Microbiological Spectrum of Infectious Keratitis: Insights from a Multi-speciality Hospital Setting

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Abstract
Microbial keratitis (MK) is a prevalent eye condition that leads to vision impairment and poses significant risks to ocular health. It occurs when bacteria, fungi, viruses, or parasites infect the cornea, causing damage through their virulence factors or triggering harmful immune responses. The incidence of infectious keratitis is notably higher in developing countries, especially in regions with low education, inadequate sanitation and poor hygiene. This study aimed to identify the microbiological profile of MK and to explore its socio-demographic components. This time-bound, cross-sectional research involved patients who attended Multi-specialty hospital between January 2022 and June 2024 (18 months). A total of 361 patients were recruited for the study. Of the 315 samples that tested positive for microbes (87.2% of the total), 188 (59.6%) had been found to have bacteria.

Among Gram-positive organisms, Coagulase-negative staphylococci (CNS), was the most predominant organism isolated, accounting for 86 (45.7%) of the cases. Of Gram-negative bacteria, Pseudomonas aeruginosa was shown to be a prominent culprit, responsible for 32 cases (17.1%) and Escherichia coli was found to be involved in 26 cases (13.8%). Out of the 127 culture positive cases, the majority of fungi were Fusarium species 54 (42.7%), Aspergillus species 46 (36.2%), Candida species 21 (16.5%), Curvularia species 3 (2.3%), Penicillium species 3 (2.2%), F. solani 42 (77.8%), F.semitectum 7 (12.9%) and F. dimerium 5 (9.3%).

Keywords: Bacterial keratitis, fungal keratitis, risk factors.

Introduction

Microbial keratitis (MK) is a common illness that can seriously impair vision, posing a serious hazard to ocular health. Numerous infections including bacteria, fungi, viruses and parasites, can cause MK[6]. These microbes directly invade the cornea and harm it by either using their virulence factors or inducing an immunological response in the host. It is more likely in the following circumstances: wearing contact lenses, injuries to the eyes, diseases of the ocular surface (dry eye, facial nerve palsy), disorders of the lacrimal apparatus (chronic dacryocystitis), corneal leukoma caused by trauma or infection; scarring from herpes simplex infections and immunosuppressive conditions (diabetic, HIV infection, steroid treatment). The rate of infections is higher in developing nations, especially in areas with low levels of education, unsanitary conditions, poor personal and environmental hygiene as well as restricted access to medical services and sanitation.

To enhance prevention, diagnostic and treatment approaches, as well as to efficiently allocate resources, a thorough understanding of the underlying causes of MK is necessary[9]. According to WHO data, the fourth-highest rate of blindness worldwide is caused by corneal diseases, with almost two million new instances of keratitis occurring year. This poses a serious public health concern[11].

In India, the prevalence of MK ranges from 44% to 47% because of the country's tropical environment and large agricultural population. It can lead to corneal scarring, perforation and abscess which can ultimately cause blindness[1].

Bacteria like *Staphylococcus aureus* and *Pseudomonas aeruginosa* have the ability to cling to and infiltrate corneal epithelial cells, leading to infections. These microorganisms create biofilms on contact lens casings which further encourage infections. A wide range of virulence factors are also present in *Pseudomonas aeruginosa* such as the type III secretion system (TTSS) which allows the bacteria to damage host cells by injecting effector proteins. Other virulence mechanisms of *Pseudomonas aeruginosa* include the TTSS, which alters host cells by introducing effector proteins. Bacterial pathogens in keratitis might be difficult to identify early and accurately[16].

While over 390 different species of filamentous fungus and yeast have been identified as potential etiological agents of FK, *Fusarium spp*. is thought to be the most frequent cause of this illness. *Fusarium species* are hyalohyphomycetes that are extensively distributed and they proliferate quickly. They may be found in soil, water, plants and vegetative detritus.

Since the features of fungal keratitis (FK) and bacterial keratitis (BK) are so similar, it is challenging to differentiate between the two using image analysis alone[7]. Furthermore, misdiagnosing fungal and BK is a common inaccuracy that occurs in over 30% of cases. This study aimed to identify the microbiological profile of MK and to explore its sociodemographic components.

