Original Article

Etiological spectrum of infectious keratitis in the era of MALDI-TOF-MS at a tertiary care hospital

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Abstract

Background: Infectious Keratitis (Ik) Is A Potential Vision-Threatening Ocular Infection Caused By A Variety Of Microorganisms. **Aim:** To Explore Risk Factors And Etiological Agents Associated With Ik. **Design:** A Prospective Cross-Sectional Study In Which Corneal Scrapings From 120 Suspected Cases Were Evaluated At A Tertiary Health Care Institute From January To December 2019. **Methods:** Scrapings Were Subjected To Direct Microscopy, Culture, And Identification By Both Conventional Methods And Maldi-Tof-Ms. The Patient's Demographic Data And Predisposing Factors, If Any Were Recorded. **Results:** The Mean Age Of Patients Was 48.9 Years And Predisposing Factors Were Documented In 46% (55/120) Of Cases. Overall, Infective Etiology Could Be Established In 51% (*N* = 61/120) Of Cases. Fungal Growth In 26% (*N* = 31/120) Of Cases And Bacterial Growth In 22% (*N* = 27/120) Of Cases Was Obtained. Growth Of More Than One Species Of Fungi Or Growth Of Bacteria Along With Fungus Were Observed In 2% (*N* = 3/120) Of Cases. Of All The Fungal Isolates Obtained (*N* = 34), The Most Common Isolate Was *Fusarium* (18/34) Followed By *Aspergillus* (8/34), *Curvularia* (4/34), *Pseudallescheria Boydii* (3/34), And *Geotrichum* (1/34). Among The Gram-Positive Bacterial Isolates (*N* = 16), *Staphyloccus Species (15/16)* Were Isolated In Maximum Number Followed By *Streptococcus Pneumoniae* (1/16). Among The Gram-Negative Isolates (*N* = 13), *Pseudomonas* Species (8/13) Were Isolated In Maximum Number Of Cases, Followed By *Acinetobacter* (3/13), *Klebsiella Pneumoniae* (1/13), And *Escherichia Coli* (1/13). **Conclusion:** For Initiating Appropriate Empirical Therapy, The Knowledge Of The Epidemiological Pattern Of Infectious Keratitis Of A Particular Geographical Region Is Crucial.

Keywords: Corneal Ulcer, Fusarium Keratitis, Infectious Keratitis

Introduction

Infectious keratitis (IK) can have a major impact on the quality of life of individuals leading to significant visual impairment or even loss of vision.[1] Corneal infections are known to be one of the most significant causes of monocular blindness in some developing countries, especially in tropics. It may be caused by a variety of microorganisms like bacteria, fungi, viruses, or parasites.[2]

Proper diagnosis of the causative organism is critical and culture is the most utilized diagnostic tool to delineate aetiology.[3] After culture growth, microbial identification by matrix-assisted laser desorption/ionization time of flight–mass spectrometry (MALDI-TOF MS) is a steadfast technique being utilized in many clinical microbiology laboratories. Implementation of this method enables conclusive organism identifications in minutes that was previously unimaginable.[4]

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Several studies have established the etiological profile of IK from various parts of India. Knowledge of etiology in a specific region is crucial for the optimal management of these infections. However, meagre data is available from the foothills of the Himalayas in particular. As it varies geographically, the purpose of the study is to determine the aetiology of IK using conventional techniques and MALDITOF-MS at a tertiary healthcare institute and to explore epidemiological risk factors.

Material and Methods

Study design, setting, duration, and population

The prospective cross-sectional study was carried out in a tertiary healthcare institute from January 2019 to December 2019. The ethical clearance was taken from the Institute Ethics Committee. The patients were clinically examined by experienced clinicians and after taking informed consent, corneal scraping samples were collected from 120 clinically suspected IK cases. Inclusion criteria were the presence of signs of IK in slit-lamp examination (i.e., epithelial defect, underlying stromal infiltrate with signs of acute inflammation, e.g., circumcorneal congestion, stromal infiltrates, hypopyon, scarring, or perforation).

Collection of samples

Corneal scrapes were obtained under topical anesthesia by scraping the leading edges and base of the ulcer under the magnification of a slit lamp, using a sterile Bard-Parker blade. The patient's age, gender, occupation, history of any predisposing factor like trauma, systemic illness, past and current use of topical medicines, and use of contact lens use were entered in the Microsoft Excel sheets. Samples were sent to the ocular microbiology section and were processed by standard procedures for the diagnosis of causative pathogens.

