Original Article

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Epidemiological and Microbiological Profile of Infective Keratitis at a Tertiary Care Centre in Central Zone of Kerala, India

Microbiology Section

REENA ANIE JOSE, VIJAYAKUMAR S, RAJINI KC, PRITHI NAIR K, REENA JOHN

ABSTRACT

Introduction: Infective keratitis is a potentially vision threatening condition caused by various microbial etiology. Due to high virulence and rapid progression of many etiological agents, any corneal inflammation should be considered a potential threat to vision, requiring immediate evaluation and treatment. Evaluation of epidemiological and microbiological profile of infective keratitis of a region can significantly contribute towards appropriate management of this ophthalmic emergency.

Aim: To study the bacterial and fungal etiological agents and the epidemiological factors associated with infective keratitis in patients attending the Ophthalmology Department of Government Medical College, Thrissur, Kerala, India.

Settings and Study Design: In this cross-sectional study, a total of 135 corneal scrapings were evaluated from patients presenting with corneal ulcers from December 2012 to May 2014.

Materials and Methods: Scrapings were subjected to Gram staining, KOH wet mount and culture for bacterial and fungal pathogens. Isolates were identified by conventional methods and their antibiotic susceptibility pattern was established.

Statistical Analysis: The results were analysed by mean, Chi-square test and Fisher's exact test.

Results: Corneal trauma was the main risk factor associated with infective keratitis (59.3%) and vegetative matter was the most common trauma type observed (36.3%). Culture was positive in 35.56% of cases, of which 18.52% were bacterial and 17.04% were fungal. Gram positive cocci were the predominant isolates (64%). *Staphylococcus epidermidis* was the most common bacterial isolate (32%), followed by *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* (16% each). *Fusarium* species was the most common fungal isolate (39.13%), followed by *Curvularia* species and *Aspergillus* species (13.04%). Unidentified dematiaceous fungi accounted for 17.39%. Sensitivity of Gram stain in bacterial and fungal detection was 56% and 39%, while specificity was 95% and 96% respectively. Sensitivity and specificity of KOH wet mount examination in the detection of fungal elements was observed as 82% and 91% respectively.

Conclusion: Our study observed corneal injury as the main risk factor associated with infective keratitis. Males were more affected than females. *Staphylococcus epidermidis* and *Fusarium* species were the commonest bacterial and fungal isolates respectively. Geographical variation persists in microbial etiology of infective keratitis.

Keywords: Corneal injury, Gram stain, KOH wet mount

INTRODUCTION

The term "keratitis" refers to "inflammation of the cornea". It is caused by infectious organisms or non-infectious agents. Infective keratitis is a potentially sight-threatening condition. Most organisms gain access via corneal epithelial defects created by mechanical, thermal or chemical injury. Such risk factors may vary with occupation. The epidemiological pattern of corneal ulceration varies significantly from region to region. The annual incidence of corneal ulceration in Madurai district in south India was 113 per 1 lakh people [1]. The present study has been focused on identifying the bacterial and fungal etiology of infective keratitis and the epidemiological trends of the disease during the period December 2012 to May 2014 in central region of Kerala.

MATERIALS AND METHODS

In this cross-sectional study, a total of 135 clinically diagnosed cases of infective keratitis, who attended the Ophthalmology Out-Patients Department of Government Medical College, Thrissur, India were studied over a period of eighteen months.

The present study was conducted after obtaining informed patient consent and approval by Institutional Ethical Committee/Institutional Review Board.

Inclusion Criteria: Clinically diagnosed cases of infective keratitis irrespective of age and prior treatment were included in the study.

Exclusion Criteria: Patients with suspected or confirmed viral or protozoal keratitis were excluded.

A detailed history of the disease with special reference to ocular injury, other predisposing local or systemic factors and history of medication prior to ophthalmic consultation were recorded. The specimens were collected by an ophthalmologist. The eye was anaesthetized with lignocaine drops. The surrounding skin was wiped with sterile gauze soaked in sterile saline. The ulcers were scraped thoroughly but gently with sterile No.23 gauge needle. Corneal scrapings were streaked directly on to a blood agar medium and Sabouraud's Dextrose agar medium without anti-fungal agents by the C streak method. Materials were also smeared on two clean, labelled microscopic glass slides. The smears were subjected to wet mount preparation with 10% potassium hydroxide (KOH) for the detection of fungal elements and Gram's staining for detection of pus cells, bacteria and fungal forms. Bacterial and fungal growth were identified by standard laboratory procedures and the antibiotic sensitivity testing was performed by the disk diffusion method as per CLSI guidelines. Statistical significance of the study was determined by Chi-square test and Fisher's exact test.