Material and Methods

This time-bound, cross-sectional research involved patients who attended in Multi-specialty hospital between January 2022 and June 2024 (18 months). The Institutional Ethical Review Board granted ethical approval. There were 361 participants in our research who had MK presenting clinical symptoms. Of the 315 samples that tested positive for microbes (87.2% of the total), 188 (59.6%) had been found to have BK and 127 (40.4%) have FK. Participants were included only after a thorough clinical and microbiological diagnosis of a corneal ulcer. The following individuals were not allowed to participate: those with healing ulcers, marginal ulcers, perforated ulcers, typical viral ulcers, sterile neurotropic ulcers, or ulcers related to autoimmune diseases. Participants in the research did not provide extra consent since the study depended on corneal scrapings taken by ophthalmologists and forwarded to laboratory.

KOH mount: 10% KOH mount was created from another slide using corneal scraping, coated with a coverslip and examined under a microscope to check for the presence of any fungal components.

Culture: Scrapings were aseptically inoculated on thioglycolate, Sabouraud's dextrose agar (SDA), blood agar, chocolate agar, MacConkey's agar and potato dextrose agar. The growth of bacterial and fungal pathogens was facilitated by the proper incubation of the culture material.

Bacterial Culture: MacConkey agar, blood and chocolate were the three bacterial growth mediums that were incubated at 37°C for the duration of the overnight. In the event that growth was not seen during the initial overnight incubation, the plates were incubated for further 48 hours. Following observation of growth along the C streaks in culture media, an automated technique was used to identify the bacterium in the end and to test for its sensitivity to antibiotics (Vitek2 Compact system, bioMerieux).

Fungal Culture: Two inoculated Sabouraud dextrose agar plates containing 0.05 mg/mL of chloramphenicol were used as the infected media and they were incubated at 37°C and 22°C for a duration of 14 days. The inoculated media were tested for signs of fungal development on the third, seventh and fourteenth days. In cases of fungal growth, the identification procedure involved both microscopic examination of the fungal morphology in lactophenol cotton blue (LPCB) mount and slide culture as well as a consideration of the features of the colony. FK was diagnosed only when both the fungal culture and the KOH mount showed positive results, or when the same growth was observed in both Sabouraud dextrose agar media.

Results

Etiology of Bacterial and Fungal Keratitis

Socio-demographic: A total of 361 patients with ocular infection were recruited for the study. In the study, males were affected more (65.9%) when compared to females (34.1%). Males were shown to be afflicted in greater numbers, but there was no overly large male preponderance This study was enrolled by patients between 10 and 60 years and around 30.4% of patients were in the range of 40–49 years, indicating that ages beyond 40 years are more prone to ocular keratitis. In the present study, most of the patient's were agriculturists 148 (41%) followed by home maker, driver, mechanics and students. Out of 361 patients, 68 (18.8%) were diabetic and 27 (7.4%) were having hypertension. Predisposing factors included vegetative matter 167 (46.3%), hay 104 (28.8%), mud and stone 70 (19.4%) and foreign bodies 20 (5.5%) (Table 1).

Bacterial keratitis: There were 361 participants in our research who had infectious keratitis presenting clinical symptoms. Of the 315 samples that tested positive for microbes (87.2% of the total), 188 (59.6%) had been found to have BK. Although it was demonstrated that more men were affected than women, there was not an excessive male preponderance. The mean age of BK was 44 years. Among Gram-positive organisms, *Coagulase-negative staphylococci* (CNS), was the most predominant organism isolated, accounting for 86 (45.7%) of the cases. This indicates that CNS plays a crucial role in the pathophysiology of keratitis. The second most common, accounting for 23 cases (12.3%), was *Streptococcus species*, underscoring its important role in the illness.

Of the Gram-negative bacteria, *Pseudomonas aeruginosa* was shown to be a prominent pathogen, responsible for 32 cases (17.1%) of keratitis, while *Escherichia coli* was found to be involved in 26 cases (13.8%). Also, a small percentage of the cases had additional Gram-negative bacteria, such as *Proteus mirabilis* 11 (5.8%) and *Klebsiella* species 10 (5.3%), which are less commonly found species.

