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Decoding microbial keratitis: Epidemiology, clinical spectrum, and treatment outcomes in a tertiary care setting

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ABSTRACT

Background: Infectious keratitis poses a significant public health challenge worldwide, with variable incidence rates reported globally. Timely diagnosis and intervention are crucial to prevent ocular morbidity and potential blindness. **Aims and Objectives:** This study aimed to study and investigate the epidemiology, risk factors, etiology, clinical progression, microbiological findings, and treatment outcomes of microbial keratitis in a tertiary care center in western India. **Materials and Methods**: We conducted a prospective observational study from July 2020 to July 2022, enrolling 90 patients with suspected microbial keratitis. Clinical data were collected, and statistical analysis was performed using SPSS 26.0, with $P<0.05$ considered statistically significant. **Results:** Most patients were males (67.80%) and laborers (27.8%) from urban areas, with fungal keratitis being more prevalent in rural regions. No statistically significant associations were found between keratitis type and demographic factors, such as age, gender, occupation, or socioeconomic status. Bacterial keratitis was more common in urban areas. Delayed presentation was linked to larger ulcers and worse visual outcomes. Fungal keratitis presented with smaller but deeper ulcers compared to bacterial keratitis. We found 8.06% positivity rate for Gram and KOH stain each. Microbiological cultures identified bacteria and fungi in 11.29% and 20.97% samples, respectively. Treatment varied from topical medications to surgery, with 26.67% of patients receiving both. **Conclusion:** Our findings underscore the importance of prompt diagnosis, targeted treatment, and comprehensive microbiological workup in managing microbial keratitis to prevent vision and eye loss. Further research is warranted to enhance understanding and optimize management strategies for this sight-threatening condition.

Key words: Microbial/infective keratitis; Fungal corneal ulcer; Bacterial corneal ulcer; Risk factors; Microbiological tests; Antimicrobial agents

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INTRODUCTION

Infectious keratitis presents a notable public health challenge, exhibiting variable global incidence rates. Notably, the United States reports 11 cases/100,000 person-years, while Nepal demonstrates a strikingly higher rate of 799 cases/100,000 person-years. India's annual incidence stands at 11.3/10,000 individuals.[1] Timely diagnosis and intervention are imperative to mitigate the risk of ocular morbidity and prevent potential blindness.

Keratitis, characterized by corneal inflammation, can stem from infectious or non-infectious causes. Microbial keratitis, a severe form, involves corneal epithelial defects and stromal infiltrates caused by bacterial, viral, fungal, or parasitic agents. Optimal therapeutic outcomes rely on prompt and targeted interventions, supported by thorough clinical evaluation and patient history assessment. Presumptive treatment of the keratitis is often begun immediately after specimens are obtained for isolation. Microbiological tests are required to establish etiological

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agents and antimicrobial susceptibility, especially in nonresponding cases to medical treatment. Recent reports of increasing antibiotic resistance among ocular pathogens are the cause of grave concern given the shift among ophthalmologists from culture-driven treatment with multiple, fortified, compounded antibiotic agents to empiric treatment with widely available commercial preparations.[2,3]

Aims and objectives

Our research aims to investigate the epidemiological characteristics, risk factors, etiology, clinical progression, microbiological findings, and treatment outcomes of microbial keratitis at the tertiary care referral center in western India.

MATERIALS AND METHODS

Our hospital-based prospective observational study was conducted from July 2020 to July 2022 in the tertiary care center in western India. Approval was obtained from the Institutional Ethics Committee and we have adhered to the declaration of Helsinki of 1964 during the study. Clinical data were collected from 90 patients with signs and symptoms of suspected microbial (infective) keratitis using consecutive sampling.

Patients unable to provide informed written consent or unwilling to participate, patients lacking signs and symptoms of infective keratitis or unable to understand the management of the same, and patients with a recent history of ocular surgery (within the past 3 months) were excluded from the study. Furthermore, patients with other ocular comorbidities such as lid/adnexa deformities and patients with suspected of endophthalmitis were excluded.

All patients underwent thorough anterior segment evaluation with slit-lamp biomicroscopy including corneal sensations and visual acuity testing using Snellen's chart. Posterior segment evaluation was conducted with ophthalmoscopy or ultrasonography if the ulcer obstructed clinical examination. Documentation included clinical drawings with color coding and, when feasible, slit-lamp photography. Corneal ulcer is defined as small (<3×3 mm), medium (>3×3 mm–<6×6 mm), or large (>6×6 mm) as per the maximum dimension. Depth of stromal infiltration was defined as superficial or deep as per the depth of stromal involvement with superficial defined as infiltration <1/3 of stroma and deep defined when infiltration is >1/3rd of stroma. Systemic history was taken for any underlying diseases and physician evaluation was advised when indicated.

