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Original Article

Changing trends in fungal and bacterial profile of infectious keratitis at a tertiary care hospital: A six-year study

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ABSTRACT

Background: Bacteria and fungi are important aetiological agents of infectious keratitis in India. This retrospective study was done to know causative fungal and bacterial species and associated risk factors in patients with suspected fungal or bacterial keratitis. The study included all cases (400) of corneal ulcer investigated and treated at a tertiary care hospital over 6 years.

Methods: Gram staining and 10% KOH wet mount were performed on corneal scrapings. Further identification of fungal and bacterial agents was done using routine methods.

Results: Fungal and bacterial pathogens were identified in 94 (23.50%) and 44 (11.00%) cases, respectively. Keratitis was significantly associated with fungal aetiology. Most frequent agent of fungal keratitis was *Fusarium* species (26, 27.66%). Fungal keratitis was associated with young and middle-aged agriculturists and labourers, and corneal injury with vegetative matter. Most frequent agent of bacterial keratitis was *Staphylococcus aureus* (22, 50.00%). Bacterial keratitis was associated with co-existing ocular diseases, diabetes mellitus, previous ocular surgery, contact lenses and metallic foreign bodies in eyes. Fungal keratitis cases had worse overall outcome compared to bacterial keratitis.

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Conclusions: Knowledge of predisposing factors and microbiological confirmation of keratitis is indispensable to initiate appropriate therapy. We found continuous rise in incidence of *Fusarium* keratitis, which is disturbing due to multidrug-resistant nature of the fungus. Routine surveillance of fungal and bacterial keratitis is needed to know the existing and emerging pattern of pathogens.

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1. Introduction

Corneal blindness is a major public health problem worldwide and infectious keratitis is an important cause.¹ While viral infections are the leading cause of corneal ulcer in the developed countries, bacteria and fungi are important aetiological agents in the developing world.² Though studies on bacterial and fungal keratitis have been carried out in various parts of India, there is scarcity of recent data on bacterial and fungal agents and the underlying factors leading to keratitis published from eastern part of Delhi. Hence this retrospective analysis was undertaken to know the various fungal and bacterial species identified as aetiological agents of keratitis, along with the associated risk factors and outcome in patients attending a tertiary care hospital in East Delhi.

2. Materials and methods

This retrospective study comprised all cases of corneal ulcer investigated and treated in the Departments of Microbiology and Ophthalmology of a 1000-bedded tertiary care hospital in East Delhi from 1st January 2010 to 31st December 2015. The patients' age, gender, residence, occupation, information on the history of the infectious process, as well as the use of topical steroids, antibiotics or antifungals, predisposing conditions like trauma, previous eye surgeries, use of cosmetic or therapeutic contact lens were meticulously recorded.

In all cases, corneal scrapings for analysis were collected directly from the base and margin of ulcers aseptically using Kimura's spatula under direct vision through slit lamp after instillation of anaesthetic eye drops (4% xylocaine). Direct microscopy was performed in 10% KOH wet mount. Smears were prepared from each sample for Gram staining for the demonstration of bacteria, hyphae, pseudohyphae and yeasts cells. For fungal cultures, samples were inoculated on plain Sabouraud's dextrose agar (SDA), as well as SDA with the antibiotics cycloheximide (500 mg/l), chloramphenicol (50 mg/l) and gentamicin (20 mg/l), and maintained at 25 and 37 °C separately over a period of four weeks. All culture media and antibiotics were obtained from Hi-media Laboratories, Mumbai, India. The characteristics considered for fungus identification were macroscopic aspects of texture, colour, growth rate and microscopic aspects, such as mycelium and conidium types, and relationship between hyphae and fruiting bodies by lactophenol cotton blue mount. Microculture on slides was the technique used for observation of filamentous fungi. The yeast isolates were identified by standard tests like germ tube, different spore production on

corn meal agar (CMA), urease production, sugar fermentation and assimilation tests.³ For bacterial cultures, samples were inoculated on Blood agar and MacConkey agar and incubated for 24 h at 37 °C. Bacteria were identified by using routine biochemical tests.⁴

The cultures were considered positive for growth if at least one of the following criteria was fulfilled:

- Same organism growing on one or more solid media.
- The growth on one medium is consistent with direct microscopic findings.²

2.1. Statistical analysis

Frequency distributions were obtained and percentages were calculated accordingly. GraphPad Inc. statistical software (2236 Avenida de la Playa, La Jolla, CA 92037, USA) was used for calculation of P value using Fisher's exact test. Statistical significance was defined as P value lesser than 0.05.