Fungal keratitis: Of the 315 microbiologically positive sample, 127 (40.4%) showed FK. 127 patients who were KOH mount and culture positive for fungal elements were included in the study. Among the 127 culture-positive cases, the majority of fungi were *Fusarium species* 54 (42.7%), *Aspergillus species* 46 (36.2%), *Candida species* 21 (16.5%), *Curvularia species* 3 (2.3%) and *Penicillium species* 3 (2.3%). Out of 54 *fusarium species*, *F. solani* were 42 (77.8%), *F.semitectum* were 7 (12.9%) and *F. dimerium* were 5 (9.3%). Among 46 (36.2%) *Aspergillus species*, *A. flavus* 33 (71.7%), *A. fumigatus* were 7 (15.3%), *A. terreus* 3 (6.5%) and *A. niger* were 3 (6.5%). In total of 21 *candida species,* 11 (52.3%) were *Candida albicans* and 10 (47.7%) were *Candida non albicans*.

Discussion

MK is a leading cause of permanent blindness associated with corneal diseases. It is a potentially sight-threatening emergency because it can progress rapidly, risking corneal perforation and loss of vision [2]. A diverse range of infections such as bacteria, viruses, fungi and protozoa, can induce MK.

Table 1
Characteristics of the patient population among those classified according to the type of keratitis—bacteria alongside fungi (n-315)

Demographics	Overall (n-315)	Bacterial Keratitis (n-188)	Fungal keratitis (n-127)	P Value
Gender				
Male	200 (63.4%)	115 (61.2%)	85 (66.9%)	0.297
Female	115 (36.6%)	73 (38.8%)	42 (33.1%)	
Age				
10-20	25 (7.9%)	19 (10.1%)	6 (4.7%)	0.026*
21-30	63 (20%)	41 (21.8%)	22 (17.3%)	
31-40	68 (21.6%)	30 (16%)	38 (29.9%)	
41-50	89 (28.3%)	53 (28.2%)	36 (28.3%)	
51-60	70 (22.2%)	45 (23.9%)	25 (19.8%)	
Occupation				
Agriculture	128 (40.6%)	77 (41%)	51 (40.2%)	0.242
Homemaker	92 (29.2%)	61 (32.4%)	31 (24.4%)	
Driver	57 (18.1%)	28 (14.9%)	29 (22.8%)	
Mechanics	20 (6.3%)	10 (5.3%)	10 (7.8%)	
Students	18 (5.8%)	12 (6.3%)	6 (4.8%)	
Diabetes				
Yes	68 (21.6%)	37 (19.7%)	31 (24.4%)	0.317
No	247 (78.4%)	151 (80.3%)	96 (75.6%)	
Hypertension				
Yes	27 (8.6%)	14 (7.4%)	13 (10.2%)	0.385
No	288 (91.4%)	174 (92.6%)	114 (89.8%)	
Pre disposing factors				
Vegetative matters	137 (43.5%)	74 (39.4%)	63 (49.6%)	0.351
Hay	104 (33%)	68 (36.2%)	36 (28.3%)	
Mud & Stone	54 (17.2%)	33 (17.6%)	21 (16.5%)	
Foreign bodies	20 (6.3%)	13 (6.8%)	7 (5.6%)	

The result is significant at $p < 0.05$.

Predisposing risk factors, socio-economic conditions and climate all have an impact on the infectious aetiology. MK is an eye emergency and a serious public health concern. In order to avoid corneal perforation and the ensuing loss of vision, prompt diagnosis and treatment are crucial[14].

In this study, male patients were predominantly affected aligning with the findings of Chauhan et al[8] (69%) and Bhagath et al[5] (64%). The reason for this pattern is that males are more likely to participate in outdoor activities which makes them more prone to injury. Men are even more at risk since they frequently make up a large share of the agricultural labour force. However, research by Hoffman et al[10] and Suwal et al[18] indicated that female patients had a greater prevalence of infection (58% and 61%, respectively) which may be related to the fact that a large number of women work in agriculture. The research also revealed that individuals over 40 years old were more commonly affected by ocular keratitis, although cases were reported across all age groups.

