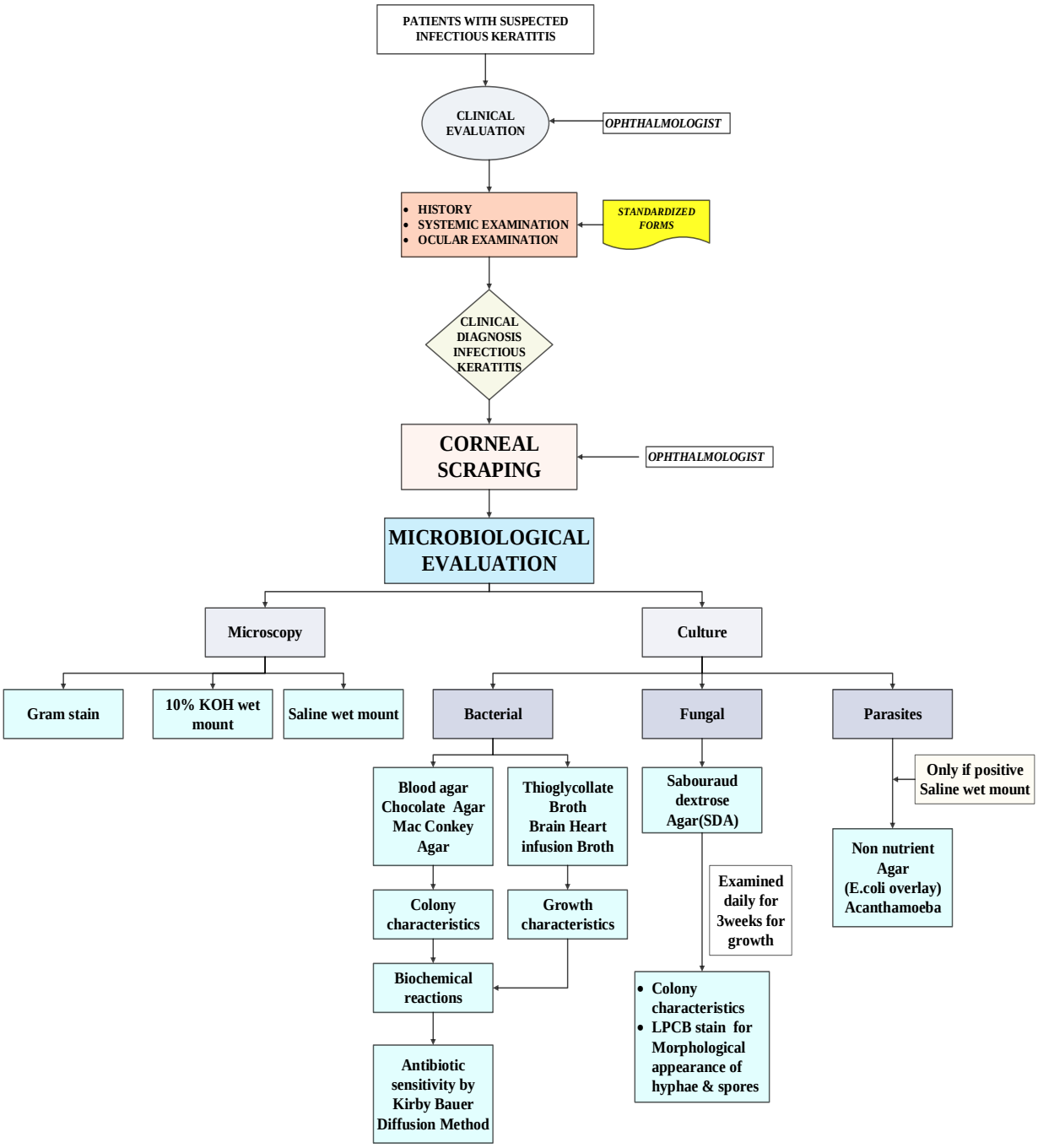



**Figure 6: Flowchart showing corneal sample processing**

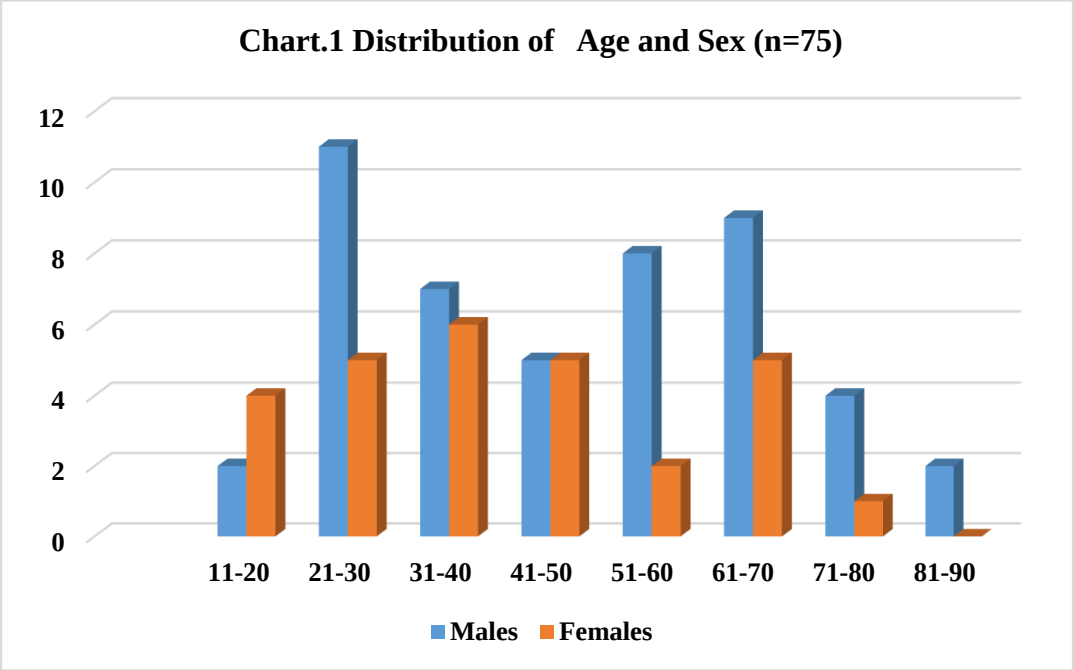
# 5. RESULTS

During the period from Jan 2013 to June 2014, corneal scrapings were collected from 75 patients with suspected infectious keratitis attending the outpatient and inpatients of RLJ Hospital and SNR hospital Kolar.

The distribution of age and sex of 75 patients is presented in Table 3 and Chart 1. There were no patients in age group in 0-10 years. Of the 75 patients studied 63(84%) belong to 21-70 years. Out of 75 patients, 48(64%) were males and 27(36%) were females.

Table 3: Age and sex distribution of 75 patients.

| Age in years | Males(n=48) | | Females(n=27) | | Total | |
|---|---|---|---|---|---|---|
| | No | % | No. | % | No | % |
| 11-20 | 2 | 4.17 | 4 | 14.81 | 6 | 8.00 |
| 21-30 | 11 | 22.92 | 5 | 18.52 | 16 | 21.33 |
| 31-40 | 7 | 14.58 | 6 | 22.22 | 13 | 17.33 |
| 41-50 | 5 | 10.42 | 5 | 18.52 | 10 | 13.33 |
| 51-60 | 8 | 16.67 | 2 | 7.41 | 10 | 13.33 |
| 61-70 | 9 | 18.75 | 5 | 18.52 | 14 | 18.67 |
| 71-80 | 4 | 8.33 | 1 | 3.70 | 5 | 6.67 |
| 81-90 | 2 | 4.17 | 0 | 0.00 | 2 | 2.67 |



The distribution of patients with corneal ulcer by occupation is presented in Table 4 and Chart2. Farmers, manual laborers and house wife's constituted 77.33% patients who presented with corneal ulcer in the study. However activities which generate small broken bits of material as carpenters, stone cutters, welders, and mechanics were also represented in those who had corneal ulcer.

Table4: Occupation distribution

| Occupation | No.(n=75) | % |
|---|---|---|
| Farmers | 30 | 40.00 |
| Manual Laborers | 20 | 26.67 |
| House wife | 8 | 10.67 |
| Student | 5 | 6.67 |
| Welder | 4 | 5.33 |
| Mechanic | 3 | 4.00 |
| Stone cutter | 2 | 2.67 |
| Bar bender | 1 | 1.33 |
| Carpenter | 1 | 1.33 |
| Driver | 1 | 1.33 |

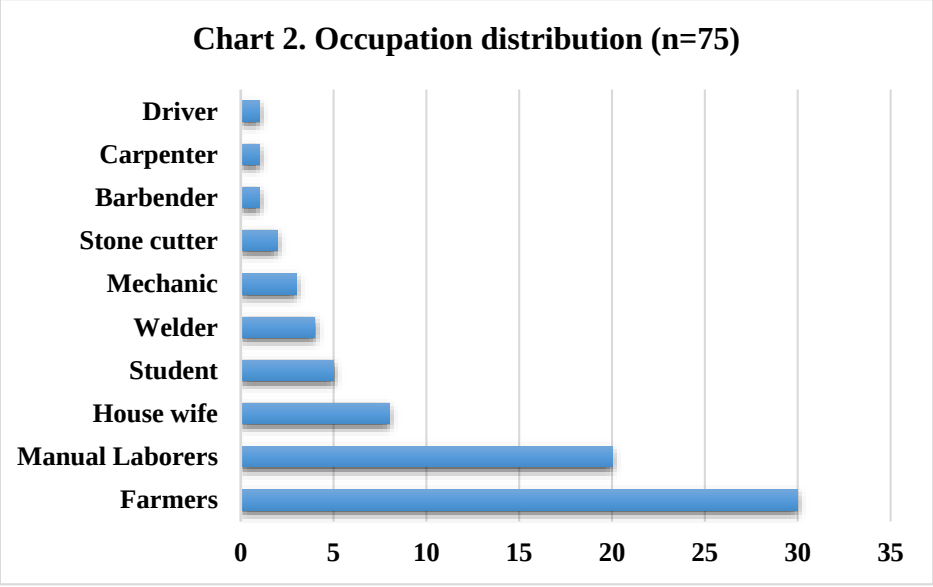
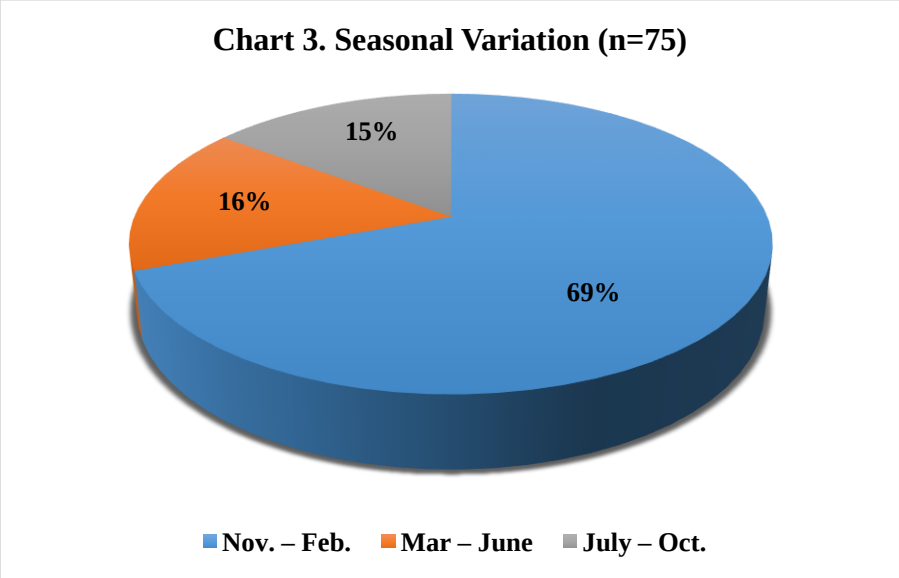


Table 5: Season wise distribution of 75 patients

| Season | No.(n=75) | % |
|---|---|---|
| Nov. – Feb. | 52 | 69.33 |
| Mar – June | 12 | 16.00 |
| July – Oct. | 11 | 14.67 |




The seasonal distribution of corneal ulcers in the study is presented in Table 5 and Chart.3.Though the corneal ulcers were encountered in all seasons, 69% of the cases were found during November and February months.

The predisposing factors for corneal ulcer is presented in Table 6.Corneal trauma was the major predisposing factor among the cases studied which accounted for 82.67%. The other factor was use of steroids which was found in only 4 patients (5.33%).

On further analysis of different modes of corneal trauma fall of vegetable matter in eye was found in 29 patients (38.67%),followed by hit by a foreign body in 19 patients(25.33%) and fall of dust was accounted for 14.67% in 11 patients

Table 6: Predisposing factors for corneal ulcers.

| **Predisposing factors** | **No.(n=75)** | **%** |
|---|---|---|
| **1.Corneal trauma** | **62** | **82.67** |
| a)Vegetable matter | 29 | 38.67 |
| b)Foreign body | 19 | 25.33 |
| c)Dust | 11 | 14.67 |
| d)Cows tail | 2 | 2.67 |
| e)Hair dye | 1 | 1.33 |
| **2.Steriod use** | **4** | **5.33** |
| **No predisposing factor** | **9** | **12.00** |

Table 7: Laterality of eyes

| **Eye** | **No.(n=75)** | **%** |
|---|---|---|
| Left eye | 39 | 52.00 |
| Right eye | 36 | 48.00 |

The laterality of eyes affected with corneal ulcer is shown in table 7. Both eyes were not affected. Out of 75 cases 39(52%) cases presented with corneal ulcer of left eye. 36(48%) cases presented with corneal ulcer of right eye.

Table 8: Location of corneal ulcers.

| **Ulcer location** | **No.(n=75)** | **%** |
|---|---|---|
| Paracentral | 55 | 73.33 |
| Peripheral | 8 | 10.67 |
| Central | 7 | 9.33 |
| Central &paracentral | 5 | 6.67 |

The location of corneal ulcers on clinical examination is shown in the Table 8. Corneal ulcers were paracentral in 55 (73.33%) and peripheral in 8(10.67%). There were

7(9.33%) central and 5(6.67%) cases with central and extending to the paracentral region.

Table 9: Hypopyon in relation to infectious keratitis.

| **Hypopyon** | **No. of cases(n=75)** | **%** |
|---|---|---|
| Present | 13 | 17.33 |

On clinical examination Hypopyon was present in 13(17.33%) patients (Table 9).

Visual acuity at the time of presentation ranged from perception of light to 6/9. Only 2(2.67%) cases presented with perception of light.

Table10: Direct microscopy of corneal scrapings

| **Method** | **Positive** | **%** |
|---|---|---|
| Gram stain | 37 | 49.33 |
| 10% KOH | 15 | 20.00 |

The results of direct microscopy of organisms among corneal ulcer patients is shown in Table 10.Out of 75 cases, Gram stain was positive in 37 (49.33%) cases.

The grading of Gram stain is presented in Table 11 and Figure 11-14.

The grading of Gram satin showed that 38 cases fell under 0 grade (50.67%) with no pus cells and no organisms. These were categorized as negative with Gram stain. Among 22(29%) which were positive in Gram stain fell under grade1+( pus cells less than1, bacteria less than 1).

Table 11: Gram stain grading

| Method | Grading | No. | % | Fungal elements in 10% KOH |
|---|---|---|---|---|
| Gram stain | 0 | 38 | 50.67 | 1 |
| | 1+ | 22 | 29.33 | 4 |
| | 2+ | 9 | 12.00 | 7 |
| | 3+ | 2 | 2.67 | 1 |
| | 4+ | 4 | 5.33 | 0 |

9 (12%) fell under 2+(pus cells 1-10,Bacteria 2-10). There were 6(8%) smears which could be graded as 3+ and 4+. (3+ pus cells 6-10, Bacteria 11-50) and 4+ pus cells greater than10, and Bacteria greater than 50). In the Gram stain, Gram positive bacteria and Gram negative bacteria could be easily detected. Fungi appeared as Gram positive filamentous thread like structures (Figure 7-10). No spores could be visualized.

Saline wet mount did not show any cysts or trophozoites.

KOH mount was positive in 15 cases (20%) which showed fungal elements and pigmented fungi. Among 15 cases, 13 were picked up by Gram stain and 2 positive by KOH were not picked up by Gram stain.

Table 12: Percentage of growth on Culture media

| Culture | Growth | % |
|---|---|---|
| Blood agar | 16 | 21.33 |
| Chocolate agar | 16 | 21.33 |
| Mac Conkey agar | 4 | 5.33 |
| SDA | 13 | 17.33 |

The percentage of growth on different media is shown in Table 12. Growth was observed in 16(21.33%) on both Blood agar and Chocolate agar. Growth on SDA was obtained from 13 (17.33%) specimens. No growth could be obtained in thioglycolate

and Brain heart infusion broth. Thus both Blood agar and Chocolate agar supported the maximum number of growth from corneal scrapings.

