MICROBIOLOGICAL PATTERN AND EPIDEMIOLOGIC TRENDS OF FUNGAL KERATITIS IN NORTH INDIA

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ABSTRACT

CONTEXT
Spectrum of fungal keratitis continues to change with geographical location and season. Microbiological and epidemiological data provide guidelines to the treating physician facilitating chances of successful treatment.

PURPOSE
To report microbiologic and epidemiologic profile of 119 culture-positive cases of fungal keratitis treated at a tertiary centre in North India.

SETTINGS AND DESIGN
All cases reporting directly or referred to the OPD of Eye Department of Rohilkhand Medical College and Hospital, Bareilly, India, diagnosed and treated as fungal keratitis during a 3-year period between March 2012 and Feb 2015.

METHODS
Retrospective analysis of clinical and microbiological data of 119 culture-positive cases of fungal keratitis. Demographic features, risk factors, clinical course and laboratory findings were reviewed.

RESULTS
All patients were residents of 11 adjoining districts of Northern India. Of the 119 patients, 76 (63.8%) were males (male: female ratio 1.79:1). 81(68%) patients were in young productive age group of 20-45 years. 87 (73%) were rural based. Ocular trauma with vegetative material, especially sugarcane leaf or dust falling in eyes were the chief precipitating factors; n = 89 (74.7%).

Microbiologically Fusarium was the predominant isolate, 64 cases (53.7%), followed by Aspergillus 34(28.6%) and Candida 11(9.2%). 2 cases of Alternaria and Curvularia and solitary cases of Acremonium and Scedosporium were reported. 4 strains remained unidentified. Mode of injury had a causal relation with fungal aetiology. Majority of Fusarium infections were caused by vegetative injuries 39(61%). Of these, 15(23.4%) were attributed to sugarcane leaves.

Soil/dust fall in eye or Surma application were responsible for bulk of Aspergillus infections; 21(61.7%). Candida infections were sporadic with a higher presenting age (Mean av 51.2 years) and a frequent association with topical steroid usage, (8 of 11 cases). Aspergillus infections were predominant in the hot and humid months of June to September; 25 of 34 cases (73.5%). Fusarium infectivity remained largely constant over the year with bimodal spurt during harvesting seasons. A wet KOH mount was effective for early diagnosis with a sensitivity of 89%.

CONCLUSION
Microbiological data of reviewed cases reveal a high preponderance of filamentous fungi as the aetiological agent, with Fusarium and Aspergillus species alone accounting for 82% of infective pathology in this rural belt of North India.

KEYWORDS
Fungal Keratitis, Epidemiology, Aetiology.

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INTRODUCTION: Fungal or Mycotic keratitis refers to a suppurative usually ulcerative mycotic infection of cornea.[1] Recent report suggests corneal scarring second only to cataract as the major blinding aetiology in developing nations.[2] Quantum of fungal infection among the prevalent microbial keratitis assumes alarming proportion.[3,4] Late diagnosis, restricted diagnostic facilities and poor response to prevailing medical treatment invariably result in poor visual outcomes. To compound the problem further, bulk of the afflicted patients are in their productive age, unlike cataract and hence harbinger great economic loss.

First reported case of fungal keratitis was by Leber in 1879.[5] Since then an increasing percentage of cases are attributed to fungal aetiology with more than 70 species of fungi having been identified as pathogenic to human cornea.[6] Aspergillus species are the single most reported aetiological agents producing fungal keratitis across the world.[1,7] However, geographical variations and climatic changes greatly affect the spectrum of aetiology.

Awareness of common fungal isolates and the attendant risk factors regionally are a great help in early diagnosis and scientific management. Epidemio-microbiological studies highlighting the demography, aetiological spectrum, risk factors and clinical course for fungal.

Keratitis, far and few in this subcontinent.[8,9,3,10,11] We present the analysis of epidemiological record and laboratory data of 119 culture-positive cases of fungal keratitis that were managed at the Department of Ophthalmology, Rohilkhand Medical College and Hospital, Bareilly in North India.

MATERIALS AND METHODS: Clinical and laboratory record of 119 culture-positive cases of fungal keratitis managed at Rohilkhand Medical College and Hospital between March 2012 and Feb 2015 were retrospectively analysed after due approval from the Institutional Ethical Board.

