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Spectrum of mycotic keratitis in north India: Sixteen years study from a tertiary care ophthalmic centre

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ABSTRACT

Introduction: To analyse the fungal culture results of patients with fungal keratitis over sixteen years and look for variations in the trends over years and distribution across ages, gender and seasons.
Materials and methods: Clinical and demographic records and microbiology reports of 18,898 patients of fungal keratitis from 2001 to 2016 were analysed.
Results: Overall fungal culture positivity was 21.5%. 67.3% were males and 32.7% were females. Maximum numbers of samples (17.9%) were received from age group 41–50 years, and maximum fungal culture positivity was seen in age group 31–40 years (30.8%). Most common fungus was *Aspergillus species* (31.1%), followed by *Fusarium species* (24.5%), *Alternaria* (10.5%), *Curvularia* (10.2%), *Helminthosporium* (5.7%), *Bipolaris* (5.4%), *Penicillium* (4.5%), *Candida* (4.4%), *Acremonium* (1.2%), *Rhizopus* (1.0%), *Paecilomyces* (0.8%), *Rhodotorula* (0.5%) and *Mucor* (0.2%). Fungal culture positivity and relative frequency of fungi remained almost stable over the study duration, except *Rhodotorula* spp, which showed a rise 2014 onwards. Highest numbers of culture proven fungal keratitis cases were seen in monsoon season.
Conclusions: To the best of our knowledge, our study is the largest compilation of epidemiological and microbiological features of fungal keratitis, throwing light on important attributes relevant to management of mycotic keratitis patients.

Introduction

Infective keratitis is the inflammation of cornea due to infection. It can be caused by bacteria, viruses, fungi or parasites. Compared to other infective corneal ulcers, fungal corneal ulcers are most challenging to diagnose, treat and are more likely to get perforated and lead to irreversible changes in the cornea. Invasion of fungi into corneal layers followed by the subsequent tissue damage that follows is particularly devastating as it can disrupt the visual axis [1]. Traditionally, fungal keratitis is a suppurative lesion with a dry, raised ulcer with crenate, speculated or pseudohyphate borders, satellite lesions and hypopyon; associated with failure to respond to antibacterial treatment [2]. However, the boundaries of clinical diagnosis become blurred in cases of partial or traditional treatments, mixed infections, antibiotic resistance etc. Therefore microbiological investigations can provide exact diagnosis.

Both filamentous fungi and yeasts are implicated as causative agents of fungal keratitis. Early and accurate diagnosis allowing timely specific treatment remains the corner stone of vision saving management; however, appropriate empirical treatment needs to be started till the time a microbiological diagnosis is made [3]. For initiating the most appropriate empirical treatment, the knowledge of clinico- epidemiological pattern of fungal keratitis is imperative. Comprehensive analysis of results obtained over a period of time from different geographical areas can go a long way in enhancing the knowledge and understanding of such patterns.

Hence, this study was undertaken to retrospectively analyse the spectrum of fungal culture isolates of patients with clinically suspected fungal keratitis paients of all ages over sixteen years (2001–2016), through all seasons.

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Materials and methods

Study setting and duration

The study was carried out in a tertiary care teaching and research eye centre of north India from January 2001 to December 2016. This was a retrospective analysis of clinical and demographic records, and microbiology reports of 18898 patients of clinically suspected fungal keratitis; whose corneal scraping samples were received in the Ocular Microbiology section for diagnosis of causative fungal pathogens in the study period.

Clinical and microbiological diagnosis

Prior to referral, the patients were clinically examined by experienced ophthalmologists. After taking informed consent, corneal scraping samples were collected from the patients and were sent to Ocular Microbiology section, where the specimens were processed by standard mycological procedures for identification of pathogenic fungi. A diagnosis of culture proven keratomycosis was made if- (a) same fungus was isolated in more than one inoculated media, (b) fungal elements were seen in primary microscopy and fungus grew at least in one fungal culture medium or (c) confluent fungal growth was obtained on the specimen inoculated in a single solid medium [4].

Demographic and clinical details of the patients and the pathogenic fungi isolated were entered in the Microsoft excel sheets.

Statistical analysis

Pearson chi square test was applied to find out the statistically significant difference between the categories. A 'p' value of <0.05 was considered to be significant.

