Original Article

Epidemiological and microbiological profile of infective keratitis in Ahmedabad

Aarti Tewari, Nidhi Sood, Mahendra M Vegad, Dipak C Mehta

Context: Study of patients attending tertiary care ophthalmology institute at Ahmedabad. **Aims**: To study the microbiological etiology and epidemiological factors associated with suppurative keratitis. **Settings and Design**: A total of 150 corneal scrapings were evaluated from patients presenting with corneal ulcers at a tertiary ophthalmology center, Ahmedabad from July 2007 to June 2008. **Materials and Methods**: Scrapings were subjected to Gram stain, potassium hydroxide preparation and culture for bacterial and fungal pathogens. Socio-demographic data and risk factors were recorded. **Results**: Ninety percent (135/150) people with corneal ulcers had trauma as predisposing factor for keratitis. Trauma due to wooden objects was the leading cause (46/135) followed by vegetable matter and stone injury (23/135). Microbial etiology was established in 59.3% (89/150) of scrapings. Out of 89 positive isolates, 65.1% (58/89) were bacterial while 34.9% (31/89) were fungal. Among the bacterial isolates, 60.3% (35/58) were Gram-positive cocci while 39.7% (23/58) were Gram-negative bacilli. The most common bacterial isolate was *Staphylococus aureus* (32.7%, 19/58) followed by coagulase-negative Staphylococci (25.8%, 15/58) and *Pseudomonas* (18.9%, 11/58). Among the 31 fungal pathogens, *Aspergillus* species was the most common (35.4%11/31), followed by *Fusarium* species (22.5%, 7/31). **Conclusion**: Trauma with wooden material is the most common predisposing factor for suppurative keratitis. Males were more affected than females. Bacterial ulcers were more common than fungal in areas in and around Ahmedabad. *Staphylococcus aureus* and *Aspergillus* were the commonest bacterial and fungal isolates respectively. Geographical variation persists in microbial etiology of suppurative keratitis.

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Keratitis is the term applied for inflammations of the cornea.[1] Corneal infections are known to be the second most significant cause of monocular blindness rated after unoperated cataract in some developing nations in particular and in the tropics in general.[2] Microbial keratitis is a common, potentially vision-threatening ocular infection that may be caused by bacteria, fungi, viruses or parasites. Emphasizing the importance of corneal ulceration [Fig. 1] as an important cause of visual loss, many studies have reported the prevalence of microbial pathogens and identified the risk factors predisposing a population to corneal infection in India[2-9] and abroad.[10,11]

The etiological and epidemiological patterns of corneal ulceration have been found to vary with the patient population, health of the cornea, geographic location and climate, and also tends to vary over time. Hence, an understanding of the epidemiological features, risk factors and etiological agents that occur in a specific region are important in rapid recognition, timely institution of therapy, optimal management and prevention of this disease. In order to start specific therapy, it is necessary to do meticulous laboratory investigations, and this includes microscopy and culture of corneal scrapings for identification of the microbial agent.

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There are several preceding studies establishing the microbial etiology of suppurative keratitis from various parts of India. However, little data is found in the technical literature from Ahmedabad in particular. Since microbial etiology varies geographically, the present study acquires significance on the backdrop of similar articles for other regions of India. The purpose of the present study was to determine microbial etiology of suppurative keratitis at a tertiary healthcare center in the city of Ahmedabad, Gujarat, India and to identify the risk factors predisposing to corneal infections.

Materials and Methods

The study was conducted on 150 patients attending Ophthalmology Institute, a tertiary care center located in Ahmedabad. The samples were collected from July 2007 to June 2008.

Ulcers suspected by the clinician to be of microbial etiology were included in the study. The following categories of ulcers were excluded: suspected viral ulcers, healing ulcers, Mooren's ulcer, marginal keratitis, interstitial keratitis and atheromatous ulcers.