3. Results

A total of 400 cases were tested for suspected fungal and bacterial corneal ulcer during the study period. Fungal and bacterial pathogens were identified in 94 (23.50%) and 44 (11.00%) cases, respectively (Table 1). However, in none of the cases were both fungal and bacterial agents present together. Keratitis was strongly associated ($P = 0.0005$) with fungal aetiology in the present study.

3.1. Fungal corneal ulcer

Out of the total 400 cases of corneal ulcers investigated (239 males and 161 females), fungal aetiology was associated in 72 (76%) males and 22 (24%) females, with a male to female ratio of 3.27:1, which was statistically significant ($P = 0.0073$) (Table 2). The most common age group affected was between 31 and 40 years in both sexes.

Of the 400 cases studied, direct microscopic examination for fungal elements was positive in 147 (36.75%), and in 94 (23.50%) cases, fungi were isolated by culture. Fifty-three (13.25%) cases revealed sterile culture for fungus in spite of positive direct microscopic findings (of which 51 were positive for hyphae and 2 were positive for yeast), in 91 cases both direct microscopic examination and also culture were positive and in 3 cases only culture was positive.

Among the identified fungi, most were hyaline. Of the 94 positive specimens, the most frequent agent isolated was

Table 1 – Annual frequency of corneal ulcer and fungal and bacterial keratitis (2010–2015).

Year	Total no. of corneal ulcer samples received with suspected fungal/bacterial aetiology	Fungal keratitis with positive cultures no. (%)	Bacterial keratitis with positive cultures no. (%)
2010	67	26 (38.81%)	6 (8.96%)
2011	49	7 (14.29%)	5 (10.20%)
2012	45	6 (13.33%)	7 (15.56%)
2013	69	16 (23.19%)	9 (13.04%)
2014	47	20 (42.55%)	8 (17.02%)
2015	123	19 (15.45%)	9 (7.32%)
Total	400	94 (23.50%)	44 (11.00%)

Table 2 – Demographic characteristics of cases of fungal and bacterial keratitis (2010–2015).

Demographics	Particulars	N (%) for fungal keratitis (total no. = 94)	N (%) for bacterial keratitis (total no. = 44)
Gender	Male	72 (76%)	31 (70.45%)
	Female	22 (24%)	13 (29.55%)
Age in years	0–10	2 (2.67%)	2 (4.55%)
	11–20	6 (6.67%)	3 (6.82%)
	21–30	18 (19.15%)	5 (11.36%)
	31–40	26 (27.65%)	6 (13.64%)
	41–50	12 (12.77%)	11 (25%)
	51–60	10 (10.67%)	13 (29.55%)
	61–70	15 (16%)	2 (4.55%)
	>70	5 (5.32%)	2 (4.55%)
Residence	Rural	75 (80%)	10 (22.73%)
	Urban	19 (20%)	34 (77.27%)
Occupation	Agriculturists	57 (60.64%)	4 (9.09%)
	Labourers	30 (32%)	3 (6.82%)
	Students	4 (4%)	4 (9.09%)
	Professionals	1 (1.3%)	4 (9.09%)
	Household	1 (1.3%)	5 (11.36%)
	Unemployed/retired	1 (1.3%)	24 (54.55%)
Predisposing factors	Corneal trauma	84 (89.36%)	1 (2.27%)
	Co-existing ocular diseases	5 (5.33%)	14 (31.82%)
	Undergone previous ocular surgeries	3 (3.19%)	5 (11.36%)
	History of wearing contact lenses	0	19 (43.18%)
	Presence of metallic foreign bodies in eyes	0	13 (29.55%)
	Diabetes mellitus	1 (1.33%)	27 (61.36%)
	Tuberculosis	1 (1.33%)	9 (20.45%)

