

# Keratomycosis: Some Epidemiological Data, Diagnosis and Drug Sensitivity

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**Abstract Purpose:** The present study was undertaken to investigate the prevalence of mycotic keratitis and the profile of the fungi responsible in this region. Special attention was given to assess the prevalence of various species of *Aspergillus*, predisposing factors for keratomycosis and drug sensitivity. **Methods:** Corneal scraping samples from 148 cases of corneal ulcer and traumatic corneal infection with clinically suspicion of having fungal infections were examined microscopically and for culture. Minimum inhibitory concentration (MIC) of itraconazole and amphotericin-B were tested against the *Aspergillus* species isolates. **Results:** *Aspergillus* species were isolated in 26 (20.15%) cases and *Aspergillus fumigatus* was the predominant species isolated in 17 (65.38%) cases. This fungus was found to be sensitive to itraconazole with MIC of 0,125 -1 µg/ml. **Conclusion:** *Aspergillus fumigatus* is more common in mycotic keratitis, and itraconazole is effective against it. Vegetative trauma is the major risk factor.

**Keywords:** aspergillosis, prevalence, risk factors, drug sensitivity

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## 1. Introduction

Mycotic keratitis differs from other mycoses in certain features: it is not life threatening unless it disseminates in the eye but is certainly sight threatening. If not properly treated, could lead to a marked diminution in vision, blindness or even loss of eyeball [1]. The incidence of keratomycosis is increasing with injudicious use of topical steroids, antibiotic agents, herbal remedies like ghee and extract of leaves [2]. Mycotic keratitis commonly occurs in elderly persons; agricultural workers, cultivators and housewives are found to be affected more. Recently it has been conclusively proved that *Aspergillus* infection of the eye is on the rise [2,3,4,5].

Keay and collaborators in 2011 reported that from a total of 733 cases of fungal keratitis identified most cases were confirmed by culture from corneal scraping (n = 693). Some 268 of 733 cases (37%) were associated with refractive contact lens wear, 180 of 733 cases (25%) were associated with ocular trauma, and 209 of 733 cases (29%) were associated with ocular surface disease. No predisposing factor was identified in the 10% of the cases. Filamentous fungi were identified in 141 of 180 ocular trauma cases (78%) and in 231 of 268 refractive contact lens-associated cases (86%) [6].

The present study was undertaken to find the prevalence of mycotic keratitis and the profile of the fungi responsible in this region. Special attention was given to assess the prevalence of various species of *Aspergillus*, predisposing factors for keratomycosis and drug sensitivity.

## 2. Materials and Methods

### 2.1. Subjects

The study was carried out in the Department of Microbiology, in collaboration with the Department of Ophthalmology, Government Medical College and Hospital, Nagpur, India. A total of 148 cases of corneal ulcer with the clinically suspicion of fungal infection were included in the present study.

### 2.2. Specimens and Culture

Corneal scraping material was collected from the patients presenting with the corneal ulcer. First, the eye was cleaned with sterile normal saline to remove all necrotic exudates. After instillation of anesthetic drops, with the help of Bark - Parker blade no. 15 scraping was done and examined for fungi by microscopic examination

of 10% KOH mount. The culture was done on Sabouraud dextrose agar with chloramphenicol and species were identified as per the standard methods [2,3]. Slide culture was carried out to identify the isolate up to the species level.

### 2.3. Antifungal Susceptibility

*Aspergillus* species susceptibility to amphotericin B and itraconazole was tested by determining minimum inhibitory concentration by agar dilution method at Ranbaxy Research Laboratory, Gurgaon, Haryana, India.

## 3. Results and Discussion

The study group comprised of total 148 cases of corneal ulcers, 105 male and 43 female in a ratio of 2.5:1. The age group ranged 15-50 years. The mean age was 44 ± 4.79. Maximum numbers of corneal ulcers 52/148 cases (35%) were in the age group 31 to 40 years followed by 38/148 (26%) in 41-50 years; 35/148 (23.64%) revealed the fungal etiology. *Aspergillus* species was isolated in 26 cases out of these 35 cases (74.28%) (Table 1). The frequency of mycotic keratitis varies from place to place [4]. Higher percentage of *Aspergillus* corneal ulcer among 40-60 years was reported [4].