All patients were examined under slit-lamp bio-microscope by an ophthalmologist. A complete slit-lamp examination was performed. Corneal scraping was performed under strict aseptic conditions by an ophthalmologist using a sterile Bard-Parker blade (No. 15).[2,3,6,7,9] Prior to obtaining the scraping, preservative-free 4% lignocaine hydrochloride was instilled. Subsequently, material was obtained from scraping of the leading edge and base of each ulcer. The material was inoculated onto blood agar, Chocolate agar, Nutrient agar and

Sabouraud dextrose agar (SDA) and smeared onto two slides—one for Gram stain and other for 10% KOH wet preparation[2,3,6,7] [Fig. 2]. The material was also inoculated in glucose broth.[3,8] All laboratory diagnosis was performed using standard protocols. All the inoculated media i.e. blood agar, Chocolate agar, Nutrient agar and glucose broth were incubated at 37° C for 24 h and if no growth was obtained, then they were incubated for a further 24 h. The criteria adopted for microbial evaluation was that the sample would be considered positive if any one of the following were met.[3,7-9]

a. The growth of the same organism was demonstrated on two or more media.
b. The same organism was grown from repeated scrapings.
c. It was consistent with clinical signs.
d. Smear results confirmed the finding from cultures.

The specific identification of bacterial colonies was performed on the basis of Gram staining by microscopy and biochemical properties using standard laboratory criteria. The inoculated SDA media were incubated at 27° C and were examined daily until three weeks for growth.[2,6-8] Fungi were identified by their colony characteristics on SDA and by the morphological appearance of the hyphae and spores in lacto phenol cotton blue stain.[2,6,7]

Results

A total of 150 patients with the clinical diagnosis of corneal ulcer with or without hypopyon were enrolled for this study. Epidemiological characteristics of the population are given in Table 1. A maximum of patients were from the age group 21–40 years followed by patients in the age-group 41–60 years [Table 1]. There was male predominance, which is evident from the table. Seventy percent (105 out of 150) patients hailed from urban areas. The occupation profile of the study group mainly consisted of housewives (21.3%), followed by farmers (16.6%), laborers (14.6%) and carpenters (11.3%).

Predisposing factors for keratitis have been listed in Table 2.

The most common predisposing factor observed in the study responsible for keratitis was trauma which was seen in 90% (135/150) of the patients. Among the reasons for corneal ulcers, trauma due to wooden objects (46 patients) was the leading cause followed by vegetable matter (24 patients) and stone injury (23 patients). Since there were no patients using contact lenses, contact lens infection was not a predisposing factor.

Microbial (bacterial and fungal) etiology was seen in 89/150 patients presenting with corneal ulcers. There was no organism isolated in rest of the 61 samples. Bacteria were isolated in 58 samples and in 31 samples fungal growth was isolated. The details of positive microbial etiology are given in Table 3.

Out of the total number of positive bacterial cultures, 35(60%) were Gram-positive cocci (GPC) and 23(40%) were Gram-negative bacilli (GNB). No Gram-positive bacilli were observed. *Staphylococcus aureus* was the most common GPC followed by coagulase-negative *Staphylococcus* and *Micrococcus*. Among GNB, the most common was *Pseudomonas* spp. followed by *Klebsiella* sp. *Enterobacter*, *E. coli*. *Proteus* and *Acinetobacter* were among the other Gram-negative isolates. A summary of the bacterial pathogens isolated during the study is shown in Table 4.

Fungal growths were observed in a total of 31 samples. A majority of the fungal isolates were filamentous fungi while a few were yeast. Four fungal growths could not be identified. Table 5 shows the detailed distribution of the samples pertaining to fungal growth.