Examined parameters were age, sex, occupational, economic and residential background, mode and month of onset of infection, prior medication, and time lag before presenting at hospital, associated systemic or ocular ailment or any other risk factor.

Documented clinical features earmarking fungal keratitis included (i) dirty white or grey surface (ii) elevated borders (iii) dry rough texture (iv) satellite lesions (v) hypopyon. (vi) ring infiltrate (vii) endothelial plaque.

Protocol for lab diagnosis comprised of subjecting each patient to corneal scraping taken from the base and advancing edge of the ulcer on a biomicroscope employing a Kimura spatula. Scrapings so obtained were subjected to Gram stain and wet 10% Potassium hydroxide mount. Scraped material was further inoculated on Sheep Blood agar, MacConkey's agar and Sabouraud Dextrose (SD) agar supplemented with 50 µg/mL gentamicin. SD agar was kept at room temperature while other medias were incubated at 37ºC. Repeat scraping was undertaken where a strong clinical suspicion remained despite an initial negative result. Positive-culture was defined as one with a positive smear or KOH mount with a confirmatory growth on culture media. Also growth of same fungus on two or more culture media or at least on one media with subsequent growth of same fungus on a medium at a later date. Fungal isolates were identified to the genus level, with Aspergillus being identified to the species level. Follow-up period was 2 weeks before a negative result was declared. Mixed infections with bacterial and fungal infections were not considered.

RESULTS: Clinical and lab records identified 119 confirmed cases of fungal keratitis. Males 76 (63.8%) were significantly more affected ($p< 0.001$) than females. Male female ratio was 1.79:1.

Average age at presentation was 34.2 (Range 4 to 76) years. Maximum patients 81(68%), belonged to the economically productive age group of 20-45 years. 87(73%) of them were rural based with agriculture related occupations. 5 of the patients were in paediatric age group (Below 14 years).

The chief precipitating factor was trauma particularly with vegetative material, noticed in 89(74.7%) of patients. 16 of these patients gave a history of accidental brushing of sugarcane leaf while at work. 4 cases gave history of animal related injuries particularly with cow tail. 12 patients gave a characteristic history of rubbing of eyes after soil or dust inadvertently going in eyes. 2 of the patients developed symptoms after putting Surma, a local medicament in the eyes. 2 of the 5 children reported, gave history of injury with bow and arrow while at play.

A distinct time lag between advent of symptoms and time of reporting was noted. Average time period was 2.5 weeks with a range of 1.3 to 7.4 weeks. At the time of presentation, majority of these patients were on multidrug therapy that included corticosteroids. Most cases, 84 (70.5%) were in advanced stage of the disease with marked visual disability (distant vision < 6/60).

The filamentous fungi dominated the aetiological spectrum accounting for 104(87.3%) of the total 119 culture-positive cases; (Refer Table-2). Majority of them comprised of the genus Fusarium; 64(53.7%), followed by Aspergillus, 34 (28.6%). Aspergillus flavus was the most frequently identified species in the Aspergillus group; 22 (64.7%) followed by Aspergillus niger; 6 (17.6%). Among the dematiaceous fungi, 2 cases each of Alternaria and Curvularia and solitary case of Acremonium were isolated. A single case of Scedosporium and 4 unidentified fungal strains were noted. Candida infections accounted for 11 cases.

8 of these gave a history of prolonged topical steroid usage prior to infection. Diabetes was reported in 5 cases and one case had rheumatoid arthritis.

Ocular surface disorder was noted in 11 eyes, of which 7 eyes had infection with candida.

2 cases had prior cataract surgery and were on topical steroids and antibiotics at the time of contacting infection. None of the cases had any history of contact lens wear.

A distinct seasonal predilection was noticed for infections with Aspergillus, favouring hot and humid months between June and September; 25 of 34 cases (75.3%). A marginal rise of incidence was also observed for Fusarium infections during late spring and early autumn.

27 (42.1%) of the total 64 Fusarium cases were reported in the months of March-April and October-November. Incidentally, this period coincided with harvesting time for sugarcane.