Routine blood and urine tests were conducted. Microbiological investigations were conducted in 62 out of 90 patients, the primary investigation being corneal scraping (58 out of 62 cases -93.55%). Other samples included bandage contact lenses (BCLs), anterior chamber (AC) tap, and corneal biopsy taken from one patient each (1 out of 62 cases- 1.61%).

Corneal scraping was done after informed consent under aseptic conditions using a sterile Bard-Parker blade (No. 15) from the edge and base of the active corneal ulcer involving the site of infiltration which was identified based on clinical examination. Scraping was usually performed on slit lamps and rarely in OT when not possible on slit lamps. Proparacaine hydrochloride (0.5%) was used as a local anesthetic. The obtained material was inoculated on blood agar, nutrient agar, Sabouraud's dextrose agar (SDA), and glucose broth and smeared on slides for Gram stain and 10% KOH wet preparation. Inoculated media were incubated at 37°C for up to 48 h, with criteria for a positive sample including growth on two or more media, consistency with clinical signs, and smear results confirming culture findings.

Bacterial identification was based on Gram staining and biochemical properties. SDA media were incubated at 27°C for up to 3 weeks for fungal identification and examined daily for growth. Fungi were identified by colony characteristics and microscopic morphology and spores in lactophenol cotton blue stain. Appropriate cultures were used for suspected *Acanthamoeba*, *Nocardia*, and atypical mycobacterial species.

After thorough clinical examination and sending out material for microbiological workup, appropriate (topical and systemic) antimicrobial agents with supportive medications such as cycloplegics, anti-glaucoma drugs, and anti-inflammatory drugs were started. Based on clinical patterns and severity with sensitivity in the local region, fluoroquinolones, cephalosporins, aminoglycosides, and vancomycin preparations were commonly considered for bacterial keratitis. Natamycin (5%), voriconazole (1%), and amphotericin B (0.15%) eyedrop preparations were the choice of drugs for fungal keratitis as per the clinical presentation and progress. In cases of viral ulcers, acyclovir 3% eye ointment was considered a topical antiviral agent. Patients were followed as per the severity of microbial keratitis and clinical response for subsequent management if required till the final resolution of the clinical infective condition.

Data collected were compiled and tabulated in an Excel sheet. Qualitative data were presented as numbers with

percentages, and quantitative data as mean with standard deviation. Statistical analysis was performed using SPSS 26.0 (IBM, SPSS, Inc.), with P<0.05 considered statistically significant.

RESULTS

The present study included 90 patients with an age range of 12–87 years and a mean age of 48.68±16.98 years. Maximum patients (23.33%) were in the 31–40 years age group, while the 81–90 years age group had the least (2.22%) cases. Only 2 patients (2.2%) out of 90 patients were <18 years of age and the rest all 88 patients (97.8%) were >18 years of age in our study. However, the type of keratitis was not significantly associated with age distribution (P=0.2731).

The summarized demographic parameters are stated in the below table (Table 1).

Fungal keratitis was predominant among farmers (52.94%, P=0.1075) and laborers (48%, P=0.7845). Bacterial keratitis was more common among retirees and business owners (P=0.0061), but no statistical relationship was found between profession and type of keratitis in other occupations.

Both eyes were equally represented in the study and no case with bilateral keratitis was observed. Our center is a tertiary care center, majority of the patients (66.7%) were referrals from other centers, and the rest primarily presented to our center. Bacterial keratitis was more prevalent in urban areas, while fungal keratitis was predominant in rural areas (P=0.0024).

In our study, we found no statistically significant association between the type of keratitis and religious affiliation or educational background, with most patients having up to primary-level education (P=0.9155).
The distribution of keratitis in the present study is as follows.