Fusarium species in 26 (27.66%) cases. The frequency of isolation of fungi was maximum in the year 2010. *Aspergillus flavus* (21, 22.34%) was the second most common followed by *Alternaria alternata* (10, 10.64%). The other fungi accounting for the rest 37 (39.36%) cases were *Aspergillus fumigatus*, *Penicillium* spp., *Rhizopus arrhizus*, *Acremonium kiliense*, *Scopulariopsis* spp., *Bipolaris hawaiiensis*, *Curvuluria lunata*, *Scedosporium apiospermum* and *Aurobasidium* spp. (Table 3). Few fungal isolates resembling *Fusarium* species were submitted to the National Reference Laboratory, Post Graduate Institute of Medical Education and Research, Chandigarh for speciation and they were identified as *Fusarium falciforme*, belonging to a larger *Fusarium solani* species complex.

Among the fungal keratitis cases, the prevalence was found to be more in young and middle-aged agriculturists (57, 60.64%) and labourers (30, 32%). History of corneal injury with vegetative matter was noted in 84 (89.36%) cases of fungal keratitis (Table 4).

The patients suffering from fungal keratitis were treated with atropine ointment 1% 8 hourly, tablet diamox 250 mg 8 hourly, topical natamycin 5% 6 hourly, topical itraconazole 1% 6 hourly and topical amphotericin B 0.15% 1 hourly. Oral itraconazole capsule 200 mg twice daily was started in severe cases. Corneal perforation occurred in 2 cases of fungal keratitis. Rest of the cases took 14–20 days (median 16 days) for re-epithelialization.

3.2. Bacterial corneal ulcer

Out of the total 400 cases of corneal ulcers investigated (239 males and 161 females), bacterial aetiology was identified in 44 (11.00%) (Table 1). Of these, 31 (70.45%) were males and 13 (29.55%) were females, with a male to female ratio of 2.39:1, which was statistically insignificant ($P = 0.0748$). The most common age group affected was between 51 and 60 years in both sexes.

Table 3 – Annual frequency of hyaline and dematiaceous fungi in 94 patients with fungal keratitis (2010–2015).

Fungi	2010	2011	2012	2013	2014	2015	Total (%)
Hyaline							
<i>Fusarium</i> spp.	6	2	2	3	6	7	26 (27.66%)
<i>Aspergillus flavus</i>	6	0	0	6	4	5	21 (22.34%)
<i>Aspergillus fumigatus</i>	3	2	2	0	0	1	8 (8.5%)
<i>Penicillium</i> spp.	0	0	0	0	4	2	6 (6.38%)
<i>Rhizopus arrhizus</i>	0	0	0	2	0	0	2 (2.12%)
<i>Acremonium kiliense</i>	0	0	2	2	2	0	6 (6.38%)
<i>Scopulariopsis</i> spp.	0	0	0	2	0	0	2 (2.12%)
Dematiaceous							
<i>Alternaria alternata</i>	2	0	0	0	4	4	10 (10.64%)
<i>Bipolaris hawaiiensis</i>	5	1	0	0	0	0	6 (6.38%)
<i>Curvularia lunata</i>	2	1	0	0	0	0	3 (3.19%)
<i>Scedosporium apiospermum</i>	1	1	0	0	0	0	2 (2.12%)
<i>Aureobasidium</i> spp.	1	0	0	1	0	0	2 (2.12%)
Total	26	7	6	16	20	19	94

Table 4 – Annual frequency of bacterial isolates in 44 patients with bacterial keratitis (2010–2015).

Bacteria	2010	2011	2012	2013	2014	2015	Total (%)
<i>Staphylococcus aureus</i>	3	3	3	5	4	4	22 (50.00%)
<i>Pseudomonas aeruginosa</i>	3	2	0	2	2	3	12 (27.27%)
<i>Klebsiella pneumoniae</i>	0	0	2	0	2	0	4 (9.09%)
Group A <i>Streptococcus</i>	0	0	2	0	0	0	2 (4.55%)
<i>Escherichia coli</i>	0	0	0	2	0	2	4 (9.09%)
Total	6	5	7	9	8	9	44

Of the 400 cases studied, direct microscopic examination for bacteria was positive in 65 (16.25%), and in 44 (11.00%) cases bacteria were isolated by culture. Twenty-one (5.2%) cases revealed sterile culture in spite of positive direct microscopic findings; in 43 cases, both direct microscopic examination and also culture were positive, and in 1 case, only culture was positive. Among the identified bacteria, the most frequent agent isolated was *Staphylococcus aureus* in 22 (50%) cases, followed by *Pseudomonas aeruginosa* in 12 (27.27%) (Table 3).