Cases with candida were uniformly distributed throughout the year; (Fig. 2). Dematiaceous fungal infections, 5(4.2%) were sporadic. A solitary case of Acremonium was related to a bow and arrow injury in a paediatric patient. All cases with Surma application contacted infection with Aspergillus.

The simple diagnostic test of wet KOH mount was found highly effective, revealing positivity in 106 of 119 (89%) corneal scrapings. Organismal growth was detected earlier for candida species in culture media, where most strains achieved sufficient growth for identification within 48 to 72 hours. In contrast filamentous fungi took at least a week to attain a reportable growth. Dematiaceous fungi did not reveal a pigmented clinical presentation in all cases and were comparatively more resistant to treatment.

Sl. No.	Risk factor	No. of Eyes (%)
I	Trauma	89(74.8%)
a)	Vegetative matter	58(48.7%)
b)	Industrial	02(1.7%)
c)	Road side	12(10.1%)
d)	Animal related	04(3.4%)
e)	Others	13(10.9%)
II	Co-existing ocular causes	26(21.8%)
a)	Ocular surface disorder	11(9.2%)
b)	Use of topical steroids	09(7.6%)
c)	Contact lens usage	Nil
III	Other systemic illness	06(5.04%)

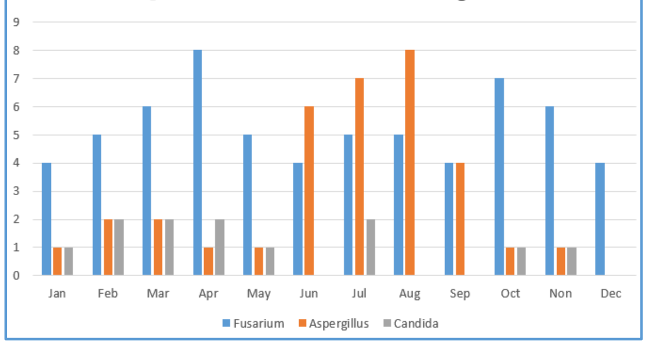
Table 1: Risk Factors for Fungal Keratitis (Total no. of cases 119)

Sl. No.	Fungal isolate	No. of isolates (%)
01	Fusarium	64 (53.8%)
02	Aspergillus	34 (28.6%)
03	Candida	11 (9.2%)
04	Curvularia	02 (1.7%)
05	Alternaria	02 (1.7%)
06	Acremonium	01 (0.84%)
07	Scedosporium	01 (0.84%)
08	Others	04 (3.4%)

Table 2: Organisms Isolated in Fungal Keratitis

Figure – 1 Spectrum of fungal isolates

Figure – 2 Seasonal distribution of fungal isolates



DISCUSSION: Even though fungal keratitis has been recognised as an important cause of unilateral blindness especially in the tropical and subtropical zone, much of its treatment remains unsatisfactory and largely empirical. This is in part because of non-availability of sufficient and reliable data regarding its aetiological characteristics and epidemiological trends. Since the causative spectrum of this condition shows marked inter-regional variation, extrapolation of data from the few studies[7,8,9,3,10,11] far and wide provide insufficient basis for a correct and definitive diagnosis.

Much of the treatment is also presumptive due to considerable overlap in the clinical presentations of bacterial, fungal and protozoal corneal infections accounting for a triage of multiple drug regimens.

In this case series, majority of patients reported 2-3 weeks, (Average 2.5 weeks) after contacting the infection. Similar delay in diagnosis and management is reported from other tertiary centres.[3,10] A multidrug regimen including steroids at presentation or misdiagnosis is noted as the usual norm.

Even though a growing number of fungal genera and species have been implicated to cause fungal keratitis, a repeatedly small number with often predictable risk factors are associated with most cases.[12]

On a simplistic basis, fungal genera are grouped as hyaline filamentous, dematiaceous, yeasts and zygomycetes, thermally dimorphic and fungi with uncertain classification.[13] Fungal infections of the cornea are frequently caused by species of Fusarium, Aspergillus, Candida and Curvularia.[12,13] Both filamentous and dematiaceous fungi are particularly common in the tropical zones while identification of yeast group of fungi is frequently reported in the cooler temperate zones.[14]