Direct microscopy and inoculation on culture media

For each patient, a portion of the corneal scrape material obtained had been used for direct microscopy (Potassium hydroxide mount/KOH mount, Gram staining or calcofluor white staining). Another portion was inoculated directly onto the following media that support the growth of bacteria and fungi: Blood agar, Chocolate agar, Sabouraud dextrose agar (SDA) (HiMedia, Mumbai). Inoculated media were incubated at temperatures of 37°C and 25°C and were examined daily for 7 days. SDA was examined twice a week for the next three weeks.

The cultures were considered positive if the growth of the same organism was demonstrated in more than one solid media, or growth on one medium was consistent with direct microscopy findings, or confluent growth was obtained on inoculated single solid medium, or direct microscopy was suggestive of non-cultivable microorganism like *Microsporidia*. The bacteria isolated had been identified by standard biochemical test methods and MALDITOF-MS (Bruker Biotyper Microflex, MA, USA). Antibiotic susceptibility was put for bacterial isolates by Kirby Bauer's disc diffusion method. Fungi isolated had been identified by its cultural characteristics on media and sporulation patterns on lactophenol cotton blue mount and slide culture.

Results

The study was conducted in the Department of Microbiology and Ophthalmology in a tertiary healthcare teaching institute in the foothills of Himalayas for 1 year. A total of 120 samples from clinically suspected IK cases were submitted. There was a male preponderance in the study, with a male to female ratio of 2:1. The mean age of patients was 48.9 years and a maximum number of patients, that is, 53% (64/120) belonged to 41–60 years' age group. Among the participants, 72% (n = 86/120) belonged to the rural background and 52% (n = 63/120) had an agricultural occupation. Table 1 shows the demographic parameters of the patients enrolled in the study and associated predisposing factors, if present.

Predisposing factors were documented in 46% (55/120) of cases. Corneal trauma was the chief predisposing factor documented in 39% (n = 47/120) of cases, followed by pre-existing illness like Diabetes mellitus type II and carcinoma with brain metastasis in 4% (n = 5/120) of cases, structural lid abnormalities in 2% (n = 2/120) of cases and use of topical steroids in 1% (n = 1/120) of cases. None of the participants gave history of contact lens use. History of trauma with a wooden stick or

Table 1: Demographic parameters of the patients (n=120) enrolled in study and predisposing factors associated

Demographics	Particulars	Number (%)
Gender	Male	80 (67)
	Female	40(33)
Age in years	<20	6(5)
	21-40	35 (29)
	41-60	64 (53)
	61 onwards	15(13)
Residence	Rural	86(72)
	Urban	34(28)
Occupation	Agricultural	63(52.5)
	Non-agricultural	57(47.5)
Predisposing factors (documented in 55/120 i.e. 46%) cases	Corneal trauma	47 (39)
	Pre-existing illness	5(4.2)
	Structural abnormality	2(1.7)
	Use Topical steroids	1(0.8)
	Contact lens	0 (0)
Traumatic agents (documented in 47/120 i.e. 39%) cases	Vegetative matter/wooden stick	32(27)
	Stone/particle	5(4)
	Chemical	2(2)
	Rubbing of eye lid	2 (2)
	Nail	2(2)
	Hair	2(2)
	Occupational hazard	1(1)
	Trauma by insect	1 (1)

vegetative matter was most common followed by injury from stone or particle, injury from chemical exposure, rubbing of eyelashes, hair, nail, insect, and injury while doing welding. Two cases of chemical exposure were noticed. One because of occupational exposure of cement and another because of a copper oxychloride containing based commercially available broad fungicide.

Clinical features

The most common complaint was pain followed by a diminution of vision, redness, and lacrimation. Clinical signs included stromal infiltrates (n = 65) in maximum number of cases, followed by circumcorneal congestion (n = 53), hypopyon (n = 38), Descemet's membrane folds (n = 13), scar (n = 8), perforation (n = 5), and impending perforation (n = 4).

Microbiological analysis

KOH mount/calcofluor white staining was positive for fungal elements in 40% of cases (n = 48/120) [Figures 1 and 2]. On gram staining, gram-variable hyphae in 19% (n = 23/120) cases, gram-positive cocci in 7% (n = 8/120) of cases and gram-negative bacilli in 2.5% (n = 3/120) of cases were seen. In one patient, on gram stain, a cluster of ovoid spore-like structures was also seen resembling *Microsporidia* species [Figure 3]. Growth on culture was obtained in 50% of cases (n = 60/120). Fungal growth in 26% (n = 31/120) of cases and bacterial growth in 22% (n = 27/120) of cases was obtained. In 2% (n = 2/120) of cases, scraping yielded significant growth of more than one species of fungi or growth of bacteria along with fungus like *Fusarium* and *Geotrichum, Fusarium and Escherichia coli, Aspergillus flavus and Klebsiella*. Overall, infective aetiology could be established in 51% (n = 61/120) of cases according to the predefined criteria. Table 2. summarizes identified causative micro-organisms responsible for the infection.