The different types of organisms including bacterial and fungal grown on each culture media is shown in the Table 13.

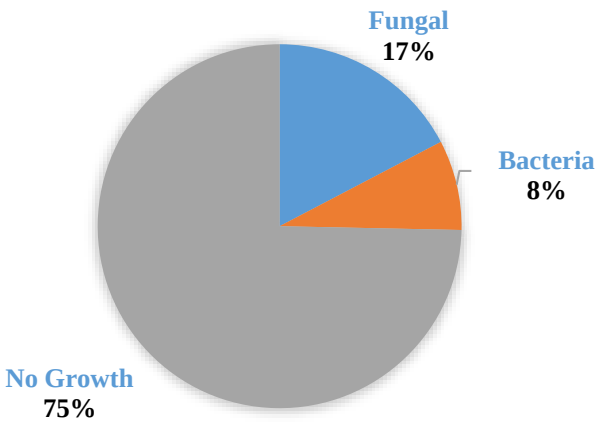
Table 13: Types of organisms grown on culture media

| **Culture** | **Type of organisms grown** |
|---|---|
| Blood agar | Streptococcus pneumoniae. Klebsiella pneumoniae Fusarium spp. Aspergillus spp. Colletotrichum spp. |
| Chocolate agar | Streptococcus pneumoniae, Klebsiella pneumoniae, Fusarium spp. Aspergillus spp. Colletotrichum spp. |
| Mac Conkey agar | Klebsiellla pneumoniae, Fusarium spp. Colletotrichum gleosporoides |
| SDA | Fusarium spp. Aspergillus spp. Colletotrichum spp, Curvularia spp. |

Table 14: Distribution of microbial isolates

| **Microbial isolates(n=75)** | No.of isolates | % |
|---|---|---|
| Fungal | 13 | 17.33 |
| Bacteria | 6 | 8.00 |
| No Growth | 56 | 74.67 |

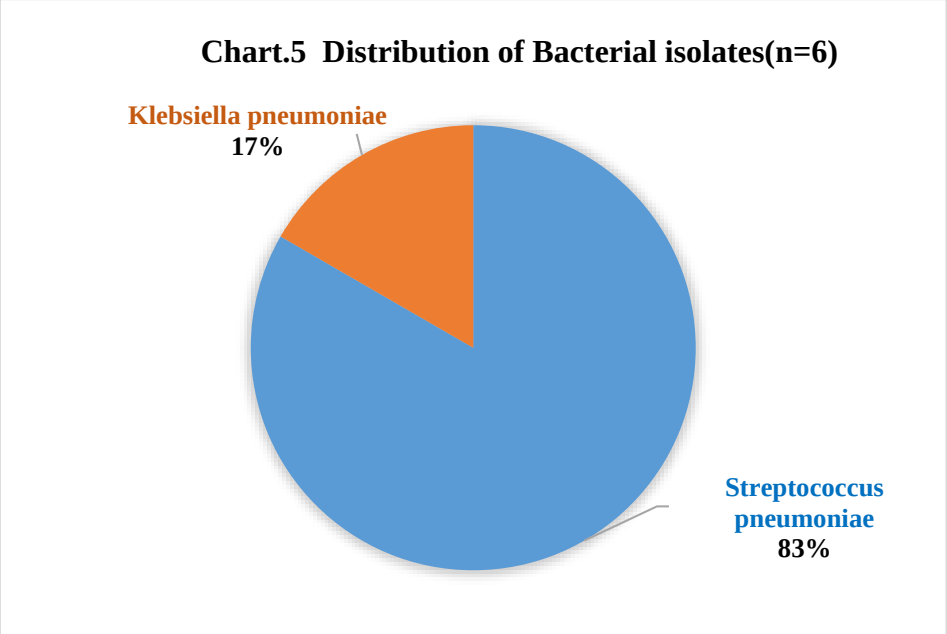
**Chart. 4 Distribution of Bacterial and Fungal isolates (n=75)**




All samples that showed on Gram stain grew organisms. SDA yielded fungal growth in 13 samples, 2 samples that showed fungal elements in KOH were negative on culture. Thus taking everything together there were 13(17.33%) fungal isolates and 6(8%) were bacterial isolates( Table. 14) There was no growth in 56(74.67%).Thus positivity rate of total microbial isolates in our study is 25.33%.(Chart 4).

Table 15: Distribution of Bacterial isolates

| **Bacterial isolates(n=6)** | **No.** | **%** |
|---|---|---|
| Streptococcus pneumoniae | 5 | 83.33 |
| Klebsiella pneumoniae | 1 | 16.67 |



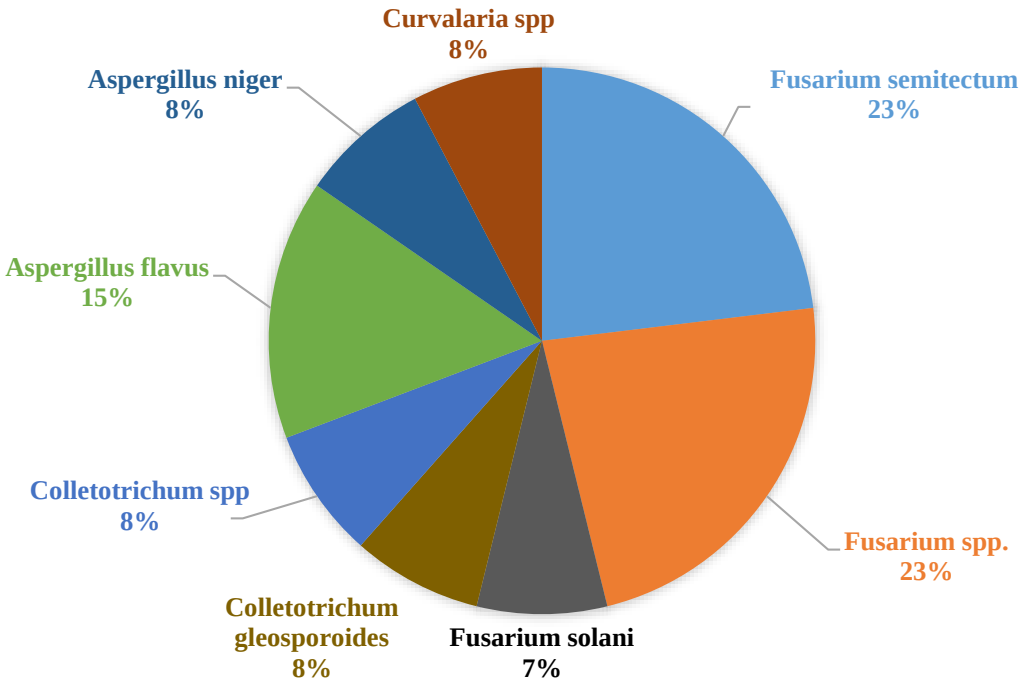

The distribution of bacterial isolates is presented in Table 15 and Chart 5. Among the bacterial isolations made in the study, 5 were *Streptococcus pneumoniae* and 1 was *Klebsiella pneumoniae.*

The distribution of fungal isolates is presented in Table 16 and Chart 6. 7(53.85%) of fungal isolates were *Fusarium* species, 3(23%) *Aspergillus* species, 2(15.38%) *Colletotrichum* species and 1(7.69%) *Curvularia* species.

Table 16: Distribution of Fungal isolates

| **Fungal** | **No.** | **%** |
|---|---|---|
| Fusarium spp. | 7 | 53.85 |
| Aspergillus spp. | 3 | 23.08 |
| Colletotrichum spp. | 2 | 15.38 |
| Curvularia spp. | 1 | 7.69 |

**Chart. 6 Distribution of Fungal isolates(n=13)**




Further speciation of *Fusarium* showed, 3(23%) belong to *Fusarium semitectum*, 1(7.69%) *Fusarium solani*, and the rest 3 species of *Fusarium* could not be identified by morphological characteristics.

*Fusarium solani* was identified by the following colony characteristics[103]: On culturing on SDA, the aerial mycelium was white to pinkish raised edges, scanty aerial mycelium towards the center but more towards periphery (Fig 31). On reverse it was tan to brown (Figure 32). The growth rate of the colony was 3.5 cm over 5 days. On microscopic examination using lactophenol cotton blue, hyphae was hyaline and septate (Fig 29). Macrconidia were abundant, curved, apical cell pointed with a beak like appearance, Basal cell was blunt with 0- 3 septa and size of the macroconidia was 12-21x 3-3.5µm. Microconida: Oval, ellipsoid, fusiform with 1 septa.Size:10.8µ x 2.7µm with long monophilades. Chalmydospores were oval rough, arranged in pairs, chains, and intercalary. Size: 9-12µm. (Fig. 35-38)

*Fusarium semitectum* was identified by the following colony characteristics[103, 127,128]: On SDA the colony appeared white to slight pinkish with aerial mycelium, on and on

reverse it was tan brown in the center. Growth rate was 2-4 cm for 7 days. On PDA the colony appeared whitish aerial mycelium in periphery with pinkish towards center and we also observed furrows radiating towards periphery. On reverse it was tan to brown. On microscopic examination hyphae was hyaline and septate. Macrconidia appeared sparse, thin walled, curved with foot cell pointed at other end, 3 septate, Size of the macroconidia measured 24µm x 3µm, and others were 21x 2.5µm. Microconidia were sparse and measured 6x3µm. Chlamydospore was solitary, smooth walled, oval shaped and measured 12µm. (Fig.19-26)

*Colletotrichum gleosporiodes* was identified by the following colony characteristics. On culturing on SDA, the colonies appeared greyish brown raised, puckered colonies Reverse brown color. On microscopy hyphae was hyaline and septate. There was dark swellings on hyphae called appresoria which were abundant, smooth walled, globose, arranged in chains and size was 18 µm. Conidia was abundant, hyaline, straight or cylindrical, single celled, smooth conidia with no septae. Size: 18µm x4µm. The cylindrical conidia can be misidentified as Fusarium spp. The absence of the septation within the conidia and the presence of the appressorias, are the two important characteristics that distinguished Colletotrichum spp. from the Fusarium spp. Fig 41-46

*Curvularia lunata* was identified based on the following colony characteristics: On culturing on SDA grayish black woolly colonies and on reverse black pigmentation was observed. On microscopic examination, dark brown septate hyphae was observed. Conidiophores were long simple or branched. Conidia showed 3 septate with 4 cells. The third cell was curved, thick darker, larger than other cells and the ends were pale brown.

*Aspergillus flavus* was identified based on the following culture characteristics: On culturing on SDA, colonies were woolly yellowish to olive green and reverse side

golden brown. Rapid growth was observed. Hyphae was septate and hyaline. Conidiophores are rough and uncolored, vesicles were globose, metulae covering the vesicle in biseriate. Conidia was globose and smooth. Fig 57-62

*Aspergillus niger* was identified based on the following colony characteristics: On culturing on SDA,dark brownish black colonies with whitish margin in periphery and on reverse side yellow color with furrows radiating from the center was observed. On microscopy, smooth long hyaline conidiophores, at the vesicle conidiophore is slight brownish in color. Vesicle is globose, conidia is black, rough and round. Biseriate metuale and phialides covered the entire vesicle.Fig 65-70

Among the Gram positive cocci, all the 5 isolates of *Streptococcus pneumoniae* was found sensitive to all drugs such as Penicillin, Erythromycin, Gentamicin, Chloramphenicol, Tetracycline, Ciprofloxacin, Linezolid, Cotrimoxozole and Vancomycin. The antibiotic sensitivity of one Gram negative bacteria, *Klebsiella pneumoniae* was found to be AmpC producer and was sensitive to ciprofloxacin and carbapenems.

Eye drops containing Natamycin (5%), Fortified tobramycin and was advised empirically and Voriconozole eye drops in some patients. 13 patients were followed up for first week and patients had shown signs of improvement in symptoms and visual acuity. In some cases hypopyon was regressing. 6 patients went against medical advice. Among the patients whose corneal ulcers yielded bacterial pathogens 3 patients with streptococcus pneumoniae showed improvement with Gatifloxacin and fortified tobramycin and ceftriaxone and 3 patients went against medical advice.

**PHOTOGRAPHS**
**GRAM STAIN SHOWING FUNGAL ELEMENTS.**

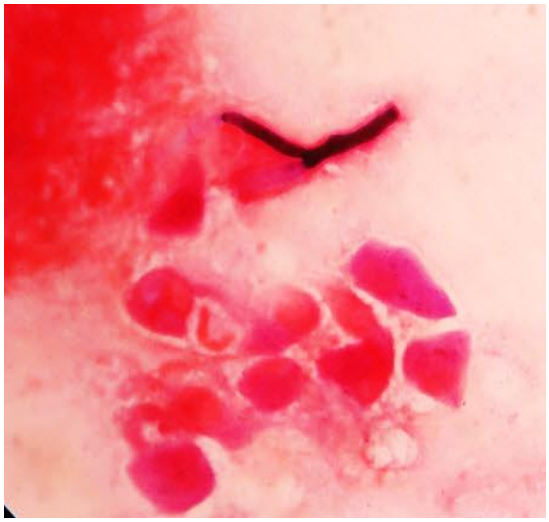


Fig 7: Few pus cells with Gram positive branching filaments in Gram stain

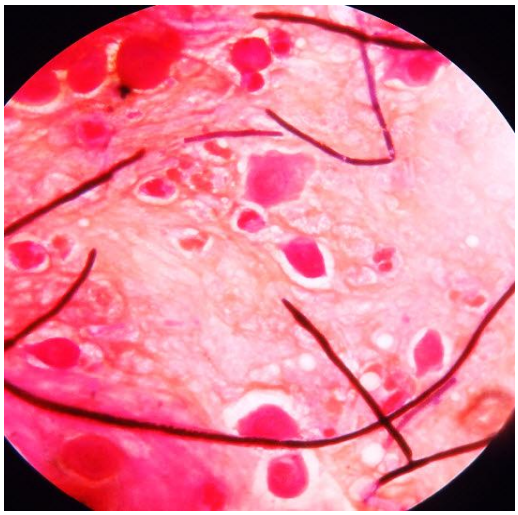


Fig 8: Moderate pus cells with Gram positive long filaments in Gram stain.

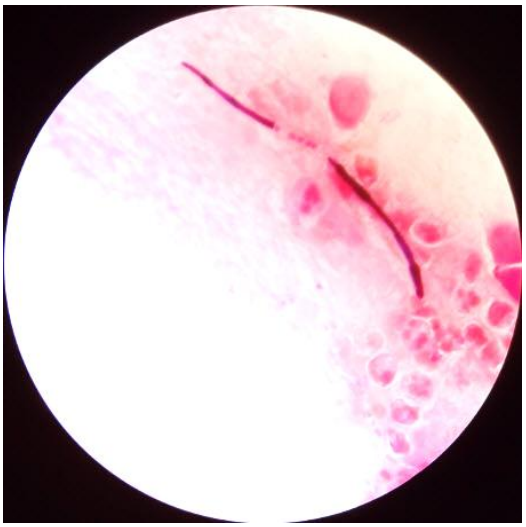


Fig 9: Numerous pus cells with Gram positive long filament

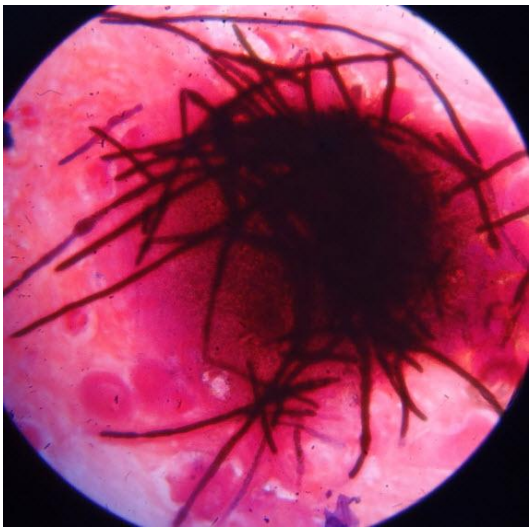


Fig 10: Numerous pus cells with Numerous Gram positive long filaments in Gram stain.