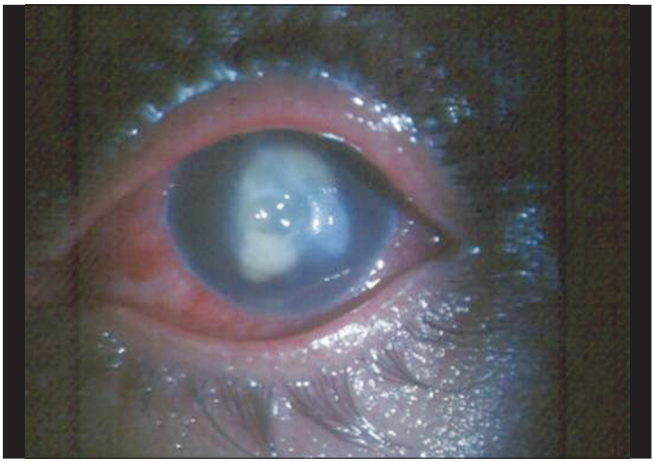

Figure 1: Photograph of eye showing suppurative corneal ulcer

Figure 2: Photograph showing *Aspergillus* growth on Sabouraud Dextrose Agar

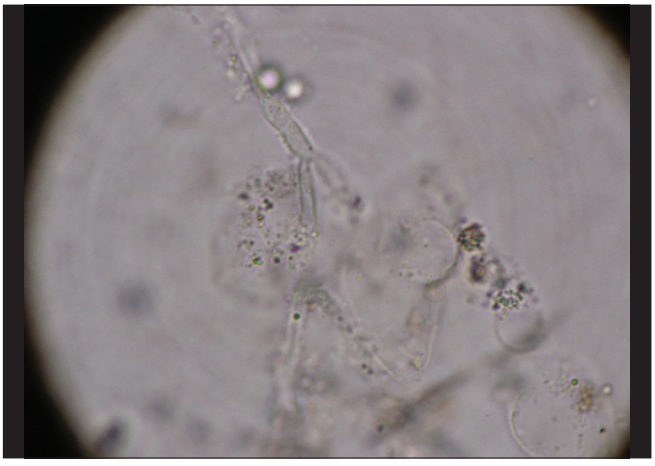

Figure 3: Photograph showing fungal hyphae in KOH mount

Table 1: Epidemiological characteristics of patients

Demographics	Indicator	No. (%)
Age (In years)	21–40	79 (52.6)
	41–60	59 (39.3)
	<20	7 (4.6)
	> 60	5 (3.3)
Sex	Male	102 (68)
	Female	48 (32)
Residence	Urban	104 (70)
	Rural	46 (30)
Occupation	Housewife	32 (21.3)
	Farmer	25 (16.6)
	Laborer	22(14.6)
	Carpenter	17(11.3)
	Workers/Merchants	30 (19.9)
	Other	25 (16.6)

Table 2: Predisposing factors in patients for keratitis

Predisposing factors	Particulars	Number (%)
Trauma	Corneal trauma	135 (90)
Corticosteroid therapy	Wooden Object	46
Diabetes	Vegetable matter	24
Preexisting Ocular diseases	Stone	23
Post-surgical	Unknown foreign	9
	body	8
	Dust	8
	Iron Particle	6
	Mud	5
	Insect	5
	Cow tail	1
	Acid	8 (5.33)
		4 (2.67)
		1 (0.67)
		1 (0.67)

Table 3: Causative micro-organisms responsible for corneal ulcers in Ahmedabad

Type of micro-organism	Number (%)
Total Isolates	89 (59.3)
Bacterial Isolates	58 (65.1)
Fungal growth	31 (34.9)
No organism isolated	61 (40.6)

The predominant fungal species was *Aspergillus* spp. [Fig. 3] followed by *Fusarium* spp. In a relatively smaller incidence *Curvularia* spp. and *Candida* spp. were also isolated. The sample distribution with respect to fungal pathogens isolated is given in Table 6.

A comparison between direct microscopy and culture positivity is depicted in Table 7. Assuming the culture method to be standard for bacteria, the smear sensitivity corresponds to 64.4% and specificity to 93.8%. For fungi, smear sensitivity is 75.6% while the specificity is 100%.

Antibiotic sensitivity patterns of Gram-positive cocci and Gram-negative bacilli are shown in Tables 8 and 9 respectively.