Similar findings were observed by Sankolli et al[15] who noted that the 41–60 age range was the most affected group. This may be as a result of the fact that people in this age range work and participate in outdoor activities. The incidence of MK is significantly influenced by environmental and occupational variables[5]. The present research showed an increased prevalence of ocular keratitis among farmers (41%). This finding aligns with the studies by Chauhan et al[9] (40.5%), Suwal et al[18] (57.8%) and Bhagath et al[5] (66.6%). The increasing incidence of vegetative trauma in agricultural areas may be connected to this correlation.

Conversely, Tewari et al[19] reported that ocular keratitis was more commonly observed among housewives (21.3%) than farmers (16.6%). This was explained by the fact that in developing nations, housewives would participate in winnowing, dusting and wood chipping. In this study, the majority of ocular keratitis-infected patients were diabetic (68 cases). Vegetative matter (167 cases) was identified as the most predominant predisposing factor.

Conversely, Al Badriyeh et al[3] identified additional risk factors for keratitis, including concurrent ocular illnesses, prolonged use of corticosteroids, improper use of antifungal drugs and corneal damage caused by vegetative matter.

Additionally, Sankolli et al[15] found that 59.6% of patients had a history of trauma, with the majority (55.8%) involving trauma from vegetable matter.

There are several pathogens that can cause MK. These microbes cause harm to the cornea by inducing an immunological response in the host or by directly invading the tissue and employing their virulence factors. The onset of MK is associated with a number of risk factors including the use of contact lenses, ocular trauma, dry eye, epithelial abnormalities, systemic diseases and immunosuppression[5,21].

In the current research work, out of 361 patients, 315 (87.2%) were microbiologically positive samples. Of them, BK (59.6%) was identified predominantly than FK (40.4%). Similar results were interpreted from the findings of Bălășoiu et al[4] who showed that BK (92.9%) was predominantly identified than FK (7.08%). In contrast, fungi (66.3%) were found to be retrieved more frequently than bacteria (33.7%), according to Chauhan et al[8].

Additionally, in their study, Srinivasan et al[17] found that there were about similar numbers of bacterial (47.1%) and fungal (46.8%) etiological agents, with 5.1% of mixed infections.

The majority of Gram-positive bacteria in this study that caused BK were *CNS* (86 cases), which were the most often isolated bacteria. These findings align with the studies by Chauhan et al[8]. Narsani et al[13] found that *Staphylococcus aureus* (14%) was the most prevalent Gram-positive organism isolated. The most frequent isolate among the Gram-negative organisms was *Pseudomonas aeruginosa* (32 instances), which is in line with the findings of Chauhan et al[8]. *Pseudomonas aeruginosa* was the most prevalent Gram-negative bacteria (4.8%). Similarly, Lam et al[12] also reported *Pseudomonas aeruginosa* as the prevalent Gram-negative bacterium in their study.

According to the current research, *Fusarium species* was the most frequently isolated fungal species from patients with FK. These findings were found to be consistent with the study conducted by Chauhan et al[8] who found that *Fusarium species* (54) was the most frequently isolated fungal species from cases of infective keratitis (28.9%). Furthermore, *Fusarium species* was the most common among individuals with FK. On the other hand, *Aspergillus species* was shown to be the most common fungus isolated from corneal ulcers in the investigations conducted by Lam et al[12].

Climate and environmental variations across different regions may be the cause of the variance in the most common fungal isolates from corneal ulcers. Spores of *Aspergillus species* are commonly present in the environment, but Fusarium species are common plant infections that can readily penetrate the cornea through traumatising lesions involving vegetative matter. In addition, fusarium-induced keratitis is more likely to be aggressive and less amenable to therapy than Aspergillus-induced keratitis [20].

Conclusion

MK is a major contributor of visual impairment, especially in developing countries such as India. The variety of causative agents both bacteria and fungi highlights the need of understanding the differences in microbiological profiles across various climates and geographic regions. As a result, it is critical to inform rural residents and farmers in particular about BK, FK and how it may endanger eyesight. Farmers have to be counselled to use safety goggles when working, to report any eye injuries right once and to follow ophthalmologists' recommended treatment plans.

The slow progression and clinical picture similar to that of other infectious agents may predispose patients to delayed presentation and treatment. The results are intended to give ophthalmologists and legislators clear information so that protocols for the efficient treatment of MK may be developed.

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