## GRAM STAIN GRADING

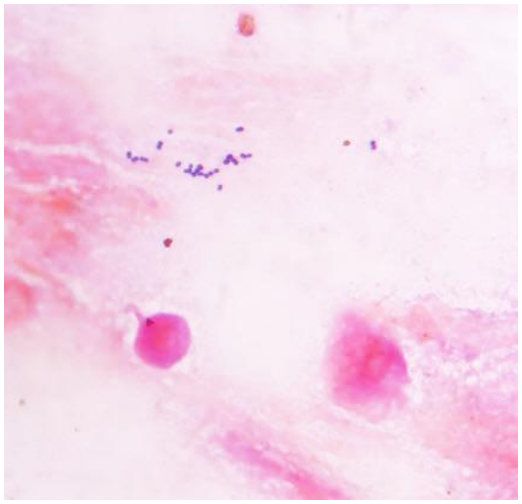


Fig 11: Few pus cells and Few Gram positive cocci in Gram stain (grading 2+)

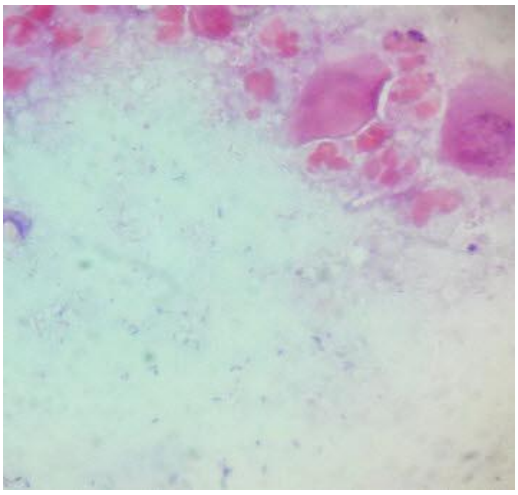


Fig 12: Moderate pus cells and Gram positive cocci in Gram stain (grading 3+)

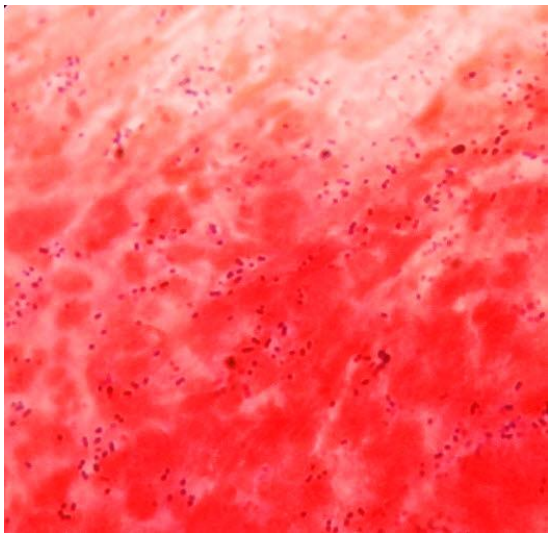


Fig 13: Numerous pus cells with Numerous Gram positive cocci in Gram stain (grading 4+)

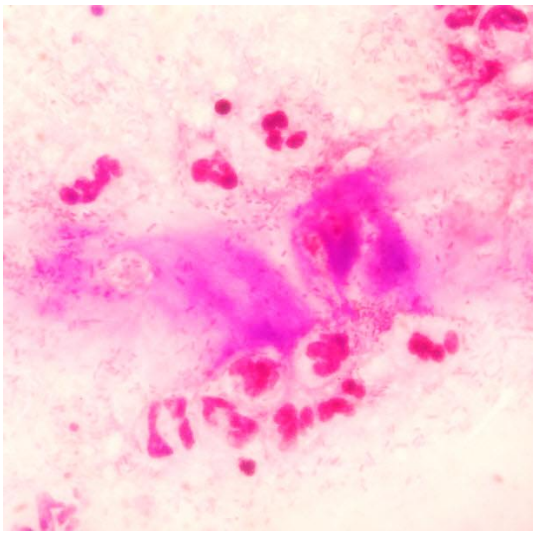


Fig 14: Numerous pus cells with Numerous Gram negative bacilli in Gram stain (grading 4+)

## FUSARIUM SEMITECTUM

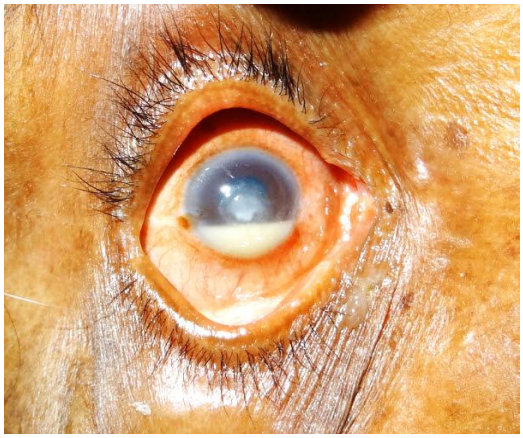


Fig 15: Central corneal ulcer with hypopyon in right eye in a female patient

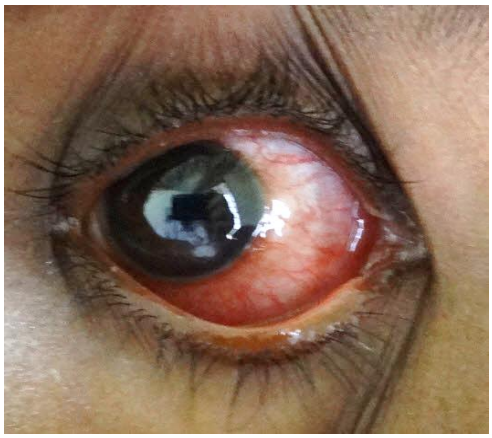


Fig 16: Paracentral corneal ulcer of Right eye in a female patient.

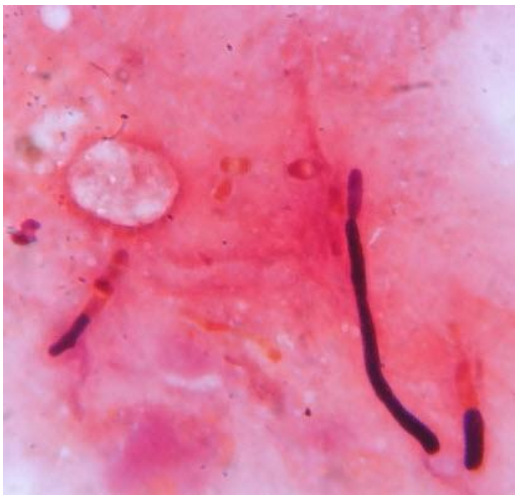


Fig 17: Gram stain showing few pus cells with Gram positive filamentous hyphae in 100x oil immersion.

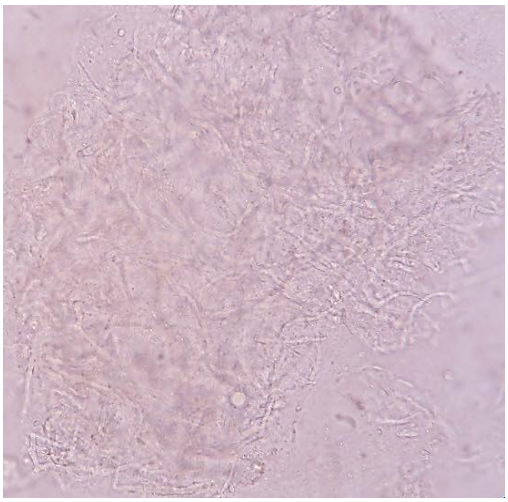


Fig 18: KOH mount showing hyaline filamentous septate fungal elements. (40x)

**FUSARIUM SEMITECTUM**
**Colony Characteristics**

Fig 19: Obverse: white to slight pinkish aerial mycelium on SDA
Growth rate: 2-4 cm for 7 days

Fig 20: Reverse is tan brown on SDA

Fig 21: Whitish aerial mycelium in periphery with pinkish towards center with furrows radiating towards periphery seen on PDA from front.

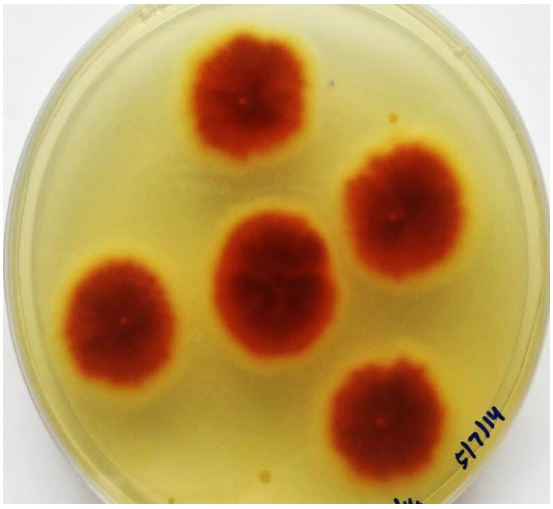


Fig 22: Tan to brown seen on reverse on PDA

**FUSARIUM SEMITECTUM**
**Microscopic Characteristics**

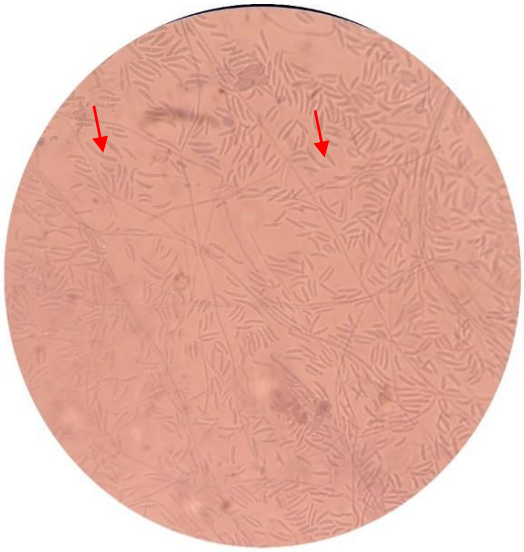


Fig 23: Hyaline septate hyphae Macrconidia curved, fusoid, pointed tip, 2-3 septae, 20-27 x 4.4-5 µm on LPCB mount in 40x.

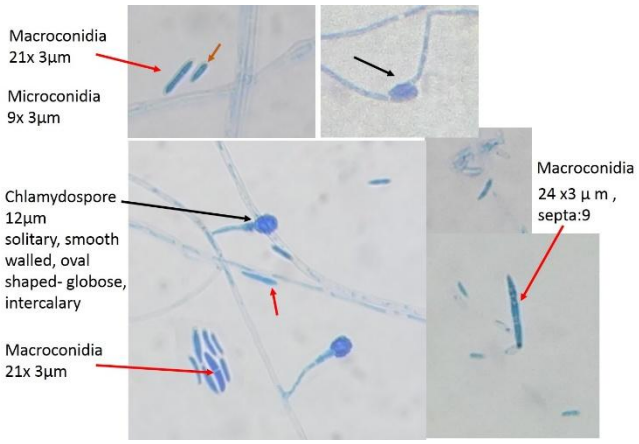



Fig 24: Macroconidia, Microconidia and Chalymdospores on LPCB mount in 40x

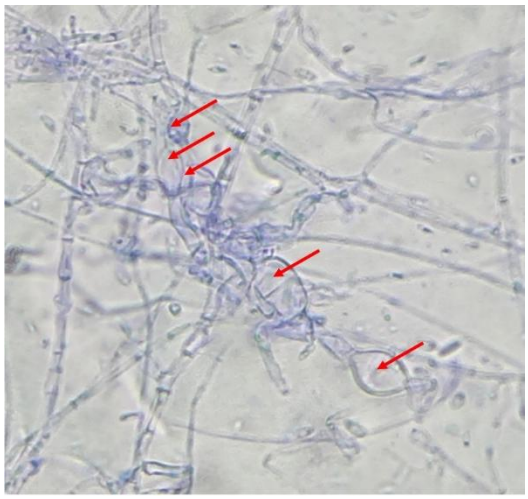


Fig 25: Chalymdospores Oval, arranged in chains, intercalary on LPCB mount 40x.Size: 9-27µm

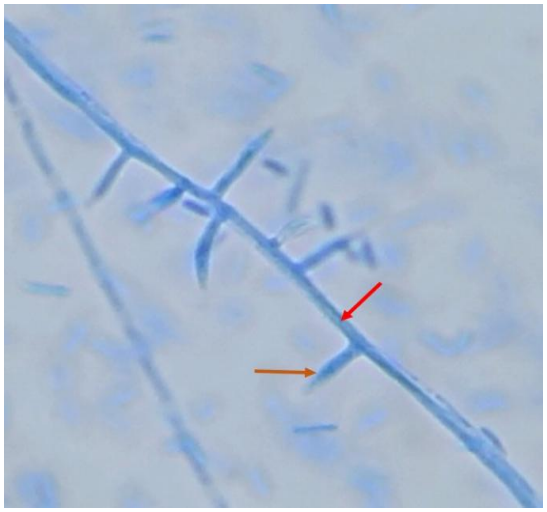


Fig 26: short lateral mono philades (Brown arrow), on the condiophore (Red arrow) on LPCB mount 40x

**FUSARIUM SOLANI**
**Microscopic Characteristics**

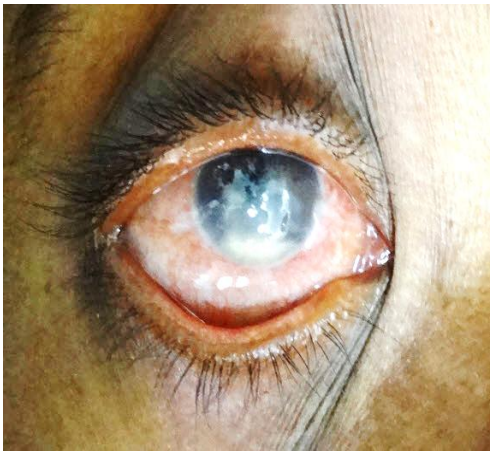


Fig 27: Paracentral corneal ulcer with Hypopyon in right eye of female patient.

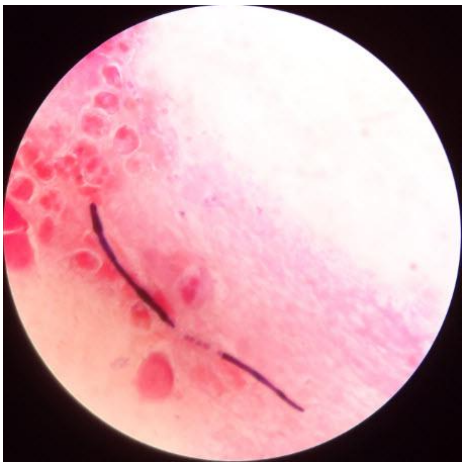


Fig 28: Gram stain showing pus cells with Gram positive long filaments

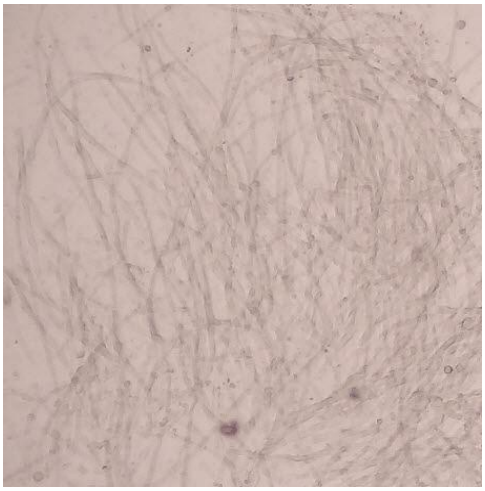


Fig 29: KOH mount showing hyaline septate hyphae in 40x.

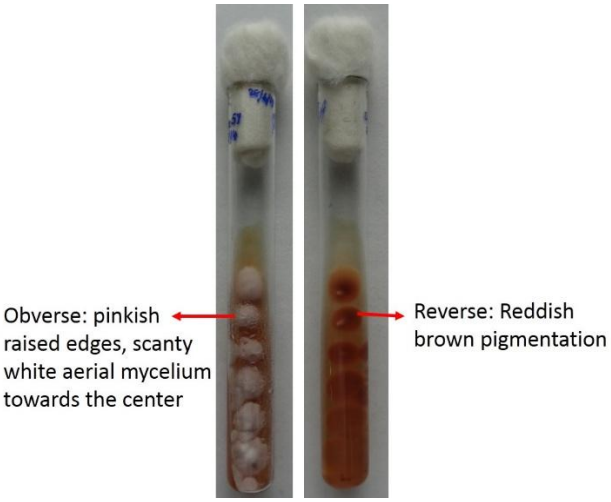



Fig 30: PDA slope showing obverse and reverse.