Results

Fungal culture positivity

From the total 18,898 clinically suspected fungal keratitis patients, (21.5%) 4069 were positive for fungal culture. The exact culture numbers and percent positivity in years through 2001–2016 are shown in Table 1.

No significant difference was observed between the number of samples received and their fungal culture positivity in these years (p value = 0.759).

Demographic profile

Out of 18,898 clinically suspected fungal keratitis patients, 12,717 (67.3%) were males and 6181 (32.7%) were females (Table 1). The respective culture positivity in males and females were 23.4% (2976/12717), and 17.7% (1093/6181) (p value <0.0001, between males and females).

The age distribution and fungal culture positivity of the patients are shown in Table 2. Maximum number of samples (3374, 17.9% of 18,898) were from patients of age group 41–50 years but maximum fungal culture positivity (30.8%) was seen in age group 31–40 years (p value <0.0001against all age groups). This was followed by culture positivity in 41–50 years group (25.4%), 21–30 years group (24.9%) and more than 70 years old patients group (23.2%). (all p values <0.05). Isolation was least among patients of age group 0–10 years (7.8%), (p < 0.000).

Spectrum of pathogenic fungi isolated from corneal ulcers

The most common fungus was *Aspergillus species*- 1266/4069 isolates (31.1%) of which there were 532 *A. flavus*, 400 *A. niger* and 334 *A. fumigatus*. The next common was *Fusarium species*, 996/4069 isolates (24.5%). Table 3 shows the different fungal isolates from the years 2001–2016.

Trends of individual fungi over the 16 years (Table 3)

Aspergillus species

Aspergillus species isolation had similar trends from 2001 to 2006. In years 2007 and 2008, significantly higher isolation was observed (p value <0.000). Years 2010, 2011, 2012 and 2014 also had higher isolation rate (p values = 0.012, <0.000, 0.002 and 0.012 respectively).

Fusarium species

Fusarium species had higher isolation in 2011 (p value = 0.005). In some years i.e 2001, 2013, 2015 and 2016; the isolation rates were very less though difference was not statistically significant.

Penicillium species and zygomycetes

The percentages of *Penicillium species* and zygomycetes (*Rhizopus* and *Mucor*) did not show much difference over the 16 years. Though some peaks and dips were observed in the line diagrams, the differences were not statistically significant.

Dematiaceous fungi

Pigmented fungi (*Alternaria*, *Curvularia*, *Bipolaris* and *Helminthosporium*) isolates in years 2001 and 2003 were significantly higher (p values 0.003 and 0.028 respectively); and were significantly lower in years 2007, 2011 and 2014 (p values 0.014 and 0.007and 0.027 respectively).

Candida species

Candida species isolation had an obvious dip in the year 2007 (0%) and an obvious high percentage in the year 2016 (7.9%); (statistically not significant).

Rhodotorula species

A significant rise in percentage was seen in years 2014 (p value = 0.028), 2015 (p value = 0.046) and 2016 (p = 0.011)

Month wise distribution of the fungal culture isolates (Table 4)

The highest percentage of fungal isolations was in September (24.3%), followed by October (23.9%), August (23.3%) and November (23.2%). Least number of isolations were in the month of June (15.5%), and May (17.4%). The difference between positivity in June was significantly lower than all other months except April and May (p value against April = 0.061, May = 0.242, March = 0.005, against all other months <0.000). The isolation in September and October was significantly higher than all other months except August and November (p values against January, February, March and December <0.05, and against April, May and June <0.000). The colder months of December, January and February had culture positivity significantly higher than the hot and dry months of May and June (p value < 0.000); and significantly lower than the humid months of September and October (p value 0.024 and 0.046 respectively).

Discussion

Keratitis is the second most common cause of blindness throughout the world and the foremost cause of visual morbidity

Table 1
Total samples received, male- female distribution and fungal culture positivity from clinically suspected mycotic keratitis cases over the study years.

Year	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	Total (2001–2016)
Corneal scrapings received for fungal culture	903	945	904	1087	993	1027	1011	1181	1336	946	1195	1498	1555	867	1780	1670	**18898**
Males	574	642	621	714	661	682	687	804	923	619	792	1030	1077	621	1189	1081	**12717** (23.4% positive)
Females	329	303	283	373	332	345	324	377	413	327	403	468	478	246	591	589	**6181** (17.7% positive)
Fungal culture positive (% of 18898)	188 (20.8)	203 (21.5)	194 (21.5)	220 (20.2)	212 (21.3)	219 (21.3)	222 (22.0)	243 (20.6)	291 (21.8)	201 (21.2)	258 (21.6)	305 (20.4)	318 (20.5)	198 (22.8)	403 (22.6)	394 (23.6)	**4069 (21.5)**

Bold values are total number of samples received, total number of samples from males, females and total positives. As the last column of each row is the total of all years, it has been made bold.