Table 1: Demographic parameters for clinical diagnosis

Category	Bacterial	Fungal	Viral	Mixed	Total (%)	P-value
Age						
<18 years	1	1	0	0	2 (2.22)	0.8805
>18 years	29	38	15	6	88 (97.7)	
Gender						
Male	22	25	11	3	61 (67.8)	0.6269
Female	8	14	4	3	29 (32.2)	
Occupation						
Farmer	3	9	2	3	17 (18.9)	0.1075
Labor worker	7	12	5	1	25 (27.8)	0.7845
Housewife	5	11	4	2	22 (24.4)	0.6644
Other (student, retired, children)	15	7	4	0	26 (28.9)	0.0061
Location						
Urban	20	16	13	1	50 (55.6)	0.0024
Rural	10	23	2	5	40 (44.4)	
Socioeconomic class						
Lower	13	24	8	5	50 (55.6)	0.2323
Middle	17	15	7	1	40 (44.4)	
Education						
Illiterate	5	8	2	1	16 (17.8)	0.9334
Primary	17	24	8	4	53 (58.9)	0.9155
Secondary	6	4	5	1	16 (17.8)	0.2510
higher secondary	2	1	0	0	3 (3.33)	0.6084
Graduate	0	2	0	0	2 (2.22)	-
Duration of presentation						
<7 days	7	14	5	0	26 (28.9)	0.0016
8–15 Days	9	12	3	2	26 (28.9)	0.8787
16–30 Days	5	4	0	1	10 (11.1)	0.3872
>30 Days	9	9	7	3	28 (31.1)	0.2742
H\O ocular injury						
None	20	9	12	1	42 (46.7)	<0.0001
Trauma	4	9	0	2	15 (16.7)	0.1331
Foreign Body	6	21	3	3	33 (36.7)	0.0129
Systemic Illness						
Diabetes	7	3	0	2	12 (13.3)	0.0449
Respiratory	1	0	0	0	1 (1.11)	-
Other (Immunocompromised Pt, Cancer, Etc.)	0	1	0	3	4 (4.44)	<0.0001

1. Bacterial (43.33% - 39 patients, 6 microbiologically proven)
2. Fungal (33.33% - 30 patients, 14 microbiologically proven)
3. Viral (16.67% - 15 patients)
4. Mixed bacterial with fungal (6.67% - 6 patients).

Of the 90 patients, 48 (53.33%) had a history of foreign body contact or trauma, with 20 out of 48 patients (41.6%) involving vegetative matter. No patient was a contact lens wearer in this study. In the present study, 30% of patients sought health care within 7 days of symptoms, while 31.1% delayed consultation for over a month. Initial treatment from either a non-ophthalmologist or self-medication (pharmacist or quacks) was found in 4.4% of patients. In addition, 33.3% were directly referred without receiving primary treatment, 62.3% (56 patients) received primary medical treatment elsewhere, in which 91.07% (51 out of 56 patients) were using only medical management (which included steroid drops in one patient), 20 patients out of these 51 patients received only topical treatments whereas rest 31 cases were using both topical and systemic treatments. The remaining 5 out of 56 (8.9%) patients were treated by nonsurgical measures in addition to topical medical treatment (BCL application and Glue with BCL).

Out of 60 patients receiving primary treatment elsewhere, 85% were compliant, while 15% were noncompliant due to affordability concerns.

On slit-lamp examination of 90 patients, eyelid edema was present in 74.4% and conjunctival and/or ciliary congestion in 95.56%. Corneal involvement included corneal ulcer in 96.67%, infiltration in 84.44% (superficial in 26.7% and deep in 57.8%), corneal thinning in 53.34% (localized in 43.3% and diffuse in 10%) which was more common in fungal etiology, and corneal vascularization in 40% (superficial in 21.1% and both superficial and deep in 18.9%). Limbal involvement was seen in 2 fungal keratitis cases (2.23%), and corneal perforation in 12.23% (11 cases) which included 6 fungal and 5 bacterial cases. Involvement of cornea was central (37 out of 90 patients, 41%), paracentral (39 out of 90 patients, 43.33%), or peripheral (14 out of 90 patients,15.5%) in location. Absent corneal sensation was noted in 16.67% of patients. Earlier presentation (<7 days) correlated with smaller ulcer size (<3×3 mm) in 22 out of 45 cases, while delayed presentation showed larger ulcer size (>6×6 mm) in 8 out of 12 cases (P<0.0001). No scleral involvement was observed. About 6.7% (6 out of 90 patients) had both AC exudates and hypopyon and in 16.7% (15 out of 90 cases), no details of AC were seen due to extensive corneal involvement (Figure 1).

Various clinical signs in different types of keratitis are summarized in the below table (Table 2).

In our study, we found no significant relationship between the duration of symptoms and type of keratitis (P=0.4655). Furthermore, no association was found between the type of keratitis and presenting symptoms.