**FUSARIUM SOLANI**
**Colony Characteristics**

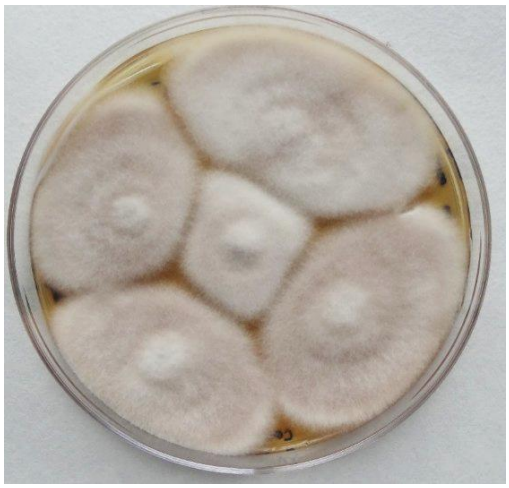


Fig 31: Obverse: white to pinkish raised edges, scanty aerial mycelium towards the center but more towards periphery on SDA plate

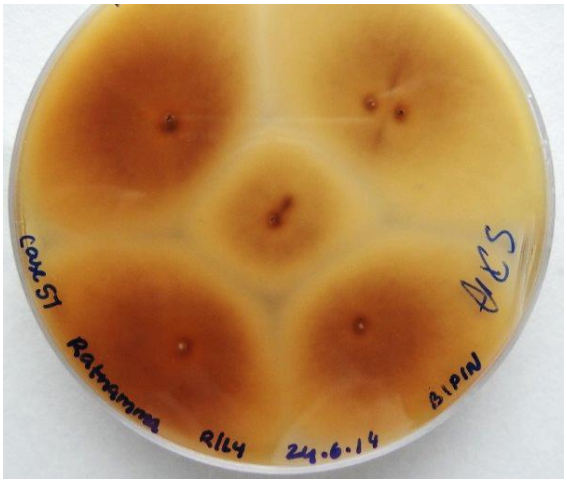



Fig 32: Reverse: tan to brown on SDA plate. Growth rate: 3.5cm for 5days.

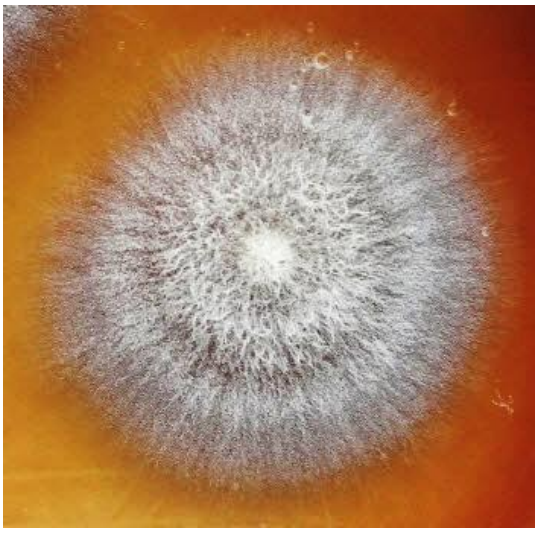


Fig 33: Blood agar plate showing greyish white aerial mycelium.

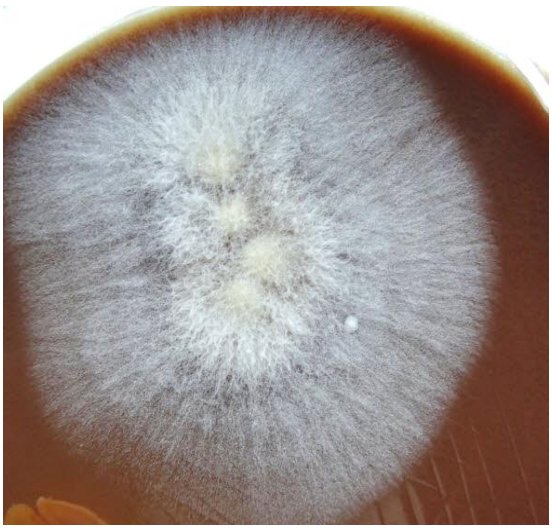


Fig 34: Chocolate agar plate showing greyish white aerial mycelium.

**FUSARIUM SOLANI**
**Microscopic characteristics on LPCB mount**

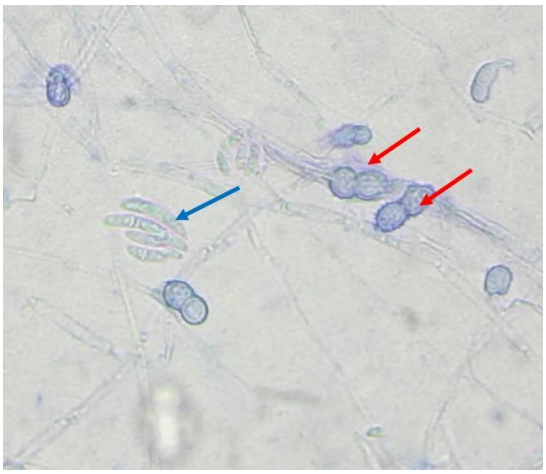


Fig 35: Macrconidia (blue arrow) abundant, curved, apical cell pointed with a beak like appearance. Basal cell is blunt.0- 3 septae. Size: 12-21x 3-3.5µm. Chlamydospores(red arrow)

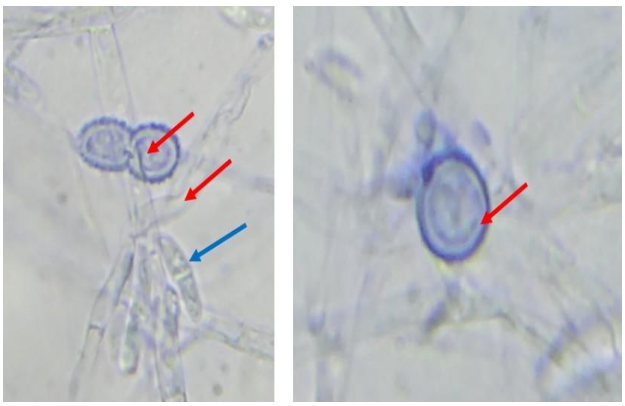


Fig 36: Chlamydospores in LPCB mount 40x.(red arrow) globose, rough walled, Single or in pairs. Size: 9-12µm

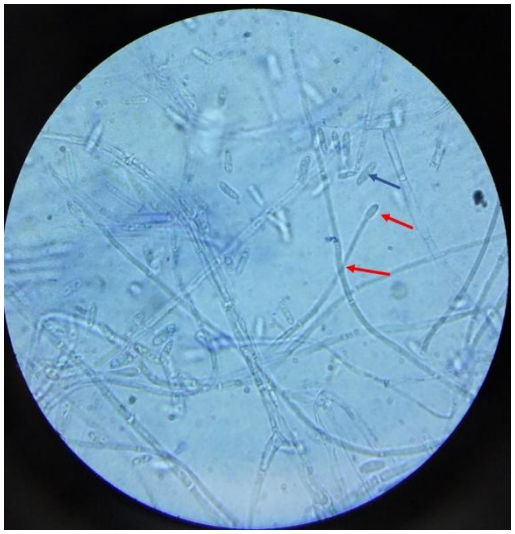


Fig 37: Microconida: Oval, ellipsoid, fusiform with 1 septa.(dark blue arrow) Size: 10.8 x 2.7µm. Long monophialide (red arrow) with microconidia.

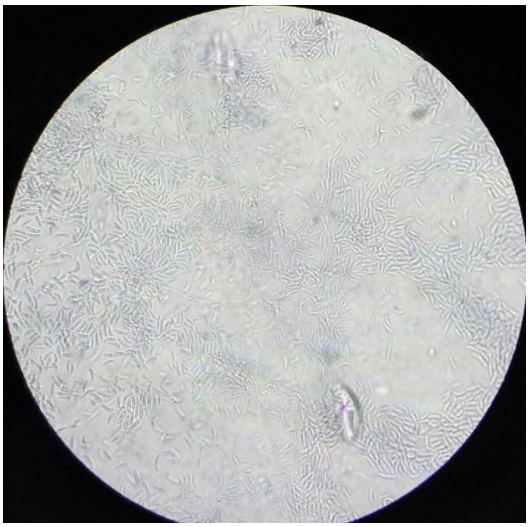


Fig 38: LPCB mount showing both abundant macroconidia and microconidia (40x)

## COLLETOTRICHUM GLOEOSPORIOIDES

### Gram stain and KOH mount

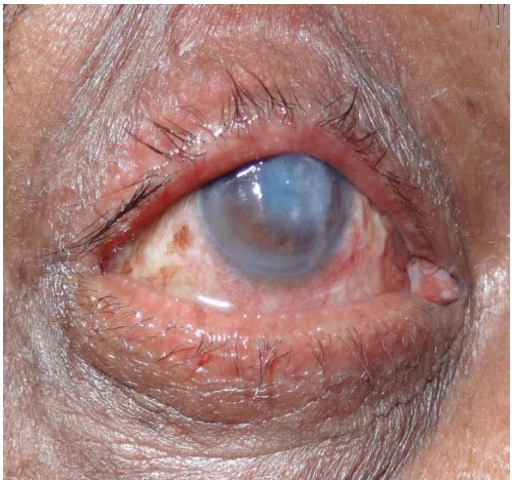


Fig 39: Right central and paracentral corneal ulcer in male patient.

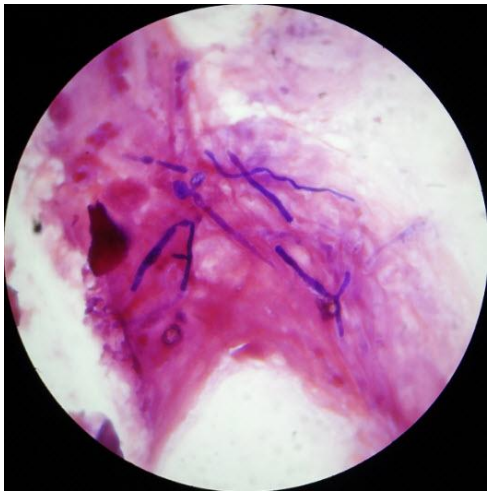


Fig 40: Grams stain showing pus cells with Gram positive filaments in 100x

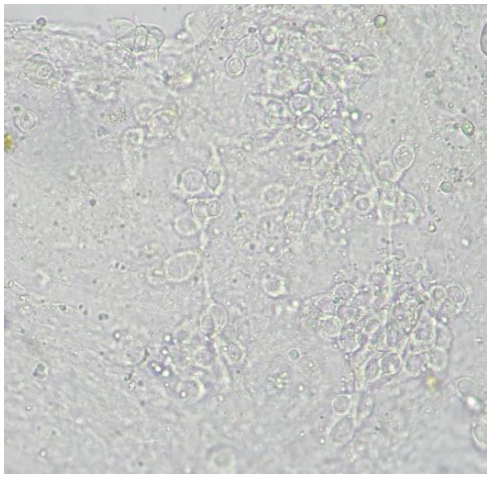


Fig 41: KOH mount showing hyaline septate with globose structures seen with Hyphae.

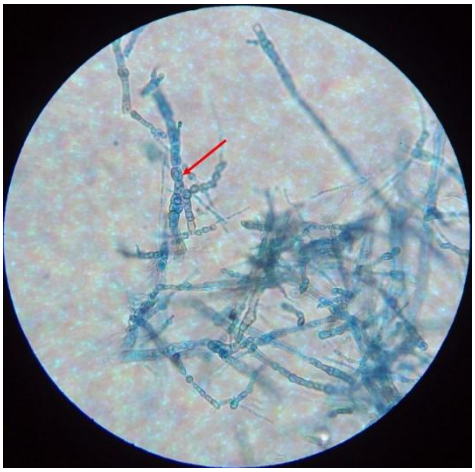


Fig 42: LPCB mount showing dark swellings on the hyphae (appresoria) (40x)

## COLLETOTRICHUM GLOEOSPORIOIDES

### Colony and microscopic characteristics

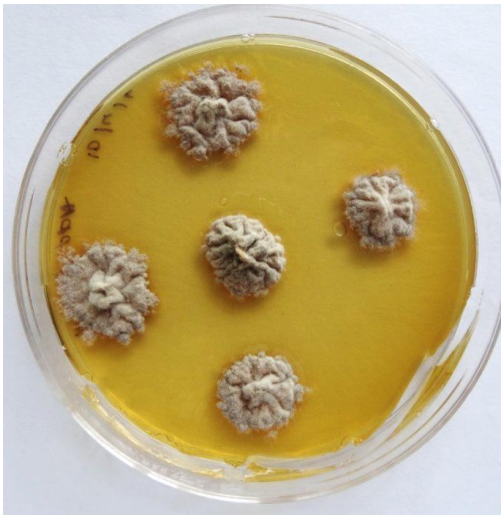


Fig 43: Greyish brown raised, puckered colonies on SDA plate.

Fig 44: Reverse is brown on SDA plate

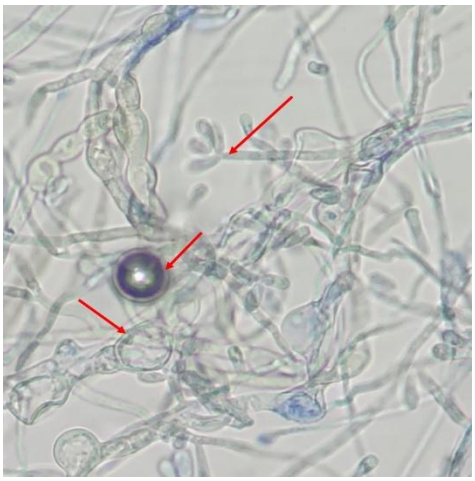


Fig 45: LPCB mount showing hyaline branching sepate with Appresoria (red arrow)abundant, smooth walled, globose, arranged in chains,
Size: 18 µm

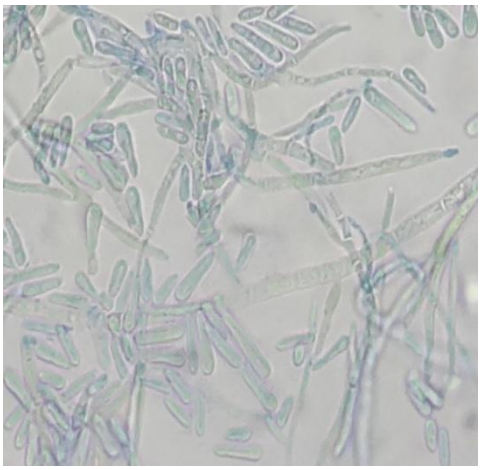


Fig 46: LPCB mount showing Abundant, hyaline straight or cylindrical, 1 celled, smooth conidia.
Size: 18µm x4µm

## CURVULARIA LUNATA

### Gram stain and KOH mount

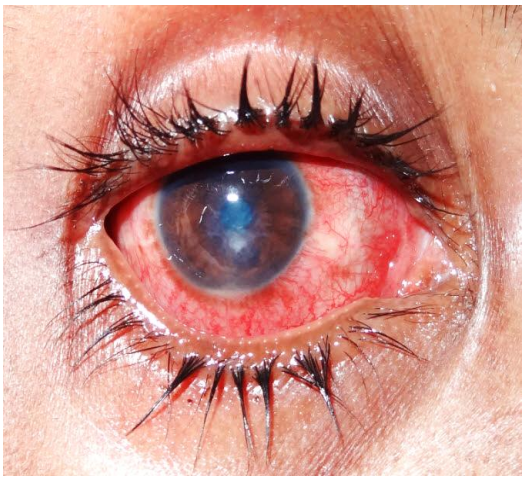


Fig 47: Paracentral corneal ulcer of Right eye in female patient.

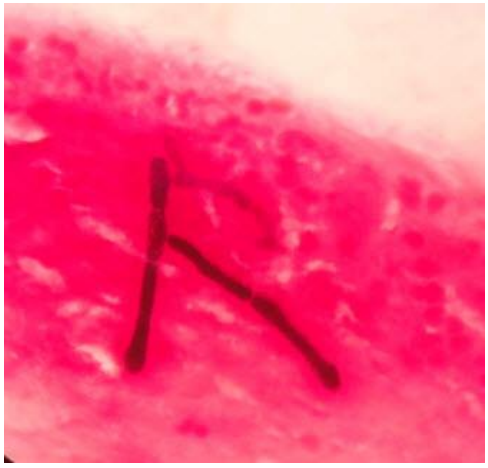


Fig 48: Gram stain shown Gram positive branching filaments with pus cells

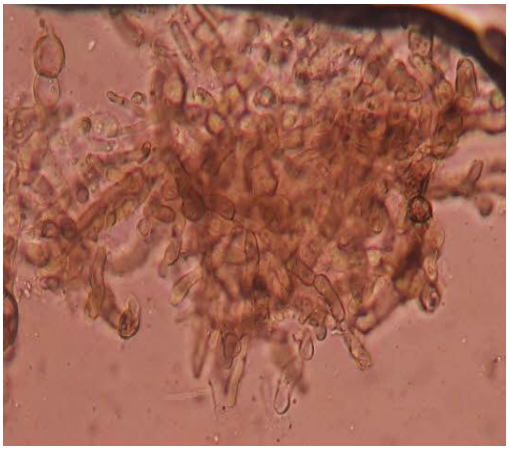


Fig 49: KOH mount showing brownish Branching septate hyphae

Fig 50: SDA showing grayish black woolly colonies

## CURVULARIA LUNATA

### Colony Characteristics

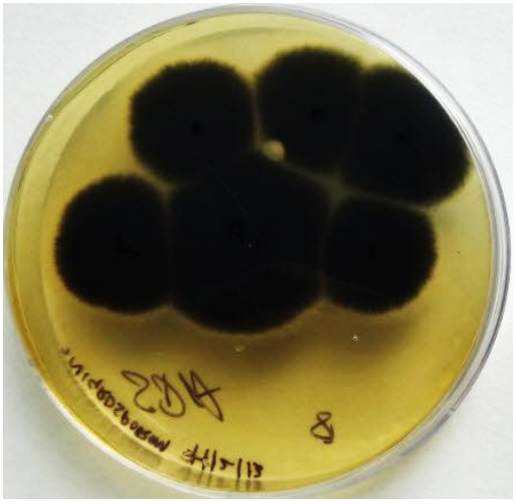


Fig 51. SDA showing black colonies on reverse side.