The prevalence of bacterial keratitis was maximum in elderly patients between 51 and 60 years of age. Most (10/76.92%) of the patients between 51 and 60 years of age had co-existing ocular diseases and few had undergone previous ocular surgery. History of wearing contact lenses and presence of metallic foreign bodies in the eyes was present in 19 (43.18%) and 13 (29.55%) patients, respectively, with all the patients being below 51 years of age. A major fraction of the patients were also diabetic (27, 61.36%) (Table 2).

The patients suffering from bacterial keratitis were treated with atropine ointment 1% 8 hourly, tablet diamox 250 mg 8 hourly, topical vancomycin 5% 6 hourly and topical cefazolin 5% 6 hourly. Systemic ciprofloxacin tablet 250 mg twice daily was started in severe cases. Treatment was administered even for the culture-negative cases that were strongly suspected to have a bacterial aetiology (sloughing, infiltrates, papilledema and mobile hypopyon). The infection resolved in every case after treatment. Corneal perforation occurred in 2 cases of fungal keratitis. Complete resolution of the infection and re-epithelialization occurred in all patients in 5–9 days (median 7 days).

4. Discussion

Scarring of cornea as a result of keratitis is one of the preventable causes of blindness, but it usually has unfavourable prognosis due to its protracted course and requirement of specific therapy. Corneal ulceration has been consistently listed as second only to cataract as major cause of blindness and visual disability. Untreated keratitis may lead to opacification and ultimately to corneal perforation.⁵ Other complications of keratitis include secondary glaucoma, corneal thinning, uveitis, severe anterior chamber reaction, severe vision loss and loss of eye.² The diversity of clinical presentation poses a diagnostic and therapeutic challenge to ophthalmologists. Proper treatment requires correct identification of the causative organism.⁵ The epidemiological pattern and causative agents for corneal ulcer vary significantly between countries, and even between regions within the same country. It is important to determine the aetiology within a given region for comprehensive strategy for the diagnosis and treatment of corneal ulcer.⁶ The prevalence of individual pathogens largely depends on geographical and climatic factors.⁷ Understanding the current status of the regional epidemiological features, risk factors, the presence of ocular and/or systemic co-morbidities, occupational status and knowledge of region-wise distribution of aetiological agents is important in the prevention and appropriate management of fungal or bacterial keratitis.⁸ Hence this study was an attempt to evaluate frequency of positive cultures in suspected cases of mycotic and bacterial keratitis, to identify

aetiological agents and to identify risk factors in patients attending a tertiary care hospital of Delhi.

In our study, fungal aetiology was significantly associated with keratitis compared to bacterial ($P = 0.0005$). This may be explained by higher frequency of risk factors for fungal keratitis (agricultural activities, etc.) in the tropical and subtropical regions than in the temperate regions.⁷ The occurrence of mycotic keratitis (23.50%) is similar to that reported from northern India.² In contrast to most of the recent studies, which have found *A. flavus* as the commonest fungal isolate,^{7,9–11} we observed higher isolation of *Fusarium* species in our study. A rising trend was observed in incidence of *Fusarium* keratitis over the 6-year study period with the species being responsible for 6 (23.08%) of 26 cases of fungal keratitis in 2010 to 7 (36.84%) of 19 cases of fungal keratitis in 2015. Occurrence of *Fusarium* species as the major agent may be attributed to global warming since *Fusarium* keratitis is known to occur more in geographical regions with hot, humid or tropical climates.¹² Nonetheless, this is particularly alarming due to the multidrug-resistant nature of *Fusarium* species.^{13,14} Multidrug-resistant *Fusarium* species, being resistant to a wide array of antifungals including natamycin, amphotericin B, echinocandins and fluconazole, may not leave many options for management and may progress to complications like endophthalmitis, corneal thinning, uveitis, severe anterior chamber reaction, severe vision loss and loss of eye.^{2,13}