We noted a significant clinical correlation between ulcer size and keratitis type, where small (<3×3 mm) corneal ulcers

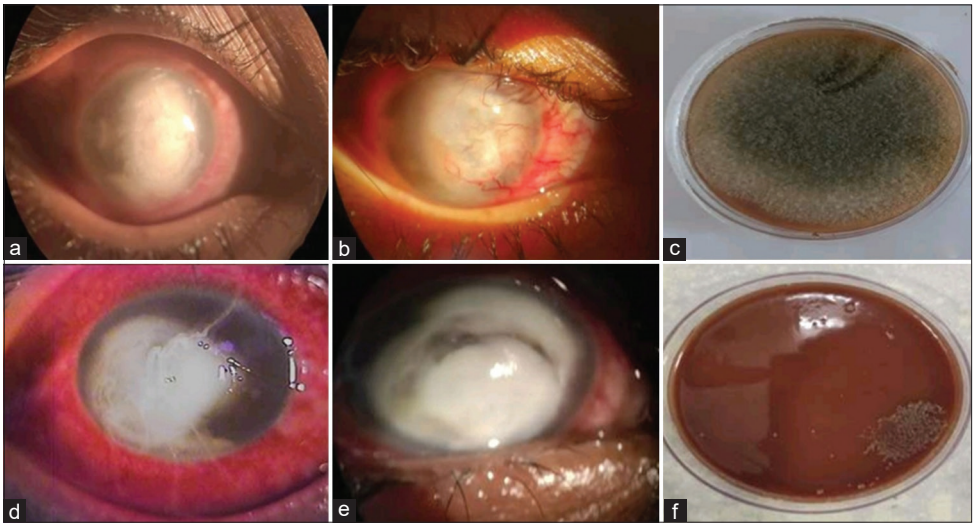

Figure 1: (a-f) Clinical and microbiological profile of infective keratitis, (a) Fungal keratitis with deep infiltration; (b) Healed fungal keratitis with corneal opacity and vascularization; (c) Growth of *Aspergillus fumigatus* on Sabouraud's dextrose agar; (d) Methicillin-sensitive *Staphylococcus epidermidis* keratitis with deep infiltration and hypopyon; (e) Corneal melting in case of fungal keratitis; (f) Growth of *Pseudomonas aeruginosa* on blood agar

Table 2: Clinical presentation based on diagnosis

Clinical presentation	Type of keratitis				Total (%)	P-value
	Bacterial	Fungal	Viral	Mixed		
Duration of symptoms						
<7 days	7	14	5	0	26 (28.9)	0.4655
8–15 days	9	12	3	2	26 (28.9)	
16–30 days	5	4	0	1	10 (11.1)	
>30 days	9	9	7	3	28 (31.1)	
Symptoms						
Pain	22	36	10	6	74 (82.2)	0.0437
Redness	25	34	14	6	59 (65.5)	0.6079
Watering	16	26	8	4	54 (60)	0.6444
Dov	22	27	10	5	64 (71)	0.8685
Signs						
Ulcer size						
<3×3 mm	11	21	13	0	45 (50)	0.0062
3×3 mm– <6×6 mm	13	15	1	4	33 (36.7)	
>6×6 mm	6	3	1	2	12 (13.3)	
Infiltration						
Superficial <1/3 of stroma	9	13	1	1	24 (26.7)	0.2185
Deep >1/3 of stroma	19	25	3	5	52 (57.8)	0.0114
Hypopyon						
<2mm	8	9	1	1	19 (21.1)	0.4581
2–5 mm	3	7	0	2	12 (13.3)	0.1452
Exudates						
Endothelial	3	9	1	0	13 (14.4)	0.2246
ANT. Chamber	2	3	3	0	8 (8.88)	0.3729

were mainly of fungal type (21 out of 45 cases, 46.7%), in larger (>6×6 mm) corneal ulcers, bacterial etiology was more prevalent (6 out of 12 cases, 50%). Fungal keratitis often presented with ulcers <3×3 mm and 3×3 mm to <6×6 mm, whereas bacterial keratitis tended to have ulcers >6×6 mm. Superficial (<1/3 of stroma) corneal involvement was consistent across all types, while deep involvement was notably more frequent in fungal keratitis (P=0.0114).