Hospital based reports on fungal keratitis from different parts of the world reflect a variable quantum of infection, accounting for 6-56% of all investigative cases of ulcerative keratitis.[4] Certain genera and species are isolated very frequently and these have a marked regional predisposition. Studies from South India have stressed the high incidence of filamentous fungal keratitis with Fusarium as the most commonly reported genus.[3,10]

Studies from tropical and subtropical climate zones such as Singapore, Nigeria, Ghana, China and Paraguay corroborate this view.[15-19] However, similar studies in Srilanka, Bangladesh and Nepal[9,11,20] report a much higher incidence of Aspergillus infections. A study in Northern India on fungal keratitis patients in paediatric age group sited an incidence of 40% for Aspergillus as opposed to 10.7% for Fusarium infections.[8] The latter demonstrated near equal pathogenicity with Alternaria (10.2%), Curvularia (7.4%) and Penicillium (7%). The picture contrasts from reports in the cooler temperate parts of the world where fungal infections are largely caused by the candida species. A study at Moorfields Hospital, London implicated Candida in nearly 61% reviewed cases of fungal keratitis.[14] Our study from Northern India differs in the reported spectrum of fungal aetiology from this geographical zone, highlighting a significantly higher incidence of Fusarium infections (53.7%) as opposed to Aspergillus (28.6%). A significantly high incidence of candida infections (9.2%) is also reported which contrasts markedly with a similar study in South India reporting negligible candida infectivity (0.7%).[10]

A close relationship has been demonstrated between fungi in the environment and those causing mycotic keratitis. Fungal species most commonly isolated from corneal ulcers are also commonly isolated from normal eyes and are prevalent in the atmosphere.[21] In a comparative study, fungi were isolated from the normal conjunctival flora of 77% agricultural workers and only 15% of city dwellers.[22] This fact underscores the special risk of rural workers to ocular mycosis.

The primacy of filamentous fungal agents in the aetio-pathogenesis of fungal keratitis in tropical and subtropical world has been acknowledged by most studies.[7,8,3,10] Our case series, reports 87% fungal keratitis cases to be related with filamentous fungi. The analysis also conjures with the recognition of ocular trauma, particularly with vegetative material as the major risk factor that is reported in literature to occur in 44-55% of patients.[3,10,15]

Since fungi are opportunistic in eye, rarely infecting healthy and intact ocular tissue, it is understandable that a traumatic abrasion or an immunocompromised status is a prerequisite for this infection.

We noted agricultural trauma in 55(46.2%) cases. Fusarium accounted for most of these infections caused due to corneal abrasions sustained with plant leaves, tree branches or grains. 16 patients gave a distinct history of sustaining injury by sugar cane leaf. 15 of these cases were culture-positive for Fusarium, highlighting the marked association of this genus with sugarcane vegetation. Affected population were young agricultural workers with no pre-existing ocular or systemic disorder. Mode of infection appeared to be either direct implantation of fungal conidia in the corneal stroma or an abraded epithelium permitting exogenous fungal elements. Fusarium species are important plant pathogens present in abundance on plant leaves and other vegetative matter.[23] Their ubiquitous presence in vegetation probably explains their high infective association with farming practices as reported with paddy, onion or sugarcane farming.

Aspergillus infections were significantly associated with history of dust fall in eyes followed by rubbing. Aspergillus species abound in the environment on a worldwide basis.

They thrive abundantly on a variety of substrates including soil and decaying vegetation.[13] A study comparing risk factors for keratitis noted ocular trauma to predispose eyes to filamentous fungal infections; most frequently to Fusarium (70%), Curvularia (11%) and Aspergillus species(5%). On a contrasting note, a study in Philadelphia reported chronic ocular surface disorder, contact lens wear and use of topical corticosteroids as the three most common risk factors.[24] The commonest fungal isolate in this study; however, was candida albicans (46%).

Our own study detected 11 cases of co-existing ocular surface disorder and no case of contact lens association. There were; however, 9 cases of topical corticosteroid usage prior to the infection, of which 8 were associated with infection due to candida. Surma, a local eye medicament attributed to 2 cases of Aspergillus infection in otherwise healthy eyes.

Abrasional injuries due to bow and arrow among children and cow tail injuries among cattle vendors accounted for 2 cases each.

Much of these facts favour the genesis that fungal keratitis remains a public health problem largely due to faulty practices that predispose healthy eyes to corneal invasional trauma.