Table 5: Fungal pathogens isolated from 31 culture-positive fungal keratitis cases

Type of Fungus	Number (%)
Filamentous	23 (74.1)
Yeast	4 (12.9)
Unidentified	4 (12.9)

Table 4: Bacterial pathogens isolated from 58 culture-positive bacterial keratitis cases

Name of the bacterial isolates	Number (%)
Total Gram-positive cocci	35 (60.3)
Staphylococcus aureus	19 (32.7)
Coagulase-negative Staphylococci	15 (25.8)
Micrococcus	1 (1.7)
Total Gram-negative bacilli	23 (39.7)
Pseudomonas spp.	11 (18.9)
Klebsiella spp.	4 (6.8)
Enterobacter spp.	3 (5.1)
E. coli	3 (5.1)
Proteus spp.	1 (1.7)
Acinetobacter spp.	1 (1.7)

Table 6: List of fungal pathogens isolated from 31 culture-positive fungal keratitis

Name of the fungal isolates	Number (%)
Aspergillus spp.	11 (35.4)
Fusarium spp.	7 (22.5)
Curvularia spp.	5 (16.1)
Candida spp.	4 (12.9)
Unidentified	4(12.9)

Discussion

At birth the eyes are sterile but soon become invaded by microorganisms. The interior structures remain sterile. Almost any bacterial species can infect the cornea if the integrity of the natural anatomic barriers or defense mechanisms is compromised.[1]

In the present study, corneal ulceration was seen in all age groups with preponderance among physically active adults; higher in males (68%) than in females (32%) as observed in Kashmir (65%),[5] South India (65%[6] and 56.7%[9]) and Ghana (69.3%).[10] Trauma was the most common predisposing factor (90% cases) matching other studies from Gangetic West Bengal (82.9%),[2] South India (92.15%)[6] and Madurai, India (65.4%)[7] but differing in Ghana (39%).[10] Corneal injury with wooden objects was the most common followed by vegetable matter and stone. However Basak *et al.*, found vegetative matter, chiefly paddy and jute, as the principal traumatic agent for the development of keratitis.[2] There was a higher incidence of keratitis among housewives (21.3%), followed by farmers (16.6%), laborers (14.6%) and carpenters (10.6%) in this study. Injury with wooden objects and vegetable matter representing farmers coincides well with other studies.[2,4,5,7] This implies a significant association between occupation and infectious

keratitis.

The observations of this study are compared in Table 10 to those in various studies. Total microbial etiology of 59.3% compares well.[2,3,5,7,10] Bacterial keratitis of 38% compares well with other studies across India[2,3,5,7,9,11] but was rather high in Nepal[3] though relatively low in Ghana.[11] Bacteria are responsible for a larger proportion of corneal ulceration in temperate climates than in tropical regions such as South India.[10]

The incidence of Gram-positive cocci (60% of bacterial etiology, 23% of total corneal ulcers) coincides with other studies.[2,3,7,9] Predominance of *Staphyloccoccus aureus* (54% of total Gram-positive cocci isolates) coincides well with the study of Gangetic West Bengal.[2] Coagulase-negative *Staphylococcus* that made up 43% of the total Gram-positive cocci was also a predominant pathogen in a study from Ghana.[10]

However, in other studies, *Streptococcus pneumoniae* was the predominant species *viz.* Kashmir[5] and South India.[3,7,9] but could not be isolated in this study which could be due to regional variation in the occurrence of corneal pathogens. Another significant reason could be that lacrimal sac pathology was not observed as a major risk factor in this study.

Gram-positive bacilli were not observed in the present study. This differs from various studies which have demonstrated a 4.0-12.5% incidence.[2,3,7,9,10] Gram-negative bacilli (39.7% of total bacterial pathogens) also correlates well with other studies.[2,3,5,7]

Pseudomonas was the most common Gram-negative bacteria in line with several studies[2,3,7,9] but was low in Kashmir.[5] *Klebsiella* (6.8%) and *Enterobacter* (5.1%) match the study from Kashmir.[5] The study from South India showed only 1.92% of *Enterobacter*.[3]

These reports vis-à-vis the findings of the present study show that there is a distinct pattern of geographical variation in the microbial etiology.