In this study, the positivity rate of bacterial keratitis was 11.00%, and of *Staphylococcus aureus*, it was 5.24%. Several other studies have shown similar results.^{6,15,16}

The most affected age group for mycotic keratitis was 31–40 years, while for bacterial keratitis it was 51–60 years, consistent with other studies.^{2,5,7,8,17,18} Features, such as the presence of ocular or systemic disease or previous ocular surgery, being more prevalent in the older age groups, are more frequently associated with bacterial keratitis.¹⁷ Other risk factors identified for bacterial keratitis in the present study were co-existing diabetes mellitus, wearing contact lenses, co-existing ocular diseases, previous ocular surgery and presence of metallic foreign bodies in the eyes. Co-existing systemic diseases like diabetes mellitus, ocular diseases and previous ocular surgery, as well as wearing contact lenses, have been reported to be associated with bacterial keratitis.^{17,19,20}

We observed that in 13.25% of fungal keratitis and 5.2% of bacterial keratitis cases, culture was sterile despite observation of microorganism on direct microscopy. Similar findings have been noted in previous studies as well.^{2,15} This was commonly observed in patients already taking topical steroids, anti-fungal agents or anti-bacterial agents prescribed by local practitioners before the corneal scrapings were collected in our tertiary care referral centre. It was only in the event of indolent and progressive nature of the ulcer that the patient was referred to our tertiary care hospital where cultures were obtained revealing the fungal or bacterial aetiology of the ulcer.

In this study, 2 cases of fungal keratitis progressed to corneal perforation while all bacterial keratitis cases resolved. Fungal keratitis cases also re-epithelialized significantly more slowly than bacterial keratitis cases (median, 16 vs 7 days, respectively; $P < 0.001$). Hence this study suggests that fungal

keratitis has a higher risk of perforation and worse outcome as compared to bacterial keratitis. Similar findings have been reported elsewhere and fungal keratitis is often considered more difficult to treat than bacterial keratitis, as well as having worse prognosis.²¹

5. Conclusions

Keratitis continues to be a cause of concern to ophthalmologists. The key element in the diagnosis of mycotic and bacterial keratitis is the clinical suspicion by the ophthalmologists followed by laboratory confirmation of the fungus or bacterium before prescribing corticosteroids and antibacterial or antifungal antibiotics. As the manifestation of keratitis is often confusing, a high degree of suspicion with sound knowledge of predisposing factors and microbiological confirmation is very essential to initiate appropriate therapy. The knowledge of common aetiological agents of bacterial and fungal keratitis is imperative for formulation of appropriate management protocol. Our study highlights the increased occurrence of *Fusarium solani* complex as the major species responsible for fungal keratitis and the multidrug-resistant character of *Fusarium* species may lead to clinical failure. A regular scrutiny of agents of fungal and bacterial keratitis thus becomes necessary for monitoring the pattern of pathogens and identifying the appropriate drug of choice in keratitis. Prompt diagnosis and early institution of treatment may limit the ocular morbidity and the sequelae of infectious keratitis.

Conflicts of interest

The authors have none to declare.

Author contributions

Concepts: Priyamvada Roy, Shukla Das, N.P. Singh. Design: Priyamvada Roy, Shukla Das, N.P. Singh, Rumpa Saha, Garima Kajla. Definition of intellectual content: Priyamvada Roy, Shukla Das, N.P. Singh, Rumpa Saha, K. Snehaa, V.P. Gupta. Literature search: Priyamvada Roy, Shukla Das. Experimental studies: Priyamvada Roy, Shukla Das, Garima Kajla. Data acquisition: Priyamvada Roy, Shukla Das, N.P. Singh, Garima Kajla, K. Snehaa, V.P. Gupta. Data analysis: Priyamvada Roy, Shukla Das. Statistical analysis: Priyamvada Roy, Shukla Das. Manuscript preparation: Priyamvada Roy, Shukla Das, Rumpa Saha. Manuscript editing: Priyamvada Roy, Shukla Das, N.P. Singh, Rumpa Saha. Manuscript review: Priyamvada Roy, Shukla Das, N.P. Singh, Rumpa Saha, Garima Kajla, K. Snehaa. Guarantor: Priyamvada Roy.

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