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## RESEARCH ARTICLE

### MICROBIOLOGICAL PROFILE OF KERATOMYCOSIS IN A TERTIARY CARE HOSPITAL: A RETROSPECTIVE STUDY

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#### ABSTRACT

**Context:** Corneal ulcer is one of the major causes of ocular morbidity in developing countries and fungi are the main etiological agents of corneal ulceration in India. Risk factors for fungal keratitis are male gender, history of ocular trauma, chronic use of corticosteroids and systemic diseases, that impair the host immunity. **Aims:** To determine microbiological profile of fungal keratitis. **Settings and Design:** Descriptive – cross sectional study. **Methods and Material:** A retrospective study was done for the period of six months. Corneal scrapings taken from the clinically diagnosed cases of fungal keratitis and processed by direct microscopic methods and standard culture techniques. **Statistical analysis used:** Statistical analysis was done by using MS Excel sheet. Descriptive statistics like percentage calculation was done in the study. **Results:** Among the 23 fungal keratitis positive cases, males outnumbered females. The maximum number of positive cases were seen in the age group of 31-40 years, followed by the age group of 51-60 years. The most common cause of fungal keratitis was found out to be *Aspergillus fumigatus* and *Fusarium* (31.25 % each), followed by *Aspergillus flavus* and *Aspergillus calidoustus* (17.39 % each), and *Acremonium* (8.69 %), *Curvularia*, *Rhizomucor*, *Aspergillus niger* (4.34% each). *Aspergillus* species were found to be isolated from 43.4% of cases of keratomycosis. Amongst the fungal keratitis cases, 91.3% cases were positive for fungal growth on culture and 8.6% cases were positive for the presence of fungal elements on direct microscopy. **Conclusions:** Lack of early diagnosis and treatment lead to the onset of complications, which may compromise the patient's vision and thereby the quality of life.

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## INTRODUCTION

Keratomycosis is an invasive fungal infection of the cornea.<sup>1,2</sup> It is one of the main reason for blindness in developing countries.<sup>3,4,5</sup> Hyaline fungi and phaeoid fungi are the majority among the agents.<sup>1</sup> Around 1.5 to 2.7 million new cases of corneal ulcer are reported annually.<sup>6</sup> There are various risk factors for fungal keratitis such as male gender, history of ocular trauma, use of corticosteroids and systemic diseases.<sup>7,8</sup> In India, *Fusarium* and *Aspergillus* species are the major ones isolated.<sup>3,9-13</sup> The appropriate therapy depends on the causative fungi isolated in order to initiate appropriate antifungal therapy.<sup>14,15</sup>

## SUBJECTS AND METHODS

A retrospective study was done for the period of six months after obtaining institutional ethical committee clearance. Corneal scrapings taken from the clinically diagnosed cases of fungal keratitis were sent to the Microbiology department for processing at Bangalore Medical College and Research Institute (BMC&RI) Bangalore, during the period of September 2021- February 2022.

#### Inclusion criteria

Samples from clinically suspected fungal keratitis cases among all age groups.

#### Exclusion criteria

Samples from cases other than fungal keratitis.

The corneal scrapings collected under aseptic precautions and were sent to the Department of Microbiology. Direct microscopic examination of the corneal scrapping was performed with 10% KOH wet mount and another portion of the collected corneal scraping samples was inoculated directly on solid culture Sabouraud's dextrose agar (SDA) with or without cycloheximide

- **Potassium hydroxide (KOH) Mount:** Wet mounts of corneal scrapings were prepared in 10% KOH. to observe the presence of fungal elements
- **Culture:** The material from corneal scrapings was inoculated directly onto The portion of the sample was inoculated onto

Sabouraud's dextrose agar (SDA) with or without cycloheximide in duplicate tubes. SDA was incubated at 25°C and 37°C for 4 weeks. Media were checked for any fungal growth daily during the first week and twice a week for the subsequent 3 weeks. Molds were identified by their colony characteristics on SDA and sporulation pattern on lactophenol cotton blue mount. Slide cultures were put up for non-sporulating molds.<sup>16</sup>

**SAMPLE SIZE: 75**

Based on previous study conducted by Alkantan H *et al*, by considering the positive fungal cultures of microbiological diagnosis of mycotic keratitis to be 3.8% , absolute precision (d) of 4.5.<sup>14</sup>

Sample size calculation is as follows

$$n = 4pq/d^2$$

$$P=3.8$$

$$q =96.2(100-p)$$

$$d=4.5$$

$$n= 4x 3.8x96.2 /(4.5)^2$$

$$= 75$$

**Statistical Analysis:** Statistical analysis was done by using MS Excel sheet. Descriptive statistics like percentage calculation was done in the study.