Table 3 illustrates the distribution of pathogens identified in the study using traditional techniques as well as MALDI-TOF. MALDI-TOF is an exemplar and rapid method for precise identification of microorganism. Of all the fungal isolates obtained (n = 34), the most common fungal isolate was *Fusarium* (18/34) followed by *Aspergillus* (8/34), *Curvularia* (4/34), *Pseudallescheria boydii* (3/34), and *Geotrichum* (1/34). Among the gram-positive bacterial isolates (n = 16), Coagulase negative *Staphyloccus species (13/16)* were isolated in maximum number followed by *Staphlyococcus aureus* (2/16) and *Streptococcus pneumoniae* (1/16). Among the gram-negative isolates (n = 13),

Table 2: Causative micro-organism responsible for infectious keratitis

Aetiology	Number (%)
Total culture positive cases	60 (50)
Fungal growth	31 (26)
Bacterial growth	27 (22)
Mixed fungal and bacterial growth	02 (2)
Microsporidia (identified in direct microscopy)	01 (1)
Cases in which no organism identified	59 (49)

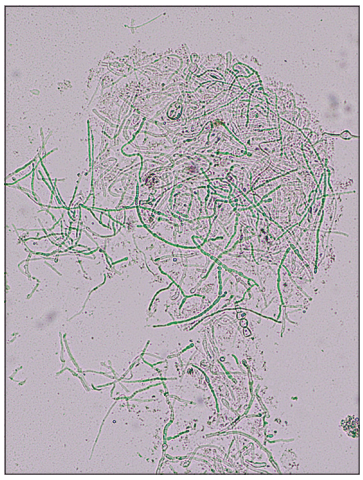

Figure 1: Direct Potassium hydroxide mount showing hyaline septate hyphae. (magnification 400×)

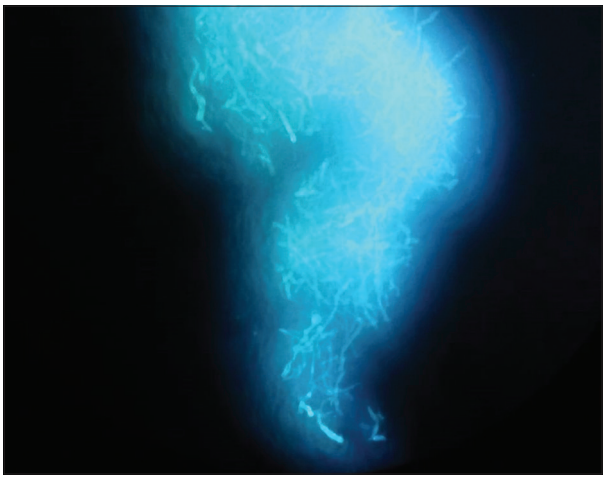

Figure 2: Calcofluor white staining mount showing fluorescing hyphae. (magnification 400×)

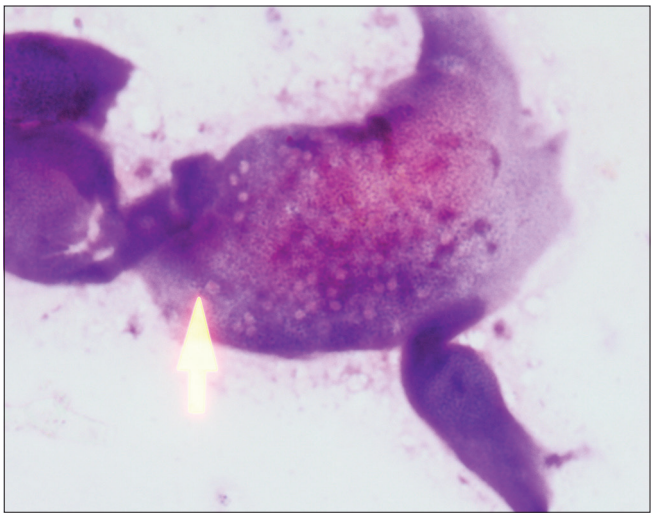

Figure 3: Gram staining smear showing cluster of ovoid spore like structures resembling Microsporidia species. (magnification 1000×)