From the antibiotic sensitivity pattern, it appears that approximately 50% patients with bacterial etiology would have responded to Ciprofloxacin monotherapy. Instead, if fortified therapy was employed, a majority of patients would respond. The authors feel that the number of samples studied is relatively small to make a definite recommendation. However, Levofloxacin may be used as first-line therapy in Ahmedabad.

Fungal growth seen in 20% of total corneal ulcers and in 34.8% of total culture positives is borne out by other studies.[3,4,6] Hagan quotes similar incidence from south Florida, Bangladesh but low from Nepal.[10] Studies from West Bengal,

Table 7: Comparison of direct smear positivity and culture positivity

Organisms	Smear Positive Culture Negative	Smear Negative Culture Positive	Smear Positive Culture Positive	Smear Negative Culture Negative
Bacteria	6	32	26	86
Fungi	0	10	21	119

Table 8: Antibiotic sensitivity patterns of Gram-positive cocci (Isolates)

Bacteria (number)	Sensitive/Resistant											
	PG	Oxacillin	Cipro	Levo	Linezolid	Vanco	Clinda	Tetra	Chloram	Azithro	Cotri	Genta
S. aureus (19)	11/8	19/0	10/9	19/0	19/0	19/0	19/0	10/9	5/14	19/0	13/6	9/10
Coagulase-negative Staphylococci (15)	12/3	15/0	10/5	15/0	15/0	15/0	15/0	11/4	10/5	15/0	8/7	12/3
Micrococci (1)	0/1	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0

PG: Penicillin G, Cipro: Ciprofloxacin, Levo: Levofloxacin, Vanco: Vancomycin, Clinda: Clindamicin, Tetra: Tetracycline, Chloram: Chloramphenicol, Azithro: Azithromicin, Cotri: Cotrimoxazole, Genta: Gentamicin

Table 9: Antibiotic sensitivity patterns of Gram-negative bacilli

Bacteria (number)	Sensitive/Resistant											
	Cipro	Levo	Tetra	Chloram	Aztreo	Pip -Taz	Cotri	Mero	Ceftaz	Genta	Amik	Cefotax
Pseudomonas (11)	6/5	9/2	4/7	6/5	11/0	11/0	3/8	11/0	11/0	6/5	11/0	10/1
	Cipro	Levo	Tetra	Chloram	Genta	Ampi/sul	Cefo/sul	Mero	Cefurox	Ceftriax	Amik	Cotri
Klebsiella (4)	2/2	4/0	2/2	2/2	2/2	4/0	4/0	4/0	0/4	4/0	4/0	0/4
Enterobacter (3)	2/1	3/0	2/1	2/1	3/0	3/0	3/0	3/0	1/2	3/0	3/0	3/0
E. coli (3)	2/1	2/1	2/1	2/1	3/0	3/0	3/0	3/0	2/1	3/0	3/0	2/1
Proteus spp.(1)	0/1	1/0	0/1	1/0	0/1	1/0	1/0	1/0	1/0	1/0	1/0	1/0
Acinetobacter (1)	0/1	1/0	0/1	1/0	0/1	1/0	1/0	1/0	0/1	1/0	1/0	1/0

*Cipro: Ciprofloxacin, Levo: Levofloxacin, Vanco: Vancomycin, Clinda: Clindamicin, Tetra: Tetracycline, Chloram: Chloramphenicol, Azithro: Azithromicin, Cotri: Cotrimoxazole, Genta: Gentamicin, Mero: Meropenem, Ceftaz: Ceftazidime, Amik: Amikacin, Cefotax: Cefotaxime, Cefurox: Cefuroxime, Cefo/sulb: Cefoperazone/sulbactum, Ampi/Sulb: Ampicillin/sulbactum, Aztreo: Aztreonam, Ceftriax: Ceftriaxone