**RESULTS**

In the present study, corneal scrapings were obtained from a total of 75 clinically diagnosed cases of fungal keratitis, out of which 61 were males and 14 were females. Out of total of 75 samples, 23 (30.6%) were positive for fungal growth. Thus the frequency of fungal keratitis in clinically diagnosed cases was 30.6 %. Among the positive cases of fungal keratitis, males (82.6%) outnumbered females (17.4%) (Table 1).

**Table 1. Age and gender wise distribution of cases of fungal keratitis (n=23)**

AGE (IN YEARS)	TOTAL NO OF POSITIVE CASES	MALE	FEMALE
≤ 30	2	1	1
31-40	9	8	1
41-50	1	1	0
51-60	7	6	1
>60	4	3	1
<b>TOTAL</b>	<b>23</b>	<b>19</b>	<b>4</b>

**Table 2. Distribution of fungal agents isolated from the cases of keratomycosis (n=23)**

FUNGAL AGENT	NUMBER OF CASES	PERCENTAGE OF CASES(%)
Aspergillus fumigatus	5	21.73
Fusarium	5	21.73
Aspergillus flavus	4	17.39
Aspergillus calidoustus	4	17.39
Acremonium	2	8.69
Curvularia	1	4.34
Rhizomucor	1	4.34
Aspergillus niger	1	4.34

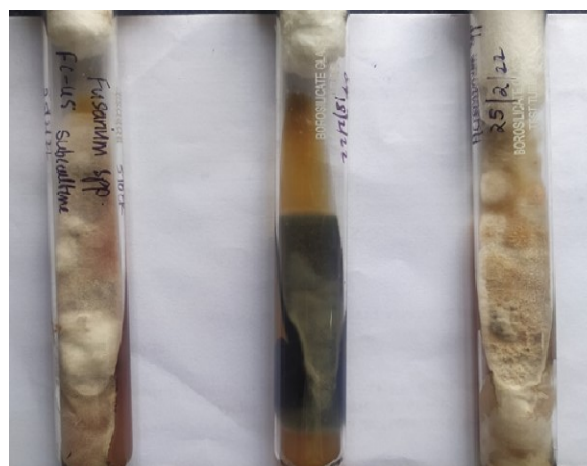
The maximum number of positive cases were seen in age group of 31-40 years, followed by the age groups of 51-60 years. (Table 1). The most common cause of fungal keratitis was found out to be Aspergillus fumigatus and Fusarium (31.25 % each), followed by Aspergillus flavus and Aspergillus calidoustus (17.39 % each), and

**Table 3. Direct microscopy versus Culture (n=23)**

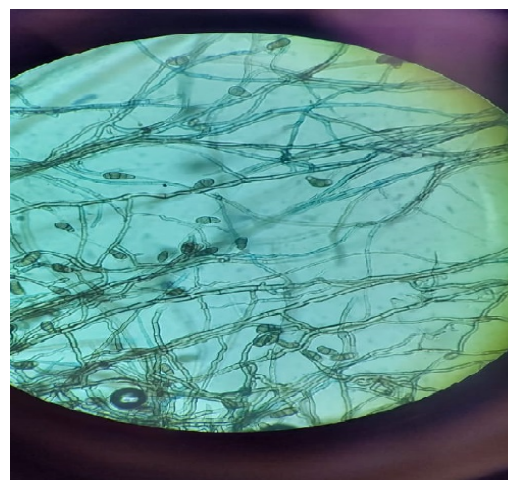
TEST	NUMBER OF POSITIVE CASES	PERCENTAGE OF POSITIVE CASES(%)
KOH	2	8.6
CULTURE	21	91.3