Table 2
Age wise distribution of samples received and fungal culture positivity from clinically suspected mycotic keratitis cases over the study years.

Age group (Years)	0–10	11–20	21–30	31–40	41–50	51–60	61–70	>70
Total	1328	1795	2859	2409	3374	3134	2843	1156
Positive (%)	104(7.8)	198(11.0)	712(24.9)	743(30.8)	856(25.4)	645(20.6)	543(19.1)	268(23.2)

and associated complications [5,6]. Microbial keratitis is more common in tropical and sub-tropical regions of the world and mycotic keratitis accounts for >50% of these [7]. The causative fungi vary between different geographical regions and climatic conditions [8].

Mycotic keratitis can occur due to filamentous fungi which is common in tropical and sub-tropical regions, or it can be due to infection by yeast (usually *Candida*). Keratitis due to filamentous fungi mostly occurs in outdoor workers; in rural setting in agricultural workers and in urban setting in construction workers. Keratitis due to *Candida* usually occurs in patients with pre-existing ocular conditions or chronic systemic diseases, superimposed on ocular virus infections or in contact lens users [9,10].

In any region, the trend also shows variation with different seasons of the year and in different age groups. India is a country with diverse geographical features and climatic conditions, varied seasonal deviations and disparate flora and fauna in different regions. The life style and occupations range from rural agrarian population to the inhabitants of mega metropolitan urban cities. The seasonal variation in fungal keratitis cases is not only attributed to the temperature and humidity of the environment, but also to the wind and harvest of crops, which play important role in ocular trauma especially in countries like India [10]. Due to the fact that mycotic keratitis is more common in outdoor workers belonging to lower socio-economic group, males of productive age group are usually affected.

It is pertinent to look deeply into the etiological agents causing fungal eye infections in different geographical regions and their variation over years, across seasons, gender and age groups. The present study was performed in a large tertiary eye care hospital in Northern India, catering to Delhi and nearby six to seven states. Therefore, the findings reflect the trends in fungal eye infections not only in Delhi but in a larger area of Northern India.

In the study population, male to female ratio was roughly 2:1, and the maximum numbers of culture proven cases were from age group 31–40 years followed by those in 41–50 years. In all the studies of mycotic keratitis- there was male preponderance, male to female ratio as high as 3.5: 1 has been reported. The most commonly reported affected age are young and middle-aged adults 16–50 years old [4,9–20].

The overall fungal culture positivity in the present study was 21.5%. The most common filamentous fungus isolated in our study was *Aspergillus species* (31.1%), followed by *Fusarium* (24.5%). Amongst yeasts, *Candida* spp. isolation was 4.4% and *Rhodotorula* spp. was isolated in 0.5% of cases. *Alternaria* (10.5%), and *Curvularia* (10.2%) were the most common dematiaceous fungi causing corneal ulcers. In our study, culture positivity was comparable over the years. There was a seasonal variation with statistically significant increase in culture proven fungal keratitis in monsoon months (August and September), and also in colder months (October–February) compared to dry months of May and June.

In a previous study spanning over a period of five years from the same institute, culture positivity was 24.3%; with *Aspergillus* spp. and *Candida* spp. being the most commonly isolated mould and yeast respectively [11]. In another study in paediatric age group, *Aspergillus* spp. was the most frequent isolate (39.5%) followed by *Fusarium* spp. (10.7%), *Alternaria* (10.2%) and *Curvularia* (7.4%). This study also reported a seasonal variation with peak incidence in months of September and October [21].

Other studies from Delhi and nearby areas have reported fungal culture positivity of 22.3%–39% [9,12,13], *Aspergillus* (25%–51%) is the most common mould reported. A study from East Delhi has reported increasing frequency of overall fungal culture positivity from 10.6% in 2000 to 38.4% in 2004 [12]. A subsequent study from same institute has reported rising trend in *Fusarium* keratitis from 23.1% in 2010 to 36.8% in 2015 [9].