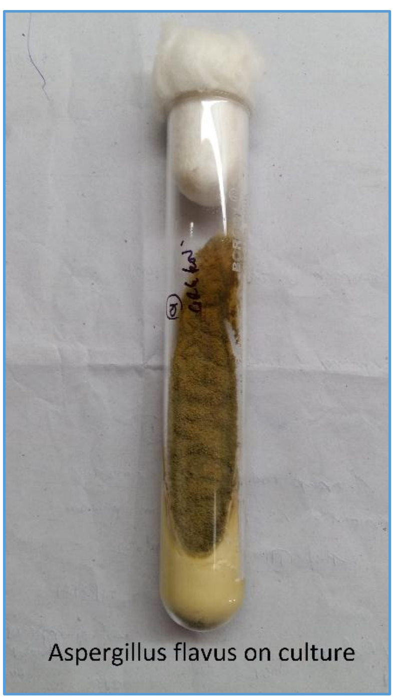
Associated factors like ocular surface disorder, systemic diseases, immune status and contact lens wear, merely facilitate the primary infecting pathology and play a more significant role in yeast group of fungi such as candida.

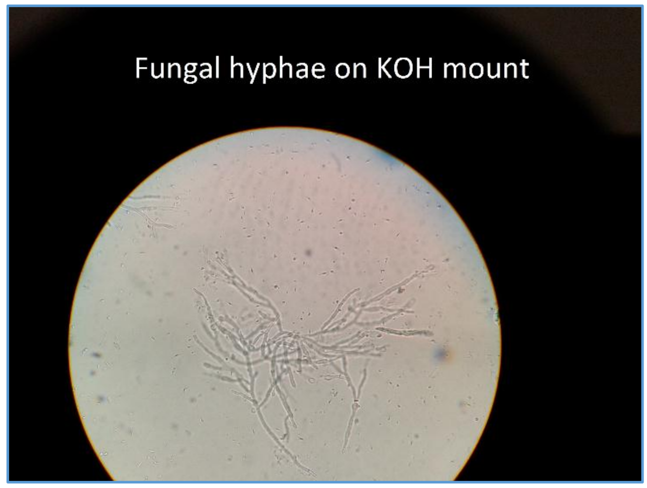
Environmental factors like wind, humidity and rainfall as well as seasonal variations have been linked to the incidence of mycotic keratitis as well as the predominant genera of fungal isolate.[25] Our study noted a near constant rate of incidence in candida and Fusarium infections. The latter; however, showed an increased incidence during harvesting months of April and October during which vegetative injuries particularly with sugarcane leaves are far more common in this region. In contrast, a striking increase for Aspergillus infections is observed in the hot and humid months of July to September, implying a relationship of this infection with sultry climatic conditions.

Diagnostically the wet KOH mount was noted as a highly sensitive method for detecting fungal infections with a sensitivity of nearly 89%. Gopinathan et al[10] while presenting their largest reported case series on fungal keratitis have corroborated a similar view. This fact assumes great importance as it suggests the possibility of early detection and initiation of treatment for fungal keratitis even at primary or secondary level.

CONCLUSION: Fungal keratitis in North India is an important infective ocular pathology leading to advanced visual impairment or uniocular blindness. Majority of affected cases comprise of young male agricultural workers who are particularly at risk due to inadvertent trauma by vegetative matter. Filamentous fungi are the most frequent causative agents causing 87% of cases. Among them, infections by fungi of Fusarium genus are the commonest (53.7%) followed by Aspergillus group (28.6%) and dematiaceous fungi (4.2%). Mode of trauma gives reliable clues regarding causative agent. Injuries with sugarcane leaves, branches of shrubs and aerial plants are prone for Fusarium infection. Soil or dust related injuries particularly in hot and humid weather conditions produce Aspergillus infections. Infections with candida are observed in older population who are on longterm topical medication especially steroids with immune compromise. Traditional topical eye medicaments like Surma may cause a fungal infection of eye particularly by Aspergillus group. A simple test of wet KOH mount is most effective in establishing the diagnosis of fungal pathology.


Aspergillus niger on culture


Aspergillus flavus on culture


Fungal hyphae on KOH mount

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