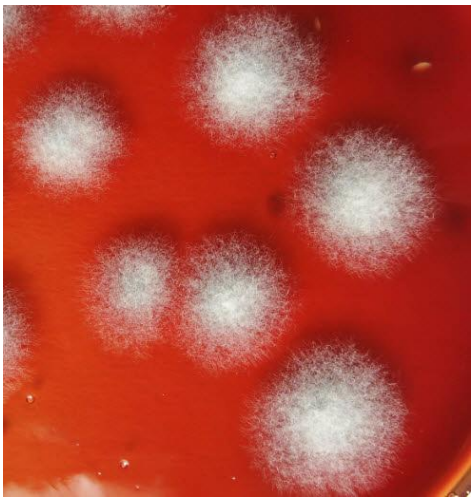


Fig 52. Blood agar plate showing greyish white woolly colonies

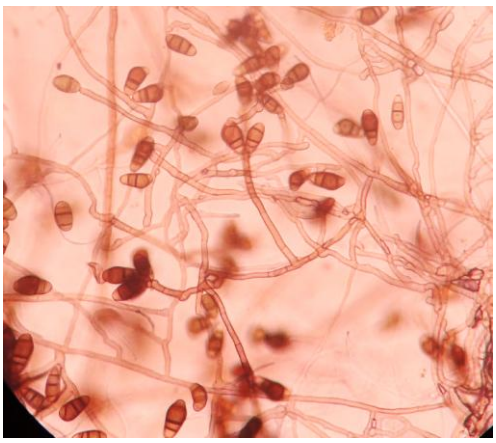


Fig 53: LPCB mount shows dark brown septate hyphae. Long Conidiophore with conidia

Fig 54: LPCB mount (40x) shows conidia 3 septate with 4 cells.3rd cell is curved thick, darker and end cells were pale brown.

## ASPERGILLUS FLAVUS

### Gram stain and KOH mount

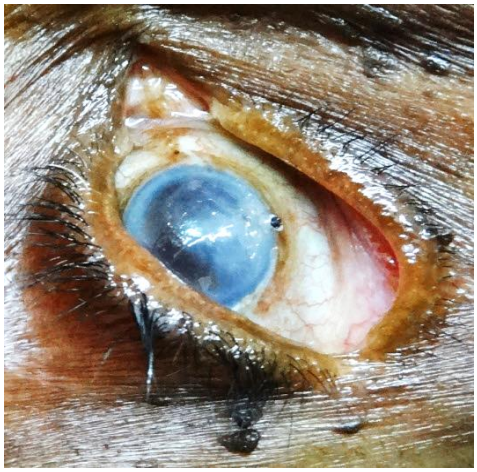


Fig 55. Pheripheral corneal ulcer with a FOB in Right eye of female patient

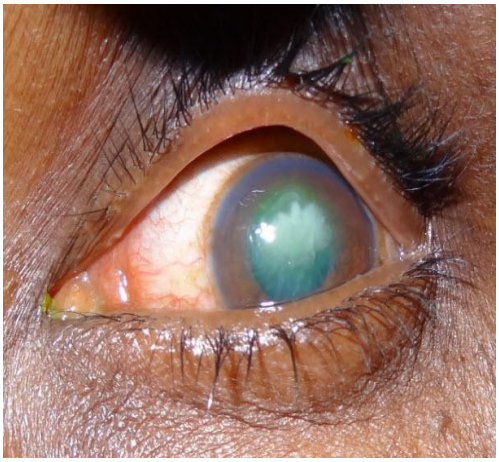


Fig 56. Paracentral corneal ulcer in left eye in a female patient

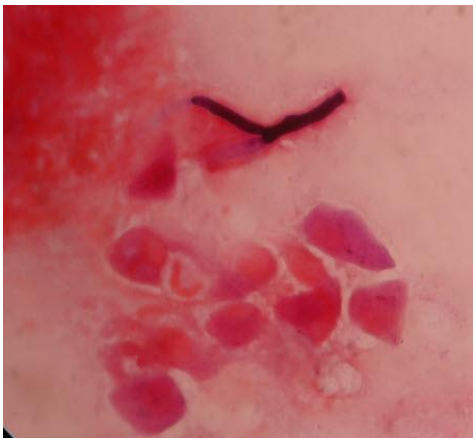


Fig 57: Gram stain showing few pus cells and branching Gram positive filaments

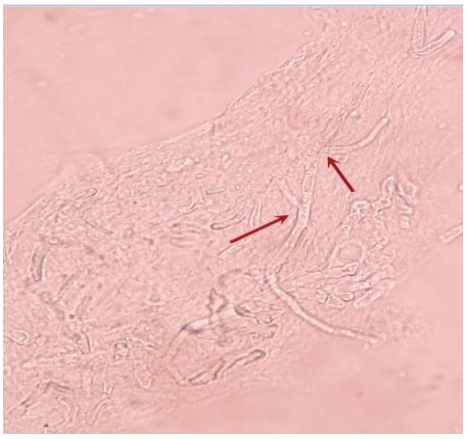


Fig 58: KOH mount (40x)showing hyaline septate, acute angle branching hyphae

## ASPERGILLUS FLAVUS

### Colony characteristics

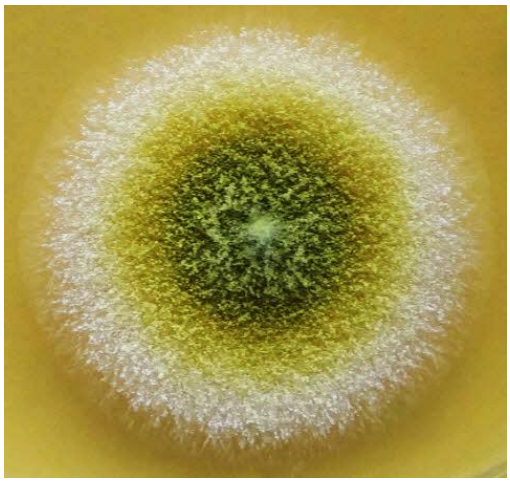


Fig 59. SDA plate showing woolly olive green colony(obverse)

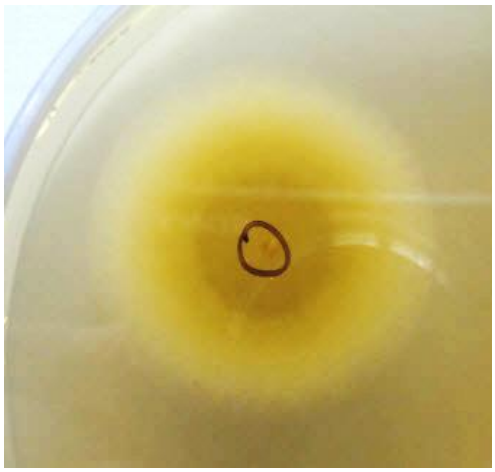


Fig 60. SDA showing golden brown on reverse.

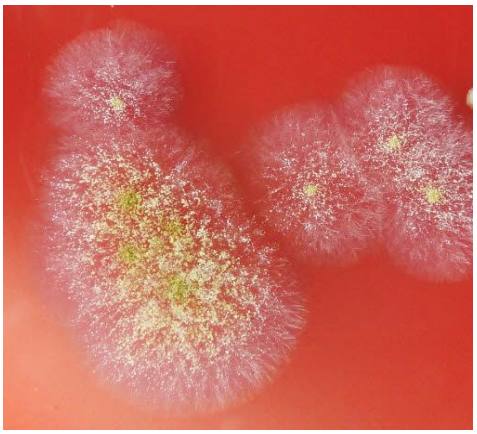


Fig 61. Blood agar showing yellowish green colonies

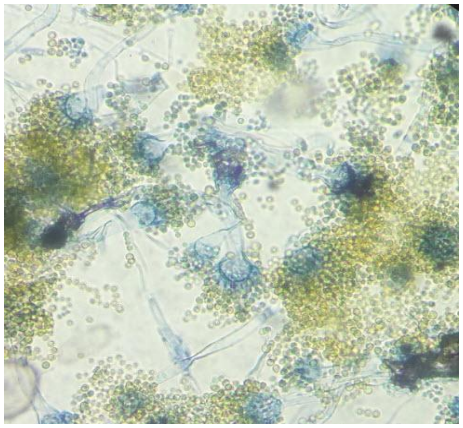


Fig 62. LPCB mount (40x) showing conidiophores, vesicle and conidia

**ASPERGILLUS NIGER**

**Gram stain, KOH Mount**

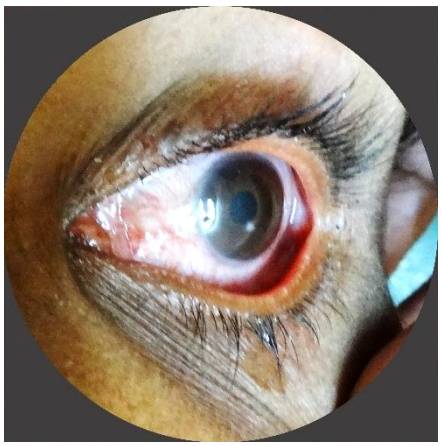


Fig 63. Para central corneal ulcer of left eye in female patient

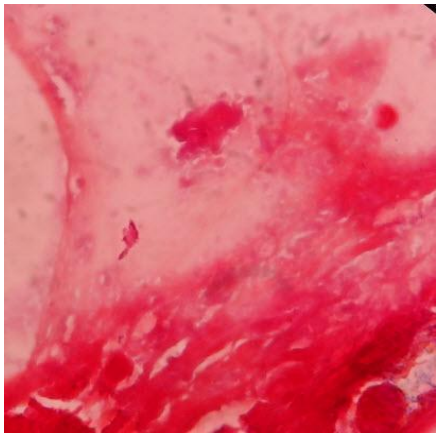


Fig 64.Gram stain shows few pus cells, no branching filaments seen.

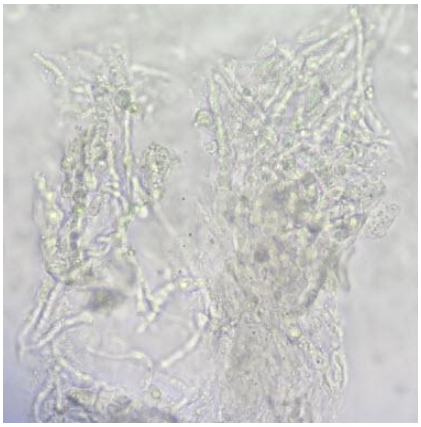


Fig 65. KOH mount (40x) shows hyaline acute angle branching septate

Fig 66. SDA shows dark brownish black colonies with whitish margin in periphery.

## ASPERGILLUS NIGER

### Colony Characteristics

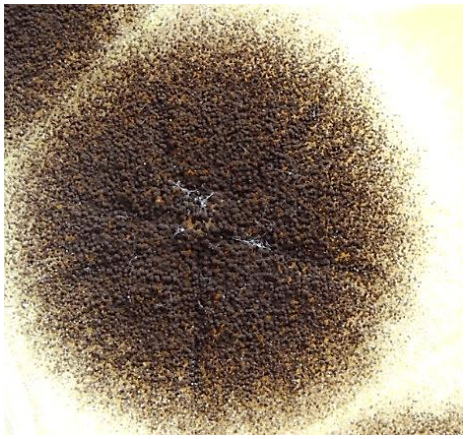


Fig 67. Close view of brownish black colonies on SDA plate.

Fig 68. SDA plate on reverse shows yellow color with furrows radiating from the center

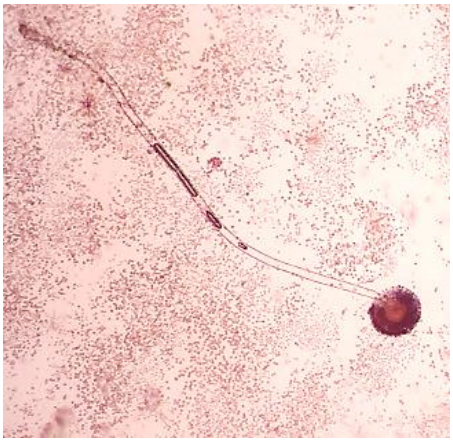


Fig 69. LPCB mount (40x) shows smooth long condiophore.

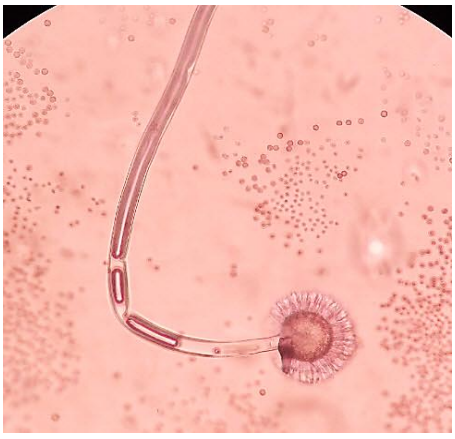


Fig 70. LPCB mount (40x) shows conidiophore with vesicle. Phialides are biseriate covering entire vesicle.

## STREPTOCOCCUS PNEUMONIAE

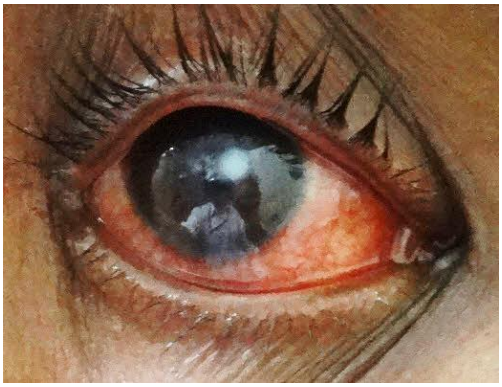


Fig 71. Paracentral corneal ulcer in right eye in a male patient.

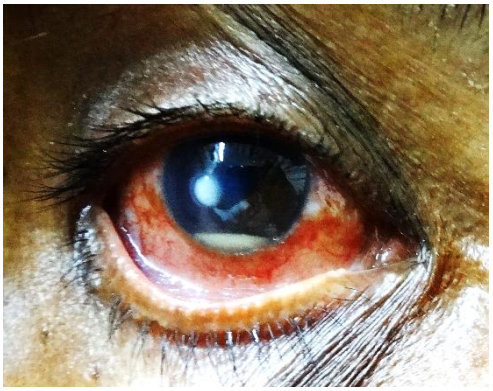


Fig.72 Paracentral corneal ulcer with hypopyon in right eye in a female patient.