There is a lot of variation in culture positivity and spectrum of pathogens from different parts of India. From central India, Pune and Mumbai have reported 25.9% and 10.07% positivity respectively, with *Aspergillus* spp. (17.6% and 57% respectively) and *Candida* being the most common isolated mould and yeast respectively [6,14].

A study from Eastern coastal state of Orissa has reported 26.4% positivity of fungal culture in suspected mycotic keratitis patients. The most common filamentous fungal isolates were *Aspergillus* (27.9%) followed by *Fusarium* (23.2%) Most common yeast was *Candida* (0.9%) [15]. A more recent study from the same state has reported similar results with 32% culture positivity, and *Aspergillus* spp. (43.8%) being the most common isolate followed by *Fusarium* spp. (25%) and *Candida* (12.5%) [22].

Two studies from southernmost state of India-Tamil nadu, have reported 34% culture proven mycotic keratitis from clinical suspects. Both studies have reported *Fusarium* to be the most common isolate (42.8% and 14.5% respectively) [10,16]. One of the studies has commented that spectrum of fungal isolates over a duration of

Table 3
Spectra of fungal pathogens isolated from clinically suspected mycotic keratitis cases over the study years.

Year	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	Total (2001–2016)
Fungal culture positive	188	203	194	220	212	219	222	243	291	201	258	305	318	198	403	394	**4069**
Aspergillus sp. (%)	49 (26.1)	46 (22.7)	49 (25.3)	65 (29.5)	65 (30.7)	57 (26.0)	93 (41.9)	98 (40.3)	83 (28.5)	68 (33.8)	97 (37.6)	106 (34.8)	106 (33.3)	67 (33.8)	113 (28.0)	104 (26.4)	**1266 (31.1)**
A.niger	12	7	13	22	19	21	35	23	27	23	29	37	37	21	39	35	**400**
A. flavus	14	24	25	26	29	27	37	43	35	26	41	42	43	29	53	38	**532**
A. fumigaus	23	15	11	17	17	9	21	32	21	19	27	27	26	17	21	31	**334**
Fusarium (%)	39 (20.7)	57 (28.1)	47 (24.2)	53 (24.1)	57 (26.9)	59 (26.9)	61 (27.5)	65 (26.7)	79 (27.1)	53 (26.4)	77 (29.8)	76 (24.9)	65 (20.4)	53 (26.8)	81 (20.1)	74 (18.8)	**996 (24.5)**
Penicillium **(%)**	6 (3.2)	9 (4.4)	7 (3.6)	8 (3.6)	9 (4.2)	7 (3.2)	17 (7.7)	9 (3.7)	11 (3.8)	13 (6.5)	11 (4.3)	17 (5.6)	15 (4.7)	11 (5.6)	17 (4.2)	15 (3.8)	**182 (4.5)**
Dematiaceous fungi (%)	**88** (46.8)	**78** (38.4)	**80** (41.2)	**78** (35.5)	**66** (31.1)	**85** (38.8)	**50** (22.5)	**59** (24.3)	**102** (35.1)	**56** (27.9)	**54** (20.9)	**85** (27.9)	**109** (34.2)	**46** (23.2)	**138** (34.2)	**119** (30.2)	**1293** (31.8)
Alternaria	38	26	17	15	19	23	25	13	43	13	15	32	39	17	51	42	**428 (10.5)**
Curvularia	25	22	32	37	19	27	7	26	27	15	11	29	33	19	48	39	**416 (10.2)**
Helminthosporium	16	19	16	17	15	18	13	11	15	15	12	13	14	7	15	15	**231 (5.7)**
Bipolaris	9	11	15	9	13	17	5	9	17	13	16	11	23	3	24	23	**218 (5.4)**
Paecilomyces	0	0	3	5	0	0	0	2	0	0	3	2	4	0	7	8	**34 (0.8)**
Acremonium	0	0	0	0	0	2	0	0	0	0	2	0	0	2	14	29	**49 (1.2)**
Zygomycetes (%)	**2** (1.1)	**3** (1.5)	**2** (1.0)	**2** (0.9)	**4** (1.9)	**0** (0.0)	**1** (0.5)	**5** (2.1)	**3** (1.0)	**2** (1.0)	**2** (0.8)	**3** (1.0)	**4** (1.3)	**3** (1.5)	**5** (1.2)	**6** (1.5)	**47** (1.2)
Rhizopus	2	3	2	2	4	0	1	3	3	2	2	3	2	2	5	4	**40 (1.0)**
Mucor	0	0	0	0	0	0	0	2	0	0	0	0	2	1	0	2	**7 (0.2)**
Candida **(%)**	4 (2.1)	9 (4.4)	6 (3.1)	9 (4.1)	11 (5.2)	9 (4.1)	0 (0.0)	4 (1.6)	13 (4.5)	8 (4.0)	12 (4.7)	14 (4.6)	15 (4.7)	13 (6.6)	23 (5.7)	31 (7.9)	**181 (4.4)**
Rhodotorula **(%)**	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.0)	1 (0.5)	0 (0.0)	2 (0.7)	0 (0.0)	3 (1.5)	5 (1.2)	8 (2.0)	**21 (0.5)**
Total (%)	**188**	**203**	**194**	**220**	**212**	**219**	**222**	**243**	**291**	**201**	**258**	**305**	**318**	**198**	**403**	**394**	**4069 (100.0)**