Table 10: Comparison with other studies across India and the world

	Present Study	Basak *et al.*[2]	Bharti *et al.*[3]	Verenkar *et al.*[4]	Bashir *et al.*[5]	Bharti *et al.*[6]	Srinivasan *et al.*[7]	Bharti *et al.*[9]	Hagan *et al.*[10]	Leck *et al.*[11]
Culture positivity	59.3	67.7	69.5		52.5		68.4		57.3	
Bacterial Keratitis	38	24.8	34.9, 63.2% in Nepal		40		32.3	32.7	25	29.3% in South India and 13.8% in Ghana
Gram-positive Cocci	60% of bacterial etiology and 23% of all corneal ulcers	71	65				79	65		
Gram-negative Bacilli	39.7	28.2	25.5		31.2, 25.4 (Ghana)		21			
Gram-positive Bacilli	NIL	+	4.15				12.5 *C. xerosis*	4.96 (Bacillus and Corynebacteria)	4.7 Corynebacterial	
S. aureus	54% of total GPC	42.6					5.4			
Cogulase-negative Staphylococci	43% of total GPC	7	16.77				10.2	17.4	Predominant 22.2	
S. pneumoniae	NIL	9.4	41.85		High		44.3	37.5	12.6	
Pseudomonas	36% of GNB and 18.9% of total bacterial Isolates	21.1	21.24		12.5		14.4	18.03		52.5
Fungal Keratitis	20% of all corneal ulcers; 34.8% of total culture +ve	62.7	32	38.9%		34.4	57	34.4	35% South Florida, 36% Ban-gladesh, 17.6% Nepal, 46.8% Ghana	37.6% in Ghana and 44% in South India
Aspergillus	35.4% of total fungal etiology	59.8	24.3	61%	40	26	16.1		15.3%	High (*Fusarium* and *Aspergillus*) 61% of all fungal infection and 83% of all fungal isolates
Fusarium	22.5% of total fungal etiology	21.2	45.85	12.5%		42.82	47.1		High 52.3% of fungal etiology	High
Candida	2.7% of all samples	1.1	NIL	NIL		NIL (reports high incidence in other parts of world)	NIL (reports high incidence in other parts of world)		0.7% of all culture +ve and 1.5% of fungal etiology	

All figures indicates in percentage

South India and Ghana showed a relatively high percentage of fungal etiology.[2,7,10,11]

Aspergillus species (35.4% of total fungal etiology) in the present study matches other studies.[3,5,6] Incidence is markedly high in Western[4] and Eastern India[2] but low in Madurai.[7]

It is noteworthy that as opposed to our findings, in South India, *Fusarium* was the most isolated fungal species.[3,6,7,10] This again may be due to regional variations. *Fusarium* incidence of 22.5% was similar in Eastern India.[2] However, it was predominant in South India and Ghana.[3,6,7,10,11] Low incidence was noted in Western India.[4] Most of these fungal growths were seen in farmers because of injury with vegetative matter.

Yeast infection was relatively higher (2.7%) in this study as compared to others. It is not readily apparent why this is so. However, two of these patients had history of diabetes.

In conclusion, this study of 150 cases carried out at Ahmedabad, India shows that trauma due to wooden objects or vegetative matter appears to be the most common predisposing factor in causing suppurative keratitis. Bacteria are found to be responsible to a greater extent than fungi with *Staphylococcus aureus* being the predominant bacterial pathogen and *Aspergillus* being the predominant fungal agent. While the findings of this study show similarities with other studies from across India and some from abroad, important differences are also observed. The microbial etiology thus exhibits regional variations and this aspect must be kept in mind while planning the management of suppurative keratitis. From the limited samples studied, Levofloxacin monotherapy may be considered as a first line of treatment in Ahmedabad.

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