Table 3: Distribution of identified pathogens from infectious keratitis cases in 1-year study period (*n*=64)

Pathogens	Identification by Conventional Method	Identification by MALDI-TOF-MS	Pure isolate in culture (number)	Mixed with other species in culture (number)
		Fungal isolates (*n*=34)		
Fusarium species	18	*Fusarium proliferatum* (10) *Fusarium oxysporum* (04) *Fusarium solani* (04)	16	2
Aspergillus flavus	5	*Aspergillus flavus (05)*	4	1
Aspergillus fumigatus	3	*Aspergillus fumigatus* (03)	3	-
Pseudallescheria boydii	3	*Scedosporium apiospermum* (03)	3	-
Curvularia geniculata	3	Not identified	3	-
Curvularia lunata	1	Not identified	1	-
Geotrichum	1	Not identified (Not in database)	-	1
		Gram positive bacterial isolates (*n*-=16)		
Coagulase negative Staphylococcus species	13	*Staphylococcus epidermidis* (13)	13	-
Staphylococcus aureus	2	*Staphylococcus aureus* (2)	2	-
Streptococcus pneumoniae	1	*Streptococcus pneumoniae* (1)	1	-
		Gram negative bacterial isolates (*n*=13)		
Pseudomonas species	8	*Pseudomonas aeruginosa* (8)	8	-
Acinetobacter baumannii complex	3	Acinetobacter lwofii (02) Acinetobacter junii (1)	3	-
Klebsiella pneumoniae	1	*Klebsiella pneumoniae* (1)	-	1
Escherichia coli	1	*Escherichia coli* (1)	-	1
		Non-cultivable microorganism (identified on basis of direct microscopy)		
Microsporidia	1	NA	-	-

Pseudomonas species (8/13) were isolated in a maximum number of cases, followed by *Acinetobacter* (3/13), *Klebsiella pneumoniae* (1/13), and *Escherichia coli* (1/13).

Fusarium forms reproductive ovoid structures *in vivo*, this phenomenon is known as adventitious sporulation.[5] This phenomena was also observed in few cases and a rapid presumptive diagnosis of *Fusarium* infection can be made while awaiting the results of the culture.

Management and Outcome

The standard protocol for the empirical treatment of cases was the topical instillation of antibiotics, antiviral and antifungals drugs, in combination with oral antifungals. Table 4 shows the antimicrobial treatment of the patients (n = 120) enrolled and their clinical outcomes. Table 5 illustrates organisms identified in culture-proven cases (n = 63) and clinical outcomes associated with them.

Discussion

IK is a potentially damaging ocular infection when the corneal epithelial barrier is breached owing to injury or trauma. Immediate diagnosis and treatment are required if vision threating outcomes are to be avoided.[6] We have explored epidemiological risk factors, clinical features, etiological agents associated with IK and described clinical outcomes.

In the study, 41–60 was the age group in which most of the keratitis cases were recorded. This is in contrast to the findings of an epidemiological study from North India which states 31–40 years as the age group with the highest number of cases. A study from Delhi for over 16 years, that is, 2010–2016 and a study from Nagpur state also states 31–40 as the most affected age group. A study from Tamil Nadu states 21–50 as the commonest age group affected and studies from Ahmedabad and Bengal describes 21–40 as the age group with highest number of cases.[2,7-11] There was a male predominance which can be attributed to outdoor work done by them. Similar findings were reported by many authors as Manikandan *et al.* and Deorukhkar S *et al.*[12,13] The majority of them belonged to the rural background, had an agricultural occupation and trauma was the most significant risk factor observed. Agricultural practices in rural areas and humid environments as in the state of Uttarakhand is favorable for the development of IK from minor trauma.[7]

Corneal trauma has always been identified as the chief risk factor behind IK.[8,9,11,14] Trauma with the vegetative matter, leaves, wood, some particle or stone was the leading predisposing factor. Interestingly, two cases of chemical injury have also been documented: one with cement fall and another with a fungicide. The patient was exposed to a commercially available copper-based broad fungicide while spraying it in fields. To the best of our knowledge, no case of keratitis has been reported after ocular exposure with copper oxychloride.