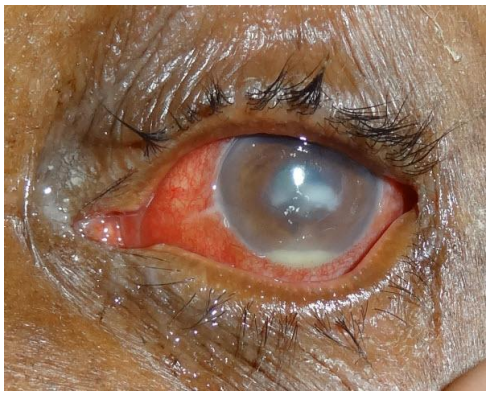


Fig 73. Central corneal ulcer in left eye with hypopyon in a male patient.
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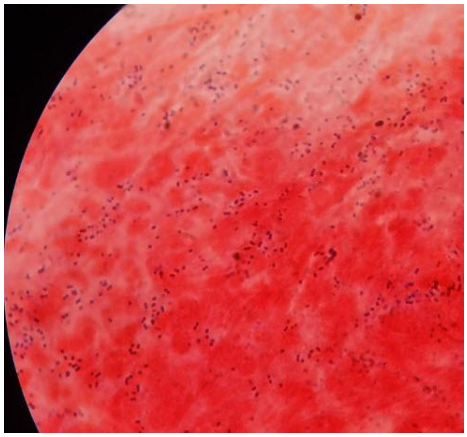

Fig 74. Gram stain shows numerous pus cells with numerous Gram positive cocci in pairs

## STREPTOCOCCUS PNEUMONIAE

### Colony Characteristics

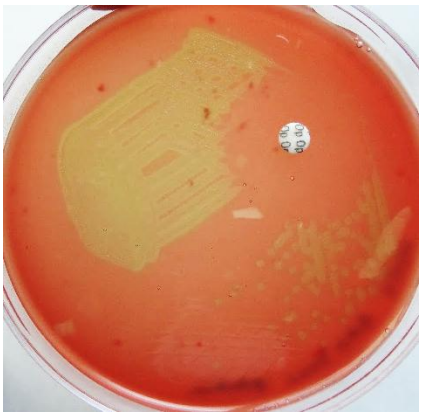

Fig 75. Blood agar showing alpha hemolytic colonies with optochin sensitivity

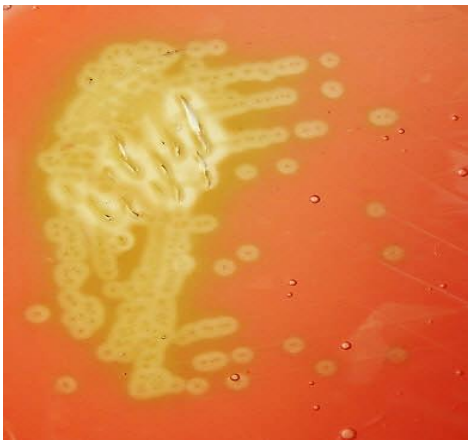

Fig 76. Blood agar showing alpha haemolytic colonies

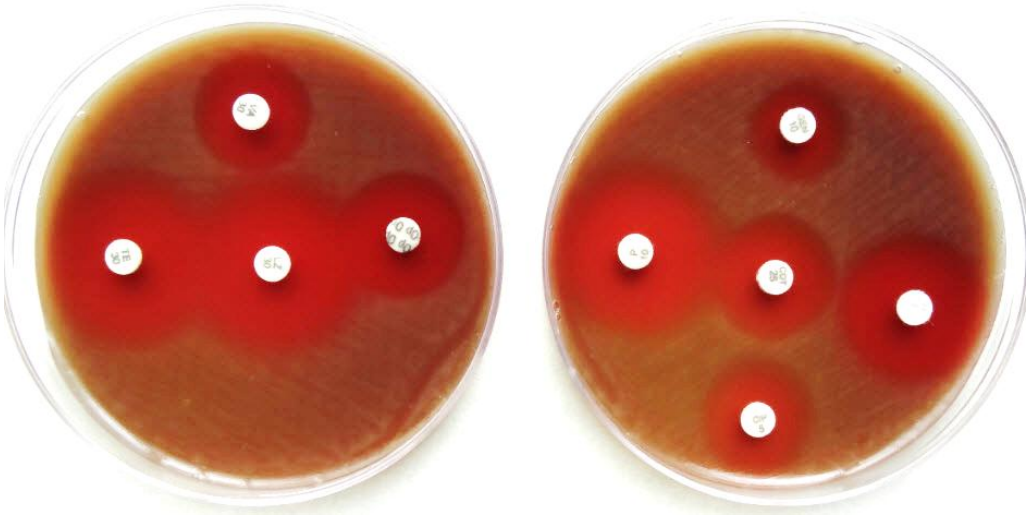

Fig 77: Antibiotic sensitivity of pneumococci on 5% blood on MHA Plate.

## KLEBSIELLA PNEUMONIAE

### Gram stain and colony characteristics

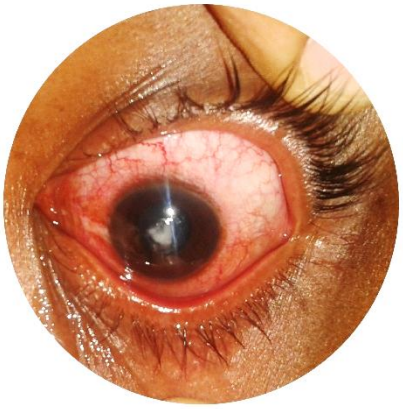

Fig 78. Central corneal ulcer in left eye in a male patient.

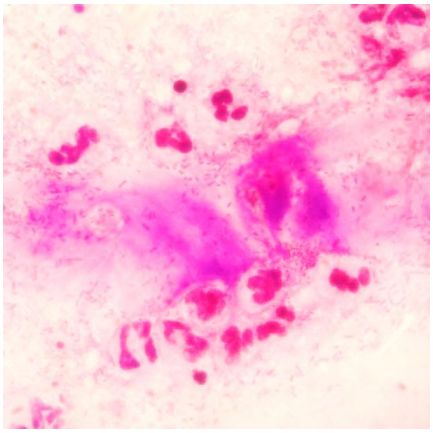

Fig.79. Gram stain showing numerous pus cells with numerous Gram negative bacilli

Fig 80: Blood agar showing large greyish white colonies.

.

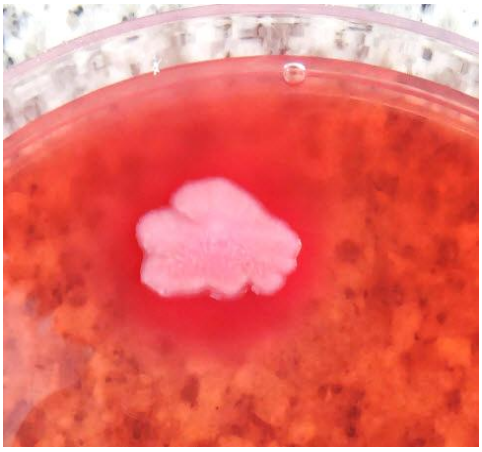

Fig 81. MacConkey agar showing large single mucoid colony

## KLEBSIELLA PNEUMONIAE

### Biochemial reactions and Antibiotic sensitivity

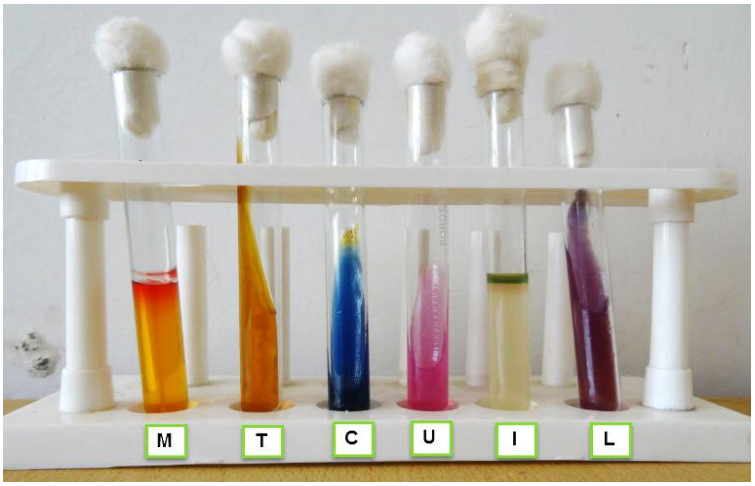


Fig 82. Biochemical reactions of Klebsiella pneumoniae **M**: Mannitol fermented, non-motile, **T**: Triple sugar Iron: A/A, **C**: Citrate+, **U**: Urease+, **I**: Indole -**L**: Lysine Iron Agar+

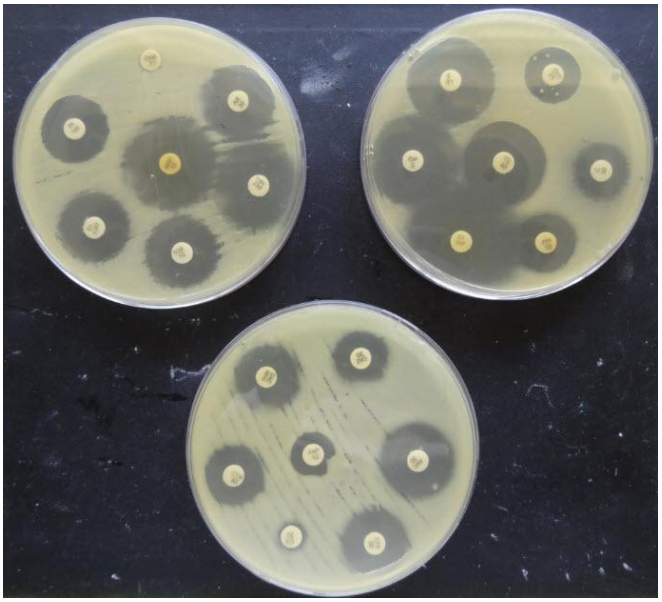

Fig 83: Antibiotic sensitivity of Klebsiella pneumoniae on MHA plate by Kirby Bauer Method showing Amp C production.

## 6. DISCUSSION

Corneal blindness is a major public health problem worldwide[1] .Corneal ulcer has been estimated to be responsible for 9% cases of the blindness in India [3]. In a population based study from south India, the incidence of corneal ulcer was reported to be as high as 1130 per million[4.] Among the causes of corneal ulcer, infectious keratitis occupies the predominant position [4].

Over the period of study from January 2013 to June 2014, we did not find any corneal ulcer in children below 10 years of age. Majority of the patients belong to age group of 21-70 years. Earlier studies[31, 67, 70,105-108] done in India showed that corneal ulcers were seen in the age group of 16-60 years. None of the studies also document corneal ulcers in children below 10 years of age. This pattern suggests that corneal ulcers are associated with activity and those who do not involve themselves in outdoor activity as children below 10 years and those above 70 years of age do not run the risk of corneal ulcers

The gender distribution of corneal ulcers in different studies across India show that male predominance with usual sex ratio of 2:1[31,67,70,105-108] .In keeping with these observations we found that out of 75 patients with corneal ulcers, 64% were males and 36% were females. Thus males were commonly affected than females with a sex ratio of 1.8:1. This higher preponderance of corneal ulcer among male may again be due to increased outdoor activities. Our findings are similar to that reported from Bangalore[105] where a sex ratio was 1.8: 1 were reported. (36%).

Corneal ulcers were related to occupational activity among 66.8-79.3% patients reported from West Bengal [70], Madurai[107], Tirunelveli[108]. These studies [31,108] also

reported that farmers were predominately affected and 80-90% of these patients were from villages or from rural background with agricultural related activities as their occupation. In contrast: a study from Ahmedabad [109] reported higher incidence of keratitis among housewives (21.3%), followed by farmers (16.6%), laborers (14.6%) and carpenters (10.6%)

Like these observations also our study found that Farmers, manual laborers and house wife's accounted for 77.33% among those who presented with corneal ulcers. As the patient population belongs to villages around Kolar town, where the population pursues agricultural activities for livelihood. The operations like harvesting, clearing the bushes, winnowing may pose risks for corneal damage. Thus 40% among those with corneal ulcers were farmers and 26.67% were manual laborers. Many of the manual laborers were also engaged with agricultural activities. Many of the activities in the kitchen undertaken by house wife seem to pose risk for corneal ulceration such as winnowing sweeping, dusting chipping wood for starting the earth may make foreign bodies to fall in the eye and subsequent rubbing of eyes may produce corneal ulceration. Earlier studies across India, reported that corneal ulcers could be documented in all seasons. However some studies from West Bengal[70], Hyderabad[67] and Tiruchirapalli,[23,110] Coastal Karnataka have bimodal distribution of fungal keratitis during the windy seasons (October to January) and monsoon seasons (June to September)

Our study showed that all though corneal ulcers were encountered in all seasons, but 69% of the cases were found during November and February months. This period coincides with the harvesting season during which the agricultural population who constitutes a sizeable population with corneal ulcers are known to engage in activities such as cutting, winnowing and stacking the grass.

Comparison of predisposing factors from different studies [31, 67, 70,105-108] showed that corneal trauma was predominant factor for corneal ulcer occurring in 35 to 95%.of patients.

The percentage of Corneal trauma reported from various places like Tirunelveli[108](92.15%), Ahmedabad[109] (90%), West Bengal (82.9%)[70], and Madurai[107] (65.4%)

Among the causative agent for corneal trauma many studies reported that vegetable matter was the most common agent as it was related to agricultural activities and the patients with corneal ulcer trauma due to vegetable mater varied from 15% to 48% [31, 67, 70,105-108] but high percentage was reported from Tirunelveli[108], (61.28%) and West Bengal[70](59.6%).

Various studies reported foreign body has one of the agents of corneal trauma and it varied from 5-35%.

Use of corticosteroids associated with development of fungal keratitis was observed in different studies from Tirunelveli[108] (7.85%) Calcutta[111] (18.5%) West Bengal (19.3%)[70] and Bangalore (25.92%)[105]

Other risk factors included systemic diseases such as diabetes mellitus, preexisting ocular disease and contact lens wear were reported in smaller numbers.

In line with these observations, in our study we found that corneal trauma was the most common predisposing factor (82.67%). This corneal trauma was caused by fall of vegetable matter into the eye. Many patients recounted fall of vegetable matter like ragi seeds, paddy husk or blades of grass while cutting the grass during harvesting.

Foreign body accounted for 25.33%. Out of which injury due to iron piece or stone piece accounted for the majority of the cases. Insect hit was another common factor causing trauma. Many times patient could not recollect the type of foreign body that hit

the eye. These traumatic factors appears to be rural in nature and specially related to agricultural operations. It was interesting to note that hit by cow's tail could also be a cause for corneal trauma. In our study we did not encounter chronic dacrocystitis, diabetes mellitus or use of contact lens as predisposing factor. Fall of hair dye into the eye and use of steroid eye drops were predisposing factors in small number of patients. These predisposing factors suggests that proper care of the eyes during agricultural operations by using goggles could prevent corneal trauma and further complications which may result in blindness.

Watering, redness, photophobia of either eyes appear to be complaints by all the patients with corneal ulcer.

Corneal ulcers were mainly observed in paracentral region and occasionally in the central and peripheral regions of the cornea. Paracentral ulcers at the time of presentation is thought to indicate good prognosis. 7 (9.33%) cases presented with central corneal ulcer, however in contrast a study from Melborne[112] reported 64.5% ulcers were central in location.

Hypopyon seems less common: found in 17.33% of the above cases. Studies[113,114] from Southern India and Northern India, Hypopyon was 66 and 55% cases respectively. Our study seemed to record lower incidence of hypopyon. Most of the patients with corneal ulcers seem to have impaired visual acuity with blindness in extreme of cases.

Traditionally the investigation for the infectious etiological agents of corneal ulcer starts with microscopic examination with Gram stain and KOH mount of the scraping taken from the corneal ulcer. Microscopic examination by both of these methods is found to be essential as bacteria are seen only in Gram stain and filamentous fungi are better appreciated in KOH mount. We found that the pus cells and bacteria or fungal

agents could be observed in 37(49.33%) cases and KOH mount positive in 15 cases (20%) with fungal elements. In different studies from Bangalore[105], Bellary[106] and Mangalore[18], Gram positivity rate was 17%, 18.39 and 23.62% and KOH positivity rate was 46%, 32.18% and 31.4% respectively. Among 15 cases, 13 were picked up by Gram stain and 2 positive by KOH were not picked up by Gram stain.

Gram stain seem to be more informative at our hands.

We investigated the utility of Gram stain grading[104] in the stained smears of corneal scrapings. No pathogens were isolated from the smears that gave 0 grade. Among those that fell under 1-4+grade, majority of the smears were found to fall under grade 1+ and 2+( 41.33%) and the yield of pathogens from the smears that showed 1+ and 2+ grade is 12%.

Gram stain grading in comparison with KOH showed that Grade 2 Gram stain picked up 7 fungal elements in KOH, and Grade 1+ Gram stain picked up 4 fungal elements and Grade 3+ and Grade 0+ of Gram stain picked up 1 each fungal elements in KOH, whereas Grade 4+ did not pick up any fungal elements. This clearly indicated the presence of moderate to heavy amounts of pus and/or the presence of bacteria on Gram stains correlated with the presence of infection.

The population were not exposed to risk factors associated with amoebic keratitis: there were no patients in our study who undertook swimming or such activities in ponds, lakes or such other watery bodies. 6(1.74%) cases of *Acanthamoeba* was reported from Kerala.[115]

We could not isolate any organisms in Liquid media as corneal scraping was inoculated with the last collected sample.

In our study the most common bacterial isolate was streptococcus pneumoniae with 5 cases and 1 case was of *Klebsiella pneumoniae*. *Streptococcus pneumoniae* (37.51%) was the predominant bacterial species in various studies from Tirunelveli[108], Madurai[107], Trichirapalli, Nepal[70] and Trivandrum[115] (26.80%). Thus our rate of isolation of *streptococcus pneumoniae* is low. This may depend upon the presence of other preocular conditions like chronic dacrocystitis. The pneumococcal infection seem to be endogenous from the flora of the nasal cavity.it is interesting to note that both bacteria causing infections of corneal ulcers are capsulated. All the 5 strains of streptococcus pneumoniae were sensitive to all groups of drugs that were routinely used in our lab

*Klebsiella sp* are rarely isolated from corneal ulcer patients, however a recent study from Trivandrum[115] documented isolation of *Klebsiella* in (10.31%) cases of *Klebsiella.* Our isolation is low and the isolate showed AmpC β lactamase production.