Of the 62 samples of Gram stain, 5 (8.06%) were positive, revealing Gram-positive cocci and bacilli in 2 samples each, along with one Gram-negative bacillus. KOH mounts detected septate fungal hyphae in 5 cases. Twenty-eight cases had no microbiological test performed as they were suspected of clinically viral etiology or were small corneal ulcers with superficial involvement not sufficient for significant microbiological assessment. Cultures and antibiotic sensitivity tests identified bacteria in 7 samples (11.29%) and fungi in 13 samples (20.97%). Bacterial cultures found methicillin-sensitive *Staphylococcus epidermidis* in 5 cases, *Pseudomonas aeruginosa* in 4, *Burkholderia* in 3, and methicillin-sensitive *Staphylococcus aureus* and *Escherichia coli* in 1 each. Sensitivity was highest to ceftazidime, piperacillin, and tazobactam (8 cases each), with ciprofloxacin effective in only 2 cases. Fungal isolates included 3 cases each of *Aspergillus fumigatus* and *Fusarium*, and 1 case of *Aspergillus niger* (Figure 1).

Among the 90 patients, all received medical treatment; 26.67% received both medical and surgical treatments, and 40 % received medical and non-surgical treatments. All patients were given topical medications, and 81 (90%) were also prescribed systemic drugs. Antiglaucoma and anti-inflammatory drugs were added as per requirement. Fortified antimicrobial drops were used in 35 patients (38.89%). Non-surgical management included BCLs in 8.9% of patients, cyanoacrylate glue with BCL in 32.2%, and therapeutic scraping in 3.3%. Cyanoacrylate glue was generally applied once, with some patients requiring multiple applications due to glue dislodgement. In 3 (3.3%) patients twice repetition and in 1 (1.1%) patient thrice repetition was required. Surgical interventions included AC wash/paracentesis (7.78%), intracameral antimicrobial injection (6.67%), tarsorrhaphy (18.89%), and tectonic/therapeutic keratoplasty (8.89%). Out of 8 cases (8.89%) which underwent therapeutic and/or tectonic penetrating keratoplasty (PK), 5 cases were of fungal etiology and 3 cases were bacterial keratitis. In the present study, no reinfection or graft infection had developed, 2 cases had central clarity in graft post-PK, and the rest cases resulted in opaque vascularized graft on final follow-up. Presenting visual acuity and final visual acuity are presented in Table 3.

Four patients were lost to follow-up. Among the remaining 86, one (1.16%) eye required evisceration due to auto-expulsion of intraocular contents secondary to large corneal perforation and two eyes (2.33%) became phthisical. Seventy-one eyes out of 86 (82.56%) had healed keratitis with variable corneal scarring (31 involving

Table 3: Presenting and final visual acuity in affected eyes

Visual acuity of the affected eye at the time of presentation		
Visual acuity (On Snellen's chart)	**Frequency of presenting visual acuity (%)**	**Frequency of final visual acuity (%)**
PL (Perception of light) negative	4 (4.44)	4 (4.44)
PR (Projection of rays) inaccurate	33 (36.67)	19 (21.11)
1/60–6/60	38 (42.22)	31 (34.44)
<6/36–6/60	1 (1.11)	8 (8.89)
6/36–6/12	8 (8.89)	19 (21.11)
6/9–6/6	6 (6.67)	5 (5.56)
Not applicable	-	4 (4.44) patients were lost to follow-up
Total	90 (100)	90 (100)

the visual axis, 40 not), 11 (12.79%) developed adherent leukoma, and one (1.16%) developed anterior staphyloma.

Among 86 eyes, 36 (41.86%) healed in over 6 weeks, another 36 in 4–6 weeks, 15.12% in 2–4 weeks, and only 1 eye in <2 weeks. There was a statistically significant relationship between healing duration and diagnosis type (P≤0.0001). Bacterial and fungal keratitis typically healed after 4 weeks, viral keratitis within 4 weeks, and mixed keratitis took over 6 weeks to heal.