IK can be caused by a variety of microorganisms like bacteria, viruses, fungi, or parasites. In this study, of all the cases with established infective aetiology, 26% of cases were attributed to fungal, 22% to bacterial, and 2% to mixed bacterial and fungal

Table 4: Antimicrobial treatment of the patients (n=120) enrolled and their clinical outcome

Treatment	Natamycin, Voriconazole, oral ketoconazole	Natamycin, Voriconazole, Moxifloxacin	Acyclovir	Antiviral, Cefuroxime, Moxifloxacin	Cefuroxime, Moxifloxacin	Moxifloxacin, Acyclovir	Moxifloxacin, Tobramycin	Azithromycin	Voriconazole, Natamycin	Natamycin, Gatifloxacin, Gancyclovir, Itraconazole	Moxifloxacin
Worse (*n*=19)	10	1	1	-	6	-	1	-	-	-	-
Symptomatically better (*n*=24)	10	3	1	-	7	-	2	-	-	1	-
Healed (*n*=61)	32	5	1	2	14	1	3	1	1	-	1
Lost to follow up (*n*=16)	7	-	3	1	4	-	-	-	1	-	-

etiology. Similarly, other studies regarding the microbiological profile of IK from Gujarat, Tamil Nadu, and Delhi, state fungal etiology as leading cause followed by bacterial aetiology. Whereas a study from Mysore, Karnataka reports bacterial aetiology as the commonest.[1,15-17]

Compared to other infective corneal ulcers, fungal corneal ulcers are difficult to diagnose as well as treat. Also, these are more likely to get perforated and the patient presents with increased ocular morbidity like scarring. Hence, rapid communication between microbiologist and ophthalmologist is of utmost significance. *Fusarium* was the most frequently isolated fungus. that is, 53% (18/34) of all fungal isolates in the study. A similar finding has been observed in many studies from the west and south India and neighbor countries with similar climate, where *Fusarium* was the commonest fungal isolate.[8,12,15,18,19] While few studies from North India have reported *Aspergillus* to be the commonest causative agent. *Fusarium* and *Aspergillus* are said to be the frequently reported agents of IK in tropical areas. Poor response to medical treatment in fungal keratitis has been documented.[10,11,14,16,20] In our study also, among all culture positive cases, poor outcome in the form of enlarged infiltrate size and perforation has been observed in 9.5% (6/63) of fungal, 3% of (2/63) gram-positive bacterial and 1.5% of (1/63) gram-negative bacterial keratitis cases.

As there are no CLSI (The Clinical and Laboratory Standards Institute)/EUCAST (The European Committee on Antimicrobial Susceptibility Testing) guidelines yet for topical ocular antimicrobial agents, sensitivity pattern coupled with clinical improvement is needed to assess the efficacy of a particular antimicrobial agent. Knowledge of causative microorganism in a particular region and judicious use of s ocular antibiotics or antifungal agent can be a pivotal step in resource limited primary health centres in the management of corneal ulcer, thereby decreasing ocular morbidities. To summarize, infective aetiology (fungal more than bacterial) could be established using conventional as well as MALDI-TOF technique in 51% cases and mixed infections can only be diagnosed using culture. Ocular trauma with vegetative material stays the most common predisposing factor for corneal ulcers.

Conclusion

For initiating appropriate empirical therapy, the knowledge of the epidemiological pattern of IK of a particular geographical region is crucial. Early and accurate diagnosis allows timely specific treatment and is the corner-stone of vision-saving management. However, relevant empirical treatment needs to be started until the time a microbiological diagnosis is made. For initiating the most appropriate empirical therapy, the knowledge of the epidemiological pattern of IK of a particular geographical region is crucial. Comprehensive analysis of results obtained over a period of time from different geographical areas can enhance the knowledge and understanding of such patterns.

Table 5: Pathogens identified in culture (*n*=63) and associated clinical outcome

	Worse *n*=9 (14%)	Symptomatically better *n*=13 (21%)	Healed *n*=31 (49%)	Lost to follow up *n*=10 (16%)
Fungal				
Fusarium species (18)	2	8	7	1
Aspergillus flavus (5)	2	1	1	1
Aspergillus fumigatus (3)	1	--	2	--
Pseudallescheria boydii (3)	--	--	1	2
Curvularia geniculate (3)	1	1	--	1
Curvularia lunata (1)	--	--	1	--
Geotrichum (1)	--	--	1	--
Bacterial				
Staphylococcus epidermidis (13)	--	--	12	1
Staphylococcus aureus (2)	--	--	1	1
Streptococcus pneumoniae (1)	--	--	1	--
Pseudomonas species (8)	2	--	4	2
Acinetobacter baumannii complex (3)	-	2	--	1
Klebsiella pneumoniae (1)	1	--	--	--
Escherichia coli (1)	--	1	--	--

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Conflicts of interest

There are no conflicts of interest.

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