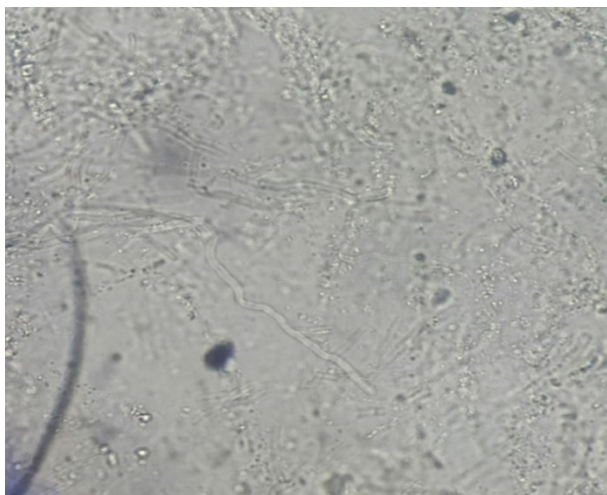
**Fig. 1. SDA culture plate and tube showing Aspergillus calidoustus**



**Fig. 2. SDA culture tubes showing Fusarium spp, Curvularia spp, Acremonium spp**



**Fig. 3. LPCB mount from slide culture showing Curvularia spp**



**Fig. 4. KOH mount showing positive for fungal elements**

Acremonium (8.69 %), Curvularia, Rhizomucor, Aspergillus niger (4.34% each). Aspergillus species were found to be isolated from 43.4% of cases of keratomycosis. Aspergillus species were found to be isolated from 56.5% of cases of keratomycosis. (Table 2). Amongst the fungal keratitis cases, 91.3% cases were positive for fungal growth on culture and 8.6% cases were positive for the presence of fungal elements on direct microscopy (Table 3).

## DISCUSSION

Fungal keratitis is a leading cause for vision impairment and blindness. In the present study, the frequency of fungal keratitis in clinically diagnosed cases was found to be 30.6 % which is comparable with the study conducted by Sardana V *et al*<sup>(16)</sup>. In India, the prevalence of fungal corneal ulcer is around 44-47%. Incidences of mycotic keratitis is varies across southern and western India with 36.7% and 36.3% northern 19-21 (7.3%), northeastern (25.6%), and eastern India (26.4%).<sup>(7)</sup> Among the positive cases of fungal keratitis, males (82.6%) outnumbered females (17.4%) as shown by (Table 1) and our data is consistent with the study done by Gupta A *et al*<sup>(7)</sup> and Chander J *et al*<sup>(17)</sup>. Male predominance can be attributed due to their more indulgence in outdoor activities. In our study, maximum number of positive cases were seen in age group of 31-40 years, followed by the age groups of 51-60 yrs (Table 1), indicating occurrence of keratomycosis in middle to old aged population. Middle aged population is involved majority because which is the most common actively working age group and followed by old age population due to challenges created by co- morbid conditions. A study conducted by Gupta A *et al*<sup>7</sup> also found that majority of fungal ulcers were seen in the middle age group. Amongst the cases of keratomycosis (n=23), 21 were positive for fungal growth on culture, out of which only 2 cases were also positive for the presence of fungal elements on direct microscopy, thus culture should be considered as superior in diagnosing fungal keratitis cases than microscopy (Table 3). Similar observations were also made by Gupta A *et al*<sup>8</sup>. In our study, the commonest causative agents of fungal keratitis was Aspergillus fumigatus and Fusarium spp followed by Aspergillus calidoustus and Aspergillus flavus . Aspergillus species were found to be responsible for 56.51% of cases of keratomycosis (Table 2). In a study done by Gupta A *et al*<sup>8</sup>, Chowdhary A *et al*<sup>18</sup> and Jadhav SV *et al*<sup>19</sup> found Aspergillus species as the most frequent cause of keratitis, followed by F. solanii . which are consistent with our observations in the present study. Hyaline fungi (Aspergillus, Fusarium species) have been reported to be commonly associated with corneal ulcers, followed by dematiaceous fungi (Curvularia).

Fungal agents causing keratitis should receive special attention due to their opportunistic behaviour and indolent course of disease. Lack of early diagnosis and treatment prelude the onset of devastating complications, which may compromise the patient's vision and thereby the quality of life.

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