AmpC β-lactamases are group C enzymes belonging to Class I of Bush's functional classification[116]. Organisms such as *Enterobacter, Citrobacter, Shigella, Morganella, Serratia*, and *Escherichia coli* possess AmpC enzymes on their chromosomes[117]

The prevalence of plasmid mediated Amp C varied widely in different parts of the world from 2% to 46%. In Indian studies, the prevalence of Amp C ranged from 8% to 47%[118.].

Fungi are very important etiological agents of infectious keratitis. The incidence of fungal keratitis in different regions of India are 77.3% in North India[119], 32% in East India, 38.9% in West India and 32% - 39.8% in Southern India[120.] In line of these observations our study showed 17.33% which is comparatively less. This may be due to geographical variations and environmental conditions.

In our study we encountered *Fusarium spp. Aspergillus spp, Colletotrichum* species and *Curvularia lunata*. Earlier studies from India have reported species of *Fusarium Aspergillus, Curvularia, Scedosporium apiospermum, Paecilomycetes*, and many other species[23]. Yeasts have been reported in small number of patients.

*Fusarium* species was found to be the most common cause of fungal corneal infections in Southern United States, Florida, Brazil, Ghana, Nigeria, Paraguay, Columbia, China, Vietnam, Hongkong, Singapore as well as from some parts of southern India.

In India, *Fusarium* is the predominate fungal pathogen in Southern India (Tamil Nadu, Madurai and Bangalore) in contrast to Aspergillus spp. which is predominant in Northern India as shown in various studies [6,23,120].

In line with these observations the commonest fungal isolates documented in our study was 7(53.85%) cases of *Fusarium* spp. followed by 3(23%) cases of *Aspergillus* species, 2(15.38%) cases of *Colletotrichum* species and 1(7.69%) case of Curvularia species.

This may be due to difference in the climate and geographical region. Majority of these filamentous fungi are present in the soil and vegetable matter and more seen in tropical and subtropical regions of the world. Most of the *Fusarium* species are plant pathogens and *Aspergillus* species are ubiquitous in nature, present in dead and decaying organic matter. Due to increased agricultural activity in the harvest season there is increase in the number of fungal corneal ulcers. This finding is evident in our study.

Out of 7 species of *Fusarium,* we could speciate 4 *Fusarium* species 3 were *Fusarium semitectum,* and 1 was *Fusarium solani*. The rest could not be speciated morphologically.

Among *Fusarium* species, *Fusarium semitectum* has been considered as uncommon fusarium[121] species causing keratitis. It has already been reported as pathogen in

corneal ulcers. A study from Madurai[122] have isolated one case of *Fusarium* semitectum. However we could isolate 3 *Fusarium semitectum* from patients with corneal ulcer. Recently *Fusarium semitectum* has been recommended to be useful for microbial control[123] of pests of silk worm (Bombyx mor), honey bee (Apis indica) and earthworm (Eisenia foetida)

Studies from China[124], India[120], UK[124] showed that *Fusarium solani* was the commonest isolate in 35.2%, 16.1% and 11% respectively. We could isolate only one *Fusarium solani*. Species of *Fusarium* appears to be important as *Fusarium solani* has been reported and uncommon *Fusarium* species like *Fusarium semitectum* has reported from Southern India.

*Colletotrichum* genus belong to class Coelomycetes, found in soil and plant vegetation. They are distributed worldwide, but more common in tropical and subtropical regions. *Colletotrichum* is now being considered as emerging fungal pathogen causing keratitis and there are less than 20 cases reported from India. Out of 5 species that cause human infections, C. dematium and C. gleosporoides [125,126] are the principal causative agents in keratitis. In our study we have isolated 2 *Colletotrichum* species one of them being C. gleosporoides and other species could be identified morphologically. *Colletotrichum* has to be differentiated from fusarium. The absence of the septation within the conidia and the presence of the appressorias, are the two important characteristics that distinguishes *Colletotrichum spp*. from the *Fusarium spp*

Dematiaceous fungi are reported to be responsible for 10–15% of all fungal keratitis and are the third most frequently encountered fungi following *Aspergillus* and *Fusarium.* They are common soil and plant saprophytes categorized on the basis of their dark pigment. Curvularia is the most common dematiaceous fungi isolated from corneal ulcers and it accounts for 4-9% all fungi isolated from patients in tropical areas[33.] In

Southern India Curvularia was the most common dematiaceous fungi isolated which represented 2.8% whereas Northern India represented 8.2%.

*Curvularia lunata* seems to be common isolate reported from various studies. We have isolated only one *Curvularia lunata.*

In our study we have not isolated candida, however the candida spp. was predominate fungi isolated from USA and Europe but in Africa and Asia candida is designated second place among the causative fungi[23].

Natamycin(5%) is effective against fusarium species, but not effective in large ulcers and in *Aspergillus* keratitis if used as monotherapy. Natamycin and in combination with azole group of drugs is found effective in keratitis due to Aspergillus, Dematacieous fungi and colletotrichum[21]. Empirically in our study Natamycin and voriconozole eye drops were administered and the improvement was seen even by the first week of follow up. However the patients could not be followed up and the final outcome could not be recorded.

# 7. SUMMARY

In this study 75 corneal scrapings from patients with suspected infectious keratitis patients during the period from January 2013 to June 2014 were studied. Males were more often affected with sex ratio of 1.8:1 Children less than 10 years were not affected. Most of the patients were farmers, manual laborers and house wives from villages around Kolar town, where the population pursues agricultural activities for livelihood. Corneal trauma with vegetable matter was the most common predisposing factor for corneal ulcer as it was related to agricultural activities and increased number of cases was seen during harvest season. All of the patients presented with redness, watering and photophobia. Both eyes were not affected. More than 70% patients presented with paracentral ulcers and hypopyon was less common. Most of the patients with corneal ulcers seem had visual acuity with blindness in extreme cases. Fungal isolates were more common than bacterial isolates. *Fusarium spp* was the commonest isolate followed by *Aspergillus spp* and *Colletotrichum spp* and 1 isolate of *Curvularia lunata*. We could speciate 4 out of 7 *Fusarium* species, 3 were *Fusarium semitectum* and 1 was *Fusarium solani*. *Streptocoocus pneumoniae* was most common isolate and all 5 isolates were found to be sensitive to Penicillin strain and one *Klebsiella pneumoniae* was found to be AmpC β lactamase producer. All patients received Natamycin, Tobramycin, Ceftriaxone and Voriconozole eye drops and the improvement in symptoms and visual acuity was seen even by the first week of follow up. Proper eye protective measures during agricultural activities could prevent corneal ulcers and subsequent complications.

# 8. CONCLUSION

1. Corneal ulcers in Kolar region are more common in the age group of 21-70 years among the population.
2. No cases of corneal ulcer in children below 10 years was recorded in our study.
3. Males were more commonly affected with corneal ulcer than females with a sex ratio of 1.8:1.
4. Patients with corneal ulcer seen in our study, most often were farmers, manual laborers or housewives.
5. Corneal trauma with vegetable matter was the most common predisposing factor.
6. We could isolate bacterial and fungal etiological agents in 25.33% of patients with corneal ulcer.
7. Fungal infections predominated accounting for 17.33%.of isolates.
8. The fungal isolates included *Fusarium, Aspergillus, Colletotrichum* and *Curvularia* species.
9. *Fusarium* species dominated among the isolates 53.85%.
10. 4 out of 7 *Fusarium* species, 3 were *Fusarium semitectum* and 1 was *Fusarium solani.*
11. Among the 6 bacterial isolates, pencillium sensitive *Streptococcus pneumoniae* in 5 cases and 1 AmpC β lactamase producing *Klebsiella pneumoniae* could be isolated.
12. We did not find: mixed infection, protozoa, or yeasts in the study.
13. Among patients who received Natamycin, Tobramycin, Ceftriaxone and Voriconozole eye drops improvement in symptoms and visual acuity was observed even by the first week of follow up.
14. Proper eye protective measures during agricultural activities could prevent corneal ulcers and subsequent complications.

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# SRI DEVARAJ URS MEDICAL COLLEGE

# TAMAKA, KOLAR

## PATIENT INFORMED CONSENT FORM

**Study title "Infectious Keratitis in Kolar region"**

I……. declared that I have been briefed and hereby consent to be included as a subject in the following dissertation.

I have been informed to my satisfaction by the attending Dr. Bipin Chandra Bhagath L, the purpose of work done and laboratory investigation that are required in the management of my case. This has been explained to me in the language I understand and I fully consent for the same.

Signature of the doctor Signature of the patient

Name of the doctor Signature of the witness

Date:

# MASTER CHART

| Sl. No | Patient Name | RLJ/ SNR | OPD No. | IP No | Date | Month | Year | Age | Sex | Occupn | Trauma | Injury of eyes | Contact lens | Eye affected | Steriod use | Hypopyon | Ulcer location | Vision | Gram stain | Grading | Gram Pus cells | GPC/GNB/FE | KOH | Saline wet mount | BA | CA | MA | SDA | LPCB | Organism isolated |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Lakshamakka | RLJ | NA | 864253 | 3 | Dec | 12 | 40 | F | Housewife | N | N | N | L | N | Y | PC | 6/24 | P | 3+ | Moderate | Numerous GPC | N | N | G | G | NG | NG | N | S.pneumoniae |
| 2 | Thipaka | RLJ | NA | 872783 | 4 | Jan | 13 | 50 | F | Housewife | Y | Vegetable matter wooden stick | N | L | N | N | PC | 6/9 | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 3 | Munivenkatappa | RLJ | 875511 | NA | 11 | Jan | 13 | 80 | M | Farmer | N | N | N | R | N | Y | PC | PL + | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 4 | Narappa | RLJ | 872208 | NA | 31 | Jan | 13 | 70 | M | Farmer | Y | Y | N | R | N | Y | PC | 6/24 | P | 1+ | Occ | No org | FE | N | G | G | NG | G | P | F.semitectum |
| 5 | Santhosh | RLJ | NA | 886271 | 18 | Feb | 13 | 23 | M | Farmer | Y | Vegetable matter Ragi husk | N | L | N | N | PC | 6/9 | P | 1+ | Occ | Occ GPCs | FE | N | NG | NG | NG | NG | N | NG |
| 6 | Abdul salim | RLJ | NA | 895588 | 22 | Mar | 13 | 30 | M | Welder | Y | FOB iron piece | N | L | N | N | C | CF 1/2 m | P | 4+ | Numerous | Numerous GNB | N | N | G | G | G | NG | N | Kleb.pneumoniae |
| 7 | Venkatamma | RLJ | NA | 361721 | 9 | Apr | 13 | 50 | F | Manual laborer | Y | vegetable matter | N | R | N | Y | PC | HM | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 8 | Venktramappa | RLJ | NA | 904343 | 23 | Apr | 13 | 65 | M | Farmer | Y | Dust falling | N | L | N | Y | PC | 6/24 | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 9 | Raghvendra | RLJ | 901838 | NA | 8 | Jun | 13 | 30 | M | Mechanic | Y | FOB Insect hit while driving 2 wheeler | N | L | N | N | PH | 6/9 | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 10 | Saraswathamma | SNR | 33437 | NA | 24 | Jun | 13 | 26 | F | Manual laborer | N | N | N | L | N | N | PH | 6/9 | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 11 | Nanjamma | SNR | 40088 | NA | 28 | Jun | 13 | 70 | F | Housewife | Y | Foreign body | N | R | N | N | PH | 6/24 | N | 0 | N | No org | FE | N | G | NG | NG | G | P | Aspergillus flavus |
| 12 | Muniyappa | SNR | 39234 | NA | 3 | Jul | 13 | 70 | M | Farmer | N | N | N | R | N | Y | PH | PL + | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 13 | Muniswamy | SNR | 42541 | NA | 5 | Jul | 13 | 28 | M | Manual laborer | N | N | N | R | N | N | PH | 6/6 | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 14 | Anappa | SNR | 53453 | NA | 8 | Jul | 13 | 42 | M | Farmer | N | N | N | R | N | N | PC | 6/18 | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 15 | Mujeeb | RLJ | 943343 | NA | 4 | Aug | 13 | 36 | M | Mechanic | Y | FOB iron piece | N | L | N | Y | C | CF 1m | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 16 | Sakappa | SNR | 82661 | NA | 11 | Sep | 13 | 66 | M | Manual laborer | N | N | N | L | N | N | PC | CF 3m | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 17 | Mujeeb-2 | RLJ | 946593 | NA | 16 | Sep | 13 | 32 | M | Mechanic | Y | FOB iron piece | N | L | N | N | PC | CF 1m | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 18 | Venkatamma | RLJ | 950933 | NA | 1 | Oct | 13 | 45 | F | Housewife | Y | vegetable matter | N | L | N | N | PC | HM | P | 3+ | Moderate | FE | FE | N | G | G | G | G | P | Fusarium sp-No sporulation |
| 19 | Prakash | SNR | 87521 | NA | 18 | Nov | 13 | 30 | M | Farmer | Y | vegetable matter | N | R | N | N | PC | 6/24 | P | 4+ | Numerous | Numerous GPC | N | N | G | G | NG | NG | N | S.pneumoniae |
| 20 | Hanumappa | SNR | 88835 | NA | 22 | Nov | 13 | 65 | M | Farmer | N | N | N | R | N | N | PC | CF | P | 1+ | Occ | Occ GPC | N | N | NG | NG | NG | NG | N | NG |
| 21 | Muniyamma | SNR | 89665 | NA | 25 | Nov | 13 | 68 | F | Housewife | Y | Dust | N | R | N | N | PC | No PL | P | 1+ | Occ | Occ GPC | N | N | NG | NG | NG | NG | N | NG |
| 22 | Illaz Khader | SNR | 89926 | NA | 25 | Nov | 13 | 40 | M | Welder | Y | FOB iron piece | N | R | N | N | PC | 6/9 | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 23 | Kauser | SNR | 88002 | NA | 25 | Nov | 13 | 20 | F | Student | Y | Dust falling | N | L | N | N | PH | 6/18 | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 24 | Kanthamma | RLJ | 966993 | NA | 26 | Nov | 13 | 27 | F | Manual laborer | Y | vegetable matter | N | R | N | N | PC | 6/9 | P | 2+ | Few | FE | FE | N | NG | NG | NG | G | P | Curvalaria sp |
| 25 | Hanumappa | SNR | 98182 | NA | 27 | Nov | 13 | 90 | M | Farmer | N | N | N | R | N | N | PC | PL + | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 26 | Girajamba | SNR | 91078 | NA | 28 | Nov | 13 | 25 | F | Farmer | N | N | N | R | N | N | PC | 6/9 | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 27 | Lakshmaiah | SNR | 91216 | NA | 28 | Nov | 13 | 63 | M | Farmer | Y | vegetable matter | N | L | N | N | PC | 6/9 | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 28 | Sonibai | SNR | 92192 | NA | 2 | Dec | 13 | 12 | F | Student | Y | Foreign body | N | L | N | N | PC | 6/12 | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 29 | Chowdappa | SNR | 92228 | NA | 2 | Dec | 13 | 60 | M | Farmer | Y | vegetable matter | N | L | N | N | PC | CF 4m | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 30 | Basavachari | SNR | 76104 | NA | 4 | Dec | 13 | 55 | M | Farmer | Y | FOB Insect hit while driving 2 wheeler | N | R | N | N | PC | HM | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 31 | Chandrashekar | SNR | NA | 14012 | 4 | Dec | 13 | 35 | M | Manual laborer | Y | Dust falling | N | R | N | N | PC | CF 2m | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 32 | Satyanna | SNR | 93315 | NA | 5 | Dec | 13 | 49 | M | Barbender | Y | Foreign body | N | L | N | N | PC | 6/12 | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 33 | Gangamma | SNR | 93637 | NA | 5 | Dec | 13 | 60 | F | Manual laborer | Y | vegetable matter | N | R | N | Y | PC | 6/9 | P | 4+ | Numerous | Numerous GPC | N | N | G | G | NG | NG | N | S.pneumoniae |
| 34 | Senappa | RLJ | 97003 | NA | 6 | Dec | 13 | 60 | M | Farmer | Y | vegetable matter | N | L | N | Y | PC | CF 4m | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 35 | Shamala | SNR | 94256 | NA | 7 | Dec | 13 | 32 | F | Manual laborer | Y | vegetable matter | N | L | N | N | PC | 6/9 | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 36 | Mulbagalippa | SNR | NA | 14766 | 9 | Dec | 13 | 80 | M | Farmer | N | N | N | L | N | N | PC | PL + | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 37 | Raniappa | SNR | 94589 | NA | 9 | Dec | 13 | 70 | M | Farmer | Y | vegetable matter | N | R | N | N | PC | No PL | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |

# MASTER CHART- continued

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 38 | Chalapathi | SNR | 95322 | NA | 12 | Dec | 13 | 30 | M | Manual laborer | Y | Dust falling | N | R | N | N | PC | CF 2m | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 39 | Kalpana | RLJ | 971825 | NA | 13 | Dec | 13 | 11 | F | Student | Y | Foreign body | N | L | N | N | PC | 6/18 | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 40 | Venkatswamy | SNR | 96851 | NA | 14 | Dec | 13 | 35 | M | Manual laborer | Y | FOB?insect hit | N | L | N | N | PC | CF | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 41 | Muniyappa | RLJ | 97244 | NA | 17 | Dec | 13 | 70 | M | Farmer | Y | FOB Insect hit | N | R | N | Y | PC | CF 2m | P | 2+ | Few | FE | FE | N | G | G | NG | G | P | Colletotrichum spp. |
| 42 | Chowdamma | SNR | 97687 | NA | 18 | Dec | 13 | 35 | F | Manual laborer | Y | vegetable matter | N | L | N | N | PC | 6/9 | P | 1+ | Occ | Occ GPC | FE | N | NG | NG | NG | G | P | Aspergillus niger |
| 43 | Narayanswamy | SNR | 85016 | NA | 19 | Dec | 13 | 45 | M | Farmer | Y | ?Dust | N | L | N | N | PC | HM | P | 1+ | Occ | Moderate GPC | N | N | G | G | NG | NG | N | S.pneumoniae |
| 44 | Chandrappa | SNR | 98484 | NA | 20 | Dec | 13 | 38 | M | Manual laborer | Y | vegetable matter | N | L | N | N | PC | 6/9 | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 45 | Kemparaju | SNR | 98513 | NA | 20 | Dec | 13 | 25 | M | Farmer | Y | vegetable matter | N | L | N | N | PC | 6/9 | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 46 | Prabhakar | SNR | 99217 | NA | 23 | Dec | 13 | 30 | M | Carpenter | Y | Dust (wood) | N | R | N | N | PC | 6/9 | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 47 | Avinash | SNR | 99882 | NA | 26 | Dec | 13 | 20 | M | Welder | Y | FOB iron piece | N | R | N | N | PC | 6/9 | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 48 | Venkatappa | SNR | 1494 | NA | 31 | Dec | 13 | 65 | M | Manual laborer | Y | vegetable matter | N | R | N | N | PC | CF 1m | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 49 | Bharathi | SNR | 1888 | NA | 1 | Jan | 14 | 21 | F | Farmer | Y | vegetable matter | N | R | N | N | PC | 6/9 | P | 2+ | Few | FE | FE | N | G | G | NG | G | P | F.semitectum |
| 50 | Shivaiah | SNR | 2087 | NA | 2 | Jan | 14 | 19 | M | Student | Y | Dust | N | L | N | N | PH | 6/9 | P | 2+ | Few | No org | N | N | NG | NG | NG | NG | N | NG |
| 51 | Asharani | SNR | 2104 | NA | 2 | Jan | 14 | 18 | F | Student | Y | vegetable matter | N | R | N | N | PC | 6/9 | P | 1+ | Occ | Occ GPC | N | N | NG | NG | NG | NG | N | NG |
| 52 | Venkatswamyappa | SNR | 3613 | NA | 7 | Jan | 14 | 45 | M | Farmer | Y | Foreign body | N | R | N | N | PH | 6/9 | P | 1+ | Occ | Occ GPC | N | N | NG | NG | NG | NG | N | NG |
| 53 | Mahadevi | SNR | 100034 | NA | 8 | Jan | 14 | 28 | F | Housewife | Y | vegetable matter | N | L | N | N | PC | 6/9 | P | 1+ | Occ | Occ GPC | N | N | NG | NG | NG | NG | N | NG |
| 54 | Muniyappa.R | SNR | 82353 | NA | 16 | Jan | 14 | 76 | M | Farmer | Y | vegetable matter | N | L | N | N | PC | CF 1m | P | 1+ | Occ | Occ GPC | N | N | NG | NG | NG | NG | N | NG |
| 55 | Sunil | SNR | 6376 | NA | 16 | Jan | 14 | 22 | M | Farmer | Y | dust Stone dust | N | R | N | N | PC | 6/9 | P | 1+ | Occ | Occ GPC | N | N | NG | NG | NG | NG | N | NG |
| 56 | Hanumanth | SNR | 6756 | NA | 17 | Jan | 14 | 28 | M | welder | Y | FOB( iron piece) | N | R | N | N | PC | 6/9 | P | 1+ | Occ | Occ GPC | N | N | NG | NG | NG | NG | N | NG |
| 57 | Ratnamma | SNR | 7667 | NA | 20 | Jan | 14 | 39 | F | Manual laborer | Y | vegetable matter | N | R | Y | Y | PC | HM | P | 2+ | Few | FE | FE | N | G | G | NG | G | P | F.solani |
| 58 | Venkatamma | RLJ | NA | 984987 | 28 | Jan | 14 | 75 | F | Manual laborer | Y | vegetable matter | N | R | N | Y | C+PC | CF 1m | P | 2+ | Few | FE | FE | N | G | G | NG | G | P | F.semitectum |
| 59 | Venkateshappa | RLJ | NA | 986108 | 30 | Jan | 14 | 60 | M | Farmer | Y | vegetable matter | N | R | N | N | PC | 6/9 | P | 2+ | Few | Few GPCs | FE | N | NG | NG | NG | NG | NG | NG |
| 60 | Krishnamma | SNR | 11410 | NA | 31 | Jan | 14 | 42 | F | Stone cutter | Y | FOB Stone splinter | N | R | Y | N | PC | HM 3m | N | 0 | N | No org | N | N | NG | NG | NG | NG | NG | NG |
| 61 | Shardamma | RLJ | NA | 9867789 | 1 | Feb | 14 | 38 | F | Manual laborer | Y | FOB Metal splinter(?iron) | N | L | Y | N | C | HM | P | 2+ | Few | Few GPCs FE | FE | N | G | G | NG | G | P | Aspergillus flavus |
| 62 | Narayanamma | SNR | 13428 | NA | 6 | Feb | 14 | 65 | F | Manual laborer | Y | vegetable matter | N | R | N | N | C+PC | 6/9 | P | 1+ | Occ | Occ GPC | N | N | NG | NG | NG | NG | N | NG |
| 63 | Krishnappa | SNR | 14497 | NA | 10 | Feb | 14 | 60 | M | Manual laborer | Y | vegetable matter | N | L | N | Y | C | HM 2m | P | 4+ | Numerous | Numerous GPC | N | N | G | G | NG | NG | N | S.pneumoniae |
| 64 | Nanjappa | SNR | 18094 | NA | 20 | Feb | 14 | 60 | M | Farmer | Y | ?dust | N | L | Y | N | PC | CF | P | 1+ | Occ | No org | N | N | NG | NG | NG | NG | N | NG |
| 65 | Shankarappa | SNR | 19737 | NA | 25 | Feb | 14 | 30 | M | Stone cutter | Y | FOB Stone hit during rock blast | N | L | N | N | C | HM 1 m | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 66 | Narayanamma | RLJ | NA | 1001497 | 15 | Mar | 14 | 45 | F | Manual laborer | Y | Vegetable matter wooden stick | N | L | N | N | C+PC | CF 3m | P | 1+ | Occ | Few GPC | FE | N | G | G | NG | G | P | Fusarium sp |
| 67 | Muniyappa | RLJ | NA | 1005672 | 2 | Apr | 14 | 55 | M | Farmer | Y | vegetable matter | N | R | N | N | C+PC | HM 0.5m | P | 1+ | Occ | FE | FE | N | G | G | G | G | P | Colletotrichum gleosporoides |
| 68 | Sundraram | RLJ | 996483 | NA | 15 | Apr | 14 | 82 | M | Farmer | N | N | N | L | N | N | PC | 6/9 | N | 0 | N | No org | N | N | NG | NG | NG | NG | N | NG |
| 69 | Lakshamamma | RLJ | 2147 | 723 | 5 | May | 14 | 70 | F | Housewife | Y | FOB insect hit | N | R | N | N | C+PC | PL + | P | 1+ | Occ | Occ GPC | N | N | NG | NG | NG | NG | N | NG |
| 70 | Malappa | RLJ | 10982014 | NA | 9 | May | 14 | 75 | M | Farmer | N | N | N | R | N | N | PC | HM 3m | P | 1+ | Occ | Occ GPC | N | N | NG | NG | NG | NG | N | NG |
| 71 | Narayanswamy | RLJ | NA | 10426 | 21 | May | 14 | 38 | M | Driver | Y | Vegetable matter hit by rice flakes in marriage function | N | L | N | N | PC | 6/9 | P | 1+ | Occ | Occ GPC | N | N | NG | NG | NG | NG | N | NG |
| 72 | Jayamma | RLJ | NA | 25430 | 4 | Jul | 14 | 55 | F | Manual laborer | Y | vegetable matter | N | L | N | N | PC | 6/60 | P | 2+ | Few | FE | FE | N | G | G | G | G | P | Fusarium spp. |
| 73 | Venkatswamy | RLJ | NA | 26197 | 7 | Jul | 14 | 50 | M | Farmer | Y | Cows tail | N | L | N | N | C | HM | P | 1+ | Occ | Occ GPC | N | N | NG | NG | NG | NG | N | NG |
| 74 | Samakka | RLJ | NA | 35709 | 1 | Sep | 14 | 65 | F | Housewife | Y | Hair dye | N | L | N | N | C | CF 2m | P | 1+ | Occ | Occ GPC | N | N | NG | NG | NG | NG | N | NG |
| 75 | Krishnappa | RLJ | NA | 52822 | 15 | Sep | 14 | 60 | M | Farmer | Y | Cows tail | N | L | N | N | PC | 6/9 | P | 1+ | Occ | Occ GPC | N | N | NG | NG | NG | NG | N | NG |

**KEY TO MASTER CHART**

| | |
|---|---|
| M | Male |
| F | Female |
| N | No |
| Y | Yes |
| FOB | Foreign Body |
| L | Left |
| R | Right |
| HM | Hand movements |
| PL | Perception of light |
| C+PC | Central +Paracentral ulcer |
| PC | Paracentral Ulcer |
| C | Central ulcer |
| PH | Peripheral ulcer |
| CF | Close to Face |
| P | Positive in Gram stain |
| N | Negative in KOH |
| FE | Fungal elements |
| No org | No organisms |
| NG | No growth |
| G | Growth |
| GPC | Gram positive cocci |
| GNB | Gram negative bacilli |
| KOH | Potassium Hydroxide (10%) |
| BA | Blood agar |
| CA | Chocolate agar |
| MA | MacConkey agar |
| SDA | Sabourauds Dextrose Agar |
| LPCB | Lactophenol cotton blue |

ANNEXURE-1

PROFORMA

**NAME:** _____ **CASE NO:** _____

**AGE:** _____ **SEX: MALE** ☐ **FEMALE** ☐ **OP/IP NO:** _____

**ADDRESS:** _____ **DOA:** _____ **DOD:** _____

_____

_____ **OCCUPATION:** _____

☎ _____ Rural ☐ Semi Urban ☐ Urban ☐

## CLINICAL DATA

**1. CHIEF COMPLAINTS:** _____

_____

_____

**2. HISTORY OF PRESENTING ILLNESS:** _____

Pain Yes ☐ No ☐ If yes, details _____

Watering from eyes Yes ☐ No ☐ If yes, details _____

Defective vision Yes ☐ No ☐ If yes, details _____

Redness of eyes Yes ☐ No ☐ If yes, details _____

Photophobia Yes ☐ No ☐ If yes, details _____

Discharge from eyes Yes ☐ No ☐ If yes, details _____

Injury Yes ☐ No ☐ If yes, details _____

(Foreign body/Dust/Vegetable matter/twig) _____

**Treatment taken**

Antibiotics Yes ☐ No ☐ If yes, details _____

Corticosteriods Yes ☐ No ☐ If yes, details _____

Others Yes ☐ No ☐ If yes, details _____

**3. PAST HISTORY:** _____

USE OF DRUGS(Antibiotics/ Corticosteriods/ Antimetabolities) Yes ☐ No ☐ If yes, details _____

_____

USE OF CONTACT LENS Yes ☐ No ☐ If yes, details _____

**4. PERSONAL HISTORY:** _____

_____

**5. FAMILY HISTORY:** _____

_____

Any history of mucocutaneous fungal infections ----------

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**6. GENERAL PHYSICAL EXAMINATION:** ----------

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**7. SYSTEMIC EXAMINATION:** ----------

**CVS:** ---------- **PA:** ----------

**RS:** ---------- **CNS:** ----------

**8. OPHTHALMIC EXAMINATION:**

| | Right | Left |
|---|---|---|
| Vision | | |
| Head posture<br>Eye brows<br>Lids<br>Lacrimal apparatus<br>Discharge | | |
| Conjunctiva | | |
| Sclera | | |
| **Details of Corneal Lesion**<br>Site<br>Size<br>Shape<br>Base<br>Color<br>Edge<br>Surface<br>Depth of Stromal Infiltrates<br>Satellite Lesion<br>Immune ring<br>KP<br>Endothelial plaques<br>Descematocele<br>Vascularization<br>Sensation<br>Rest of cornea | | |
| Anterior Chamber<br>Depth<br>Hypopyon<br>Aqueous Flare /Cells /KPs | | |
| Iris | | |
| Pupil | | |
| Lens | | |
| Fundus | | |
| Slit Lamp examination | | |

**9. OTHER EXAMINATION:**

**ENT CHECK UP** ----------

**DERMATOLOGY CHECK UP** ----------

**10. INVESTIGATIONS:**

**Corneal staining with Fluorescein** ----------

**Double Lacrimal sac patency** ----------

**11. IMPRESSION:**

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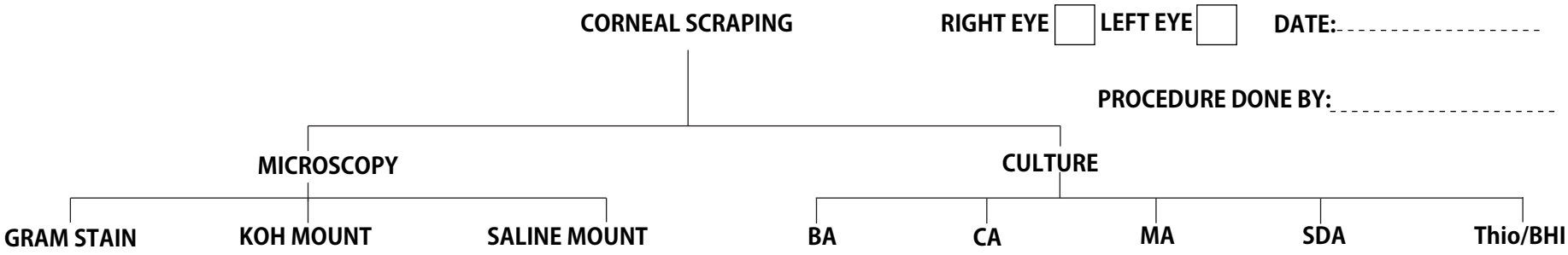
NAME:_____ AGE:_____ SEX: MALE ☐ FEMALE ☐ OP/IP NO:_____ RLJH ☐ SNR ☐



## SANSKRIT TEXT ON CORNEAL ULCER FROM SUSRUTA-SAMHITA

निमग्नरूपं हि भवेत्तु कृष्णे सूच्येव विद्धं प्रतिभाति यद्वै।
स्रावं स्रवेदुष्णमतीव रुक् च तत् सव्रणं शुक्र(क्ल)मुदाहरन्ति ॥4॥
दृष्टेः समीपे न भवेत्तु यच्च न चावगाढं न च संस्रवेद्धि।
अवेदनावन्न च युग्मशुक्रं तत्सिद्धिमाप्नोति कदाचिदेव ॥5॥
विच्छिन्नमध्यं पिशितावृतं वा चलं सिरासक्तमदृष्टिकृच्च।
द्वित्वग्गतं लोहितमन्ततश्च चिरोत्थितं चापि विवर्जनीयम् ॥6॥
उष्णाश्रुपातः पिडका च कृष्णे यस्मिन् भवेन्मुद्गनिभं च शुक्रम्।
तदप्यसाध्यं प्रवदन्ति केचिदन्यच्च यत्तित्तिरिपक्षतुल्यम् ॥7॥

## AKISPAKATYAYA (KERATITIS)

संच्छाद्यते श्वेतनिभेन सर्वं दोषेण यस्यासितमण्डलं तु ॥9॥
तमक्षिपाकात्ययमक्षिकोपसमुत्थितं तीव्ररुजं वदन्ति ।

# INTRODUCTION

# OBJECTIVES

# REVIEW OF LITERATURE

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# DISCUSSION

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