Only three types of fungi were isolated in the present study, all well known to cause mycotic keratitis, of which *Aspergillus* species was overwhelmingly common. The profile of fungal isolates is shown in Table 2. Of the 26 *Aspergillus* isolates, 17 (65.38%) were *Aspergillus fumigatus* and 9 (34.61%) were *Aspergillus niger*. Similar type of observations has been reported by other studies [7].

For the diagnosis of fungal etiology, fungal culture is more reliable than microscopy, but positive microscopy should be correlated to culture findings to exclude

contamination. In the present study, as shown in Table 2 where trauma is the most common underlying condition of corneal ulcer and 22 out of 23 fungal isolates were obtained from these patients with trauma.

**Table 1. Characteristics of the study group with corneal ulcers (n=148)**

Characteristics	Incidence (number & percent)
Gender- Male	105
Female	43
Ratio	2.5:1
Age group in years with corneal ulcers:	
Mean age of the study group	44±4.79
Incidence of corneal ulcers:	
<20	27 (18.24)
31-40	52 (35.13)
41-50	38 (2.67)
>50	31 (20.94)
Fungal etiology	35 (23.64)

**Table 2. Profile of fungal isolates from corneal ulcers (n=35)**

Isolates	Number (%)
<i>Aspergillus</i> species:	26 (74.28)
<i>Aspergillus fumigatus</i>	17/26 (65.38)
<i>Aspergillus niger</i>	9/26 (34.61)
<i>Candida albicans</i>	6 (17.14)
<i>Fusarium</i> species	3 (8.57)

In this study the antifungal susceptibility testing indicated that amphotericin B and itraconazole were useful drugs as depicted in Table 3. Only a few types of fungi are more prevalent with *Aspergillus* species being substantially common amongst them. The routine testing of all corneal ulcers for fungal etiology is important as the management of mycotic disease differs from the other causes.

**Table 3. Risk factors leading to Fungal infection in corneal ulcer**

S.NO	Predisposing factors	Corneal ulcer cases (n=148)	<i>Aspergillus</i> species (n=26)	<i>Candida albicans</i> (n= 6)	<i>Fusarium</i> species (n=3)	Fungal growth present (n= 35)
1.	<b>Trauma</b>					
	Only Trauma.	80	7	2	0	9
	Trauma + Topical steroid/antibiotic drop.	24	12	3	1	16
	Trauma + Diabetes+ Topical steroid / antibiotic drop.	8	3	1	0	4
	With Herbal juices instillation.	4	1	0	0	1
	With Oil drops.	3	0	0	0	0
	With Diabetes mellitus.	4	3	0	0	3
	<b>Total Trauma</b>	123	26	6	1	33
2	<b>Contact lens</b>	10	0	0	2	2
3	<b>No specific factor</b>	15	0	0	0	0
	TOTAL (1+2+3)	148	26	6	3	35

**Table 4. Antifungal susceptibility of *Aspergillus* species isolates**

<i>Aspergillus</i> species	Amphotericin B MIC Range (µg/ml)		Itraconazole MIC Range (µg/ml)	
	0.5 – 2	>2	0.125 - 1	>1
<i>A. fumigatus</i>	15	2	17	0
<i>A. niger</i>	9	0	7	2
<b>Total</b>	24	2	24	2

MIC range- Minimum Inhibitory Concentrations.

Nayak and collaborators reported in 2011 that 160 *Aspergillus* isolates from the corneal scrapings of patients

with keratitis were tested for susceptibilities to amphotericin B by broth microdilution method. Of the 160 isolates, 84 (52.5%) showed low minimum inhibitory concentration (MIC) values ( $\leq 1.56 \mu\text{g/ml}$ ) and were designated as amphotericin B-sensitive. Similarly, 76 (47.5%) had high MICs ( $\geq 3.12 \mu\text{g/ml}$ ) and were categorized as amphotericin B-resistant. *A. niger* and *A. flavus* isolates had higher MICs compared to *A. fumigatus*, suggesting a high index of suspicion for amphotericin B resistance [8]. The present work showed that the isolated fungi were both itraconazole- and amphotericin B-sensitive.

Amphotericin B-resistance was correlated significantly with proteinase synthesis, which seems to be an important virulence marker of filamentous fungi in mycotic keratitis [9]. The authors did not compare their data with other centers in India or other geographical areas in India. This should be done and supported with literature.

#### 4. Conclusion

*Aspergillus fumigatus* was the most common fungal species that caused mycotic keratitis and itraconazole was more effective against it. Vegetative trauma was the major risk factor.

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