"Dematiaceous fungi" consist of Alternaria, Curvularia, Helminthosporium and Bipolaris, hence the row of Dematiaceous fungi has been made bold. Also the "zygomycetes" consist of Rhizopus and Mucor, hence that column has been made bold.

Table 4
Month wise distribution of samples received and fungal culture positivity from clinically suspected mycotic keratitis cases over the study years.

	Total	Positive	Positive percentage
January	1523	321	21.1%
February	1349	289	21.4%
March	1289	257	19.9%
April	1183	219	18.5%
May	1157	201	17.4%
June	1075	167	15.5%
July	1492	324	21.7%
August	2069	483	23.3%
September	2118	514	24.3%
October	2076	496	23.9%
November	1743	405	23.2%
December	1824	393	21.5%
	18898	4069	21.5%

10 years has been stable [16]; the other has shown a non-significant increase in culture isolation during monsoon [10].

In a small study from Kerala (West coast India), 36% of clinically suspected cases of fungal keratitis were culture positive; *Aspergillus* spp (24%) and *Candida* spp (8%) were the most common isolates reported [17].

Another study from Hyderabad- a city in South India has reported 39.8% positivity of fungal culture with *Fusarium* (37.2%) being the most common isolate followed by *Aspergillus* (30.7%). Also, increase in positivity has been reported during monsoon and winter months [4].

Studies from outside India also show a great deal of dissimilarities in fungal pathogens associated with keratitis.

From neighbouring Nepal, overall 38.7% fungal culture positivity was reported, with a non-significant rise in monsoon season [18].

A study from Riyadh, Saudi Arabia has reported a very low fungal culture positivity of 3.8% in clinically suspected mycotic keratitis patients. *Candida* and *Aspergillus* (27.3% each) were the most common isolates and the spectrum was stable over the study years [19].

Another study from Egypt showed 43.3% culture proven mycotic keratitis with *Aspergillus* (35%) and *Candida* (3%) being the most common filamentous fungus and yeast respectively [20].

In a study from London, most common fungal isolates were *Candida* (32.9%) and *Fusarium* (32.9%), followed by *Aspergillus* (8.9%). The authors, while comparing their two studies of years 1994 to 2006, and 2007 to 2014 have reported statistically significant increase in annual number of culture positive fungal keratitis in the latter study. Also, they found that in the earlier study, 59.3% isolates were yeasts and 40.7% were moulds; however, in the second study- 70% were moulds and 33% were yeasts (3% being mixed infections of moulds and yeasts) [23].

There are a few limitations of the study largely because of it being a retrospective analysis. Although, fungal pathogens were looked for only in clinically suspected cases of mycotic keratitis, we could not prospectively co-relate the results with clinical manifestations and response to treatment. Also, we have not analysed the factors responsible for origin of the mycotic keratitis in our patients.

Conclusion

This is a very large compilation of epidemiological and microbiological features of fungal keratitis from a tertiary ophthalmic care centre, comprehensive updates from different regions and understanding of variations from other regions, over different age groups, and in different seasons are vital for better management of mycotic keratitis; which will in turn facilitate better visual outcome in the patients.

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Competing interests

None declared.

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