DISCUSSION

In some tropical poor nations, corneal infections are the second leading cause of monocular blindness after untreated cataracts.[4-6] The World Health Organization (WHO) identifies cataracts, glaucoma, and corneal disorders as major causes of vision loss globally.[7-9]

In this study, microbial keratitis prevalence was higher in males than females, consistent with findings by Ranjini and Waddepally[10] and Gopinathan et al.[11] The most affected age group was 31–60 years due to greater outdoor exposure, echoing Srinivasan et al.'s findings.[12] Males were more commonly affected (67.80%) than females (32.20%), with a 2:1 ratio, similar to Bharathi et al.'s study[13] (65.1% males, 34.9% females), though another study reported a 1:1 ratio.[14]

In the present study, 55.6% of patients were from urban areas and 44.4% from rural areas. The occupations included laborers (27.8%), housewives/retired people (24.4%), farmers (18.9%), and students (1.11%). Jose et al.[15] similarly reported higher keratitis rates among housewives (27.41%) and manual laborers (20.74%) compared to farmers (11.85%). Sedhu et al.[16] also found more housewives affected (21%) than farmers (16.9%). The high risk of corneal trauma in our region is due to exposure to foreign bodies, such as sand, stone, cement, and metal pieces with manual labor being a major occupation.[16] Education and practice of protective measures for eye injuries should be reinforced in potential candidates and likely fields.

The present study observed 55.6% of the cases from the lower socioeconomic class, and 44.4% from the middle class, echoing the findings of Sharma et al.[17] Frequency of presenting symptoms found in the present study was comparable with the study done by Jose et al.[15]

Rautaraya et al.[18] observed most cases of fungal keratitis, followed by bacterial and viral, similar to the infective etiology observed in the present study.

Certain clinical characteristics of corneal ulcers may suggest specific pathogens, but reliable diagnosis requires microbiological investigations. In the present study, corneal scraping was positive in only 5 patients out of 62 (8.06%) g and KOH stains each. In contrast, Upadhyay et al.,[4] and Dunlop et al.,[19] reported its effectiveness as 80% and 81.7%, respectively.

Gram stain results matched culture reports in 38.46% of cases in the present study, compared to 63% in Williams et al.'s study.[20] The correlativity of KOH mount with culture reports was 38.46% in the present study, contrasting with Vajpayee et al.'s[21] finding of 94% correlation. High negative smear and culture results may be attributed to prior use of antimicrobial agents elsewhere and advanced cases with late presentation. This warrants the earliest microbiological tests or timely referral to the centers which are equipped for microbiological investigation and management of severe cases of microbial keratitis. The increasing resistance of common pathogens to conventional antimicrobial agents and limited availability of the commercial formulation of higher antimicrobial preparations warn us against the injudicious use of antimicrobials which are costly as well for the economic profile of the affected group of patients.[22]

In this study, only 1 (1.16%) eye required evisceration, 1 eye (1.16%) developed anterior staphyloma, 2 eyes (2.33%) became phthisical., while the rest (82 cases) healed with corneal opacity and adherent leukoma indicating successful treatment. Duration of healing significantly correlated with the type of infective agent (P≤0.0001). However, despite

proper therapy, nearly 59.99% of patients had vision worse than 6/60. In other studies, time of presentation had a greater bearing on final outcomes. Chidambaram et al.[23] reported low vision in 34% of cases. This could be because the patients did not present right away. Research by Hooi and Hooi[24] and Laspina et al.[25] also concluded that the length of time to presentation following the beginning of ocular symptoms influences the outcome for vision. In addition, *Aspergillus* species isolates and hypopyon at presentation were also linked to worse outcomes.[23]

Optical keratoplasty was advised for patients with corneal scarring obscuring visual improvement due to the same in the current study.

In developing countries, corneal blindness has a significant impact in terms of social, psychological, and economical aspects in the life of the affected individual. The present research provides targeted data from a tertiary eye care center of Western India which might help to know the local demographic prevalence of microorganisms and their appropriate management. This research information may add to the existing knowledge to address the various aspects of microbial keratitis to eventually win the battle against corneal blindness due to the same.

Limitations of the study

The present study has limitations of small sample size and lack of use of higher diagnostic modalities such as confocal microscopy or polymerase chain reaction analysis for microbiological workup due to lack of availability and access to these facilities, however, this is the only study in the western part of the country with information emphasizing on demographic aspects, etiological diagnosis, proper microbiological workup and showing its various outcome over the course of the disease.

CONCLUSION

Infectious keratitis following trauma is common, particularly in men with the most active years of their life. The nature of the organism and time of presentation significantly impact outcomes, especially in large ulcers often caused by bacteria or fungus leading to vision impairment or loss. Thorough clinical examination is of diagnostic value but subjecting the corneal ulcer to microbiological assessment is crucial for instituting appropriate treatment, especially in non-responding cases. Early detection and timely management of infective keratitis are advocated to decrease the overall burden of corneal blindness due to the same in developing countries.

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