

Fungal Keratitis; New Horizons

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Fungal keratitis remains most challenging to understand, diagnose and treat for ophthalmologists. Terminology of fungus is confusing and adds to the difficulty. Current management is still based on traditional diagnosis and limited options of treatment. Fortunately, there are newer ways to diagnose and treat fungal keratitis which I call new horizons to look for in fungal keratitis.

For an ophthalmologist, fungi should be classified on the basis of microscopic characteristics, which make it easy to understand and remember. On microscopy, fungi can be seen as **unicellular yeasts** or as **multicellular molds** which are in the form of filaments/hyphae/mycelium or branches. Most common fungi are fusarium and aspergillus (both molds) in developing countries with candida (yeast) as less common while in developed countries candida is more common because of contact lens use. In following classification first three are multicellular molds and fourth one is the unicellular yeast.

1. Filamentous septate non pigmented (Hyaline): means molds with filaments which have septa and are non-pigmented. Fusarium and aspergillus are most common species.
2. Filamentous septate pigmented (Dematiaceous/phaeoid) means molds with filaments and same as first one except pigmented and now second

Common cause of fungal keratitis in developing world. Curvalaria and Alternaria are most common species

3. Filamentous non septate: These are molds with filaments but no septa and most common species is mucor causing mucormycosis.
4. Yeast which is unicellular and common species is Candida and zygomycetes.

Diagnosis is based on clinical picture, traditional microscopy, culture and latest technologies with my emphasis on later.¹ These newer techniques are practically possible and not that expensive as normally thought. Initial diagnosis of fungal keratitis is mainly clinical.² Clinically fungal keratitis can have one or more of peculiar features² like satellite lesions, infiltrate, feathery or hyphate margins, spikes on surface, elevated edges, rough surface, gritty appearance on scrapping, endothelial or posterior plaque, immobile convex cheesy hypopyon, greyish brown pigmentation, collar button appearance and Wessely immune ring.³ Traditionally and most commonly fungi are diagnosed by simple microscopy by using Gram, Giemsa and KOH staining and culture. Microscopy can easily differentiate between filamentous septate, filamentous non septate and unicellular yeasts. Cultures like sebouraud dextrose agar can be used to grow fungi but it takes many days to grow and keratitis gets worse during this time.

One should be careful in taking sample for microscopy and culture, as fungi are usually deep in cornea. Superficial exudates should be removed before taking sample. In case of negative results but strong clinical suspicion, corneal biopsy can be taken and sent to laboratory. I am very strong advocate of small ophthalmic laboratory in ophthalmic department for microscopy slides and culture media inoculation at least.

Confocal microscopy is another way to diagnose fungi.⁴ It can differentiate between molds and yeast

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and also can diagnose acanthamoeba but not good for bacteria. Other newer techniques⁵ like Polymerase Chain Reaction (PCR)⁶, Metagenomic Deep Sequencing (MDS)⁷ and Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI TOF MS)⁸ can diagnose keratitis in up to 2 hours though these tests do not give drug sensitivity. These techniques not only differentiate between bacteria, fungus and amoeba but can also diagnose exact form of fungus so exact and proper treatment can be started same day. MALDI TOF and MDS are expensive though MDT can identify any organism in sample but PCR is more economical and practical in developing countries.

PCR is perhaps most practical and relatively economical test as compared to other new tests. Advantages of PCR are handling of small samples, high sensitivity, high specificity and quick results. Its particular advantage in ophthalmology is that it amplifies the pathogen. So, even very tiny specimens give positive results; actually, sensitivity is so high that theoretically even single organism can be detected. Because of high specificity we can detect exact genotype. Speed of test is well known and results can be as quick as 2 hours. PCR is considered expensive but after establishment of service, it is cost effective because of its advantages. It does need well-trained and dedicated staff. At times one may have false positive results because of amplification.

In house PCR is more versatile and economical though there is initial high cost. PCR Laboratory not only gives quick results to ophthalmologist for keratitis and endophthalmitis but also helps other department to diagnose conditions like meningitis. One can design range of primers of common pathogens and within two hours, diagnosis of exact species of fungus, bacteria, virus or acanthamoeba can be made.

Treatment was mainly topical drops for long time and even that with limited options. Now newer antifungals have added to armory. In addition, ophthalmologists now have other non-surgical and surgical options.

Two main classes of antifungals are polyenes and azoles. The polyenes are Natamycin and Amphotecin B while Azoles are further divided into Amidazoles and Trizoles. Imidazoles are Ketaconazole, and Miconazole while Trizoles are Fluconazoles, Voriconazole and Posaconazole. Other new treatments

include antimicrobial peptides (AMPs), Terbinafine, Micafungin (MCFG), Caspofungin, immunosuppressant like Tacrolimus (FK506) and Vitamin D receptor agonist (VDRA).⁹

Natamycin works well against molds but only available in topical form and does not penetrate to deeper layers of cornea. Voriconazole is effective against both molds and yeast. Fluconazole is mainly effective against candida but it is also effective against molds in high concentration. Amphotericin B acts mostly against Candida with variable action against molds.

Fluconazole and Voriconazole drops might have been a leap forward but Mycotic Ulcer Treatment Trial 1 (MUTT 1) showed that even the topical Voriconazole was not more effective than topical Natamycin. In Mycotic Ulcer Treatment Trial 2 (MUTT 2) Voriconazole was not of any benefit as oral adjuvant to topical therapy. Systemic antifungal therapy has limited role in fungal keratitis but intrastromal and intracameral use of antifungals are showing promising results. Voriconazole is the most commonly used intrastromal antifungal because of its wide spectrum against molds and yeasts but Amphotericin B and Fluconazole are equally good if causative agent is known. Doses and techniques of intrastromal and intracameral antifungals can be downloaded from OSP app on your mobile phone, which is developed by Ophthalmological society of Pakistan.

Corneal cross linking (CXL) was developed for treating corneal ectasia particularly keratoconus.¹⁰ Ultraviolet light used in CXL is known for its microbicidal effect and this effect was used to treat microbial keratitis. This was later named as photoactivated chromophore for infectious keratitis – corneal collagen cross linking (PACK-CXL). The antimicrobial effect of CXL is because of ultraviolet (UV) light and riboflavin used in procedure. UV light can directly damage DNA and RNA in microbes including fungi. On other hand, riboflavin can release reactive oxygen species (ROS) when activated, which then interacts with cell membranes and nucleic acids of microbe. The combined effect of UV light and riboflavin increases effect 10 folds as compared to UV light alone. In addition, photoactivated collagen fibers become more resistant to enzymatic degradation by microbes. However, PACK-CXL has been shown to be more effective for superficial ulcer and more effective for bacterial than fungal keratitis.

Randomized clinical trials also showed that antimicrobial treatment and PACK-CXL had similar results as the antimicrobial treatment alone. However, majority of evidence shows that PACK-CXL improves outcome along with antimicrobial treatment.

Other surgical options are partial keratectomy, amniotic membrane, conjunctival flap and penetrating keratoplasty (PK). PK should be reserved as the last resort as chances of graft infection are very high.

In conclusion most common fungi in mycotic keratitis are *Fusarium* and *Aspergillus* in developing countries with *Candida* as less common. Clinical diagnosis can be made in 60% cases, which should be confirmed by diagnostic test like PCR being more specific and quicker. New drugs, intrastromal injections, PACK-CXL have improved management of fungal keratitis. Tertiary care centers should invest in setting up PCR laboratories and CXL facility.

Conflict of Interest

Authors declared no conflict of interest.

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