RESULTS

A total of 135 cases with clinical diagnosis of infective keratitis were studied during a period of eighteen months. Epidemiological characteristics of the patients are enrolled in [Table/Fig-1]. Mean age was 47.83±19.55 (ranging from 6 years to 85 years). Maximum number of patients were in the age group 40-59 years [Table/Fig-1]. Males were predominant (89/135, 66%) . With regard to occupation, 27.41% (37/135) of the patients with infective keratitis were house wives (involved in outdoor activities in their home) and 20.74% (28/135) were manual laborers.

Ocular trauma was present in 59.26% (80/135) of the patients. Nature of the material that caused corneal injury is listed in [Table/Fig-2]. Injury with vegetative matter was the most common type (29/80 cases) followed by injury with sand/dust (14/80 cases).

Demographics	Indicator	Number (%)
Age (in years)	20-39	38 (28.15)
	40-59	45 (33.33)
	60-79	36 (26.67)
	<20	9 (6.67)
	>80	7 (5.18)
Gender	Male	89 (66)
	Female	46 (34)
Occupation	House wives	37 (27.41)
	Farmers	16 (11.85)
	Manual Labourers	28 (20.74)
	Welders, Blacksmith	7 (5.19)
	Students	11 (8.15)
	Drivers	11 (8.15)
	Others	25 (18.51)

[Table/Fig-1]: Epidemiological characteristics of patients.

Material	Number (%)
Vegetative Matter	29 (36.25)
Sand/Dust	14 (17.5)
Broomstick/Splinter	11 (13.75)
Finger Nail	7 (8.75)
Mud	5 (6.25)
Stone Piece	4 (5)
Metal Piece	4 (5)
Cow Tail	2 (2.5)
Lead Pencil	2 (2.5)
Cloth	2 (2.5)

[Table/Fig-2]: The nature of material that caused corneal injury (in 80 cases).

Microbial (bacterial and fungal) etiology was seen in 48 out of 135 patients presented with corneal ulcers. Bacteria was isolated in 25 samples (18.52%), while fungi was isolated in 23 (17.04%).

Apart from ocular trauma, other ocular and systemic factors which contributed to infective keratitis are listed in [Table/Fig-3].

Bacterial and fungal pathogens isolated from corneal ulcer are listed in [Table/Fig-4]. Out of the 25 bacterial isolates, 16 were Gram positive cocci (64%) and 9 were Gram negative bacilli (36%). *Staphylococcus epidermidis* was the most common bacterial isolate (8/25). *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* were isolated from 4 cases each.

Fusarium species was the most common fungal isolate (9/23 samples). Of the fungal isolates, 14 were hyaline filamentous fungi, while 9 were dematiaceous. No yeast forms were isolated in this study.

During the period (2012-13'), maximum number of patients

Factors	Bacterial	Fungal	Culture negative	Total (%)
Trauma	18	21	41	80 (59.26)
Previous Keratoplasty	1	0	0	1 (0.74)
Chronic Dacryocystitis	3	1	7	11 (8.15)
Dry Eyes	4	2	4	10 (7.41)
Trichiasis	1	2	0	3 (2.22)
Ectropion	1	1	1	3 (2.22)
Systemic Factors				
• Diabetes Mellitus	12	11	23	46 (34.07)

[Table/Fig-3]: Predisposing ocular and systemic factors in infective keratitis (135 cases).

Isolates	No. of samples	Percentage (%)
Bacterial	25	18.52
Gram Positive Cocci	16	64
• *Streptococcus pneumoniae*	4	16
• *Enterococcus faecalis*	1	4
• *Staphylococcus aureus*	3	12
• *Staphylococcus epidermidis*	8	32
Gram Negative Bacilli	9	36
• *Pseudomonas aeruginosa*	4	16
• *Chromobacterium violaceum*	1	4
• *Escherichia coli*	1	4
• *Klebsiella pneumoniae*	1	4
• *Enterobacter aerogenes*	1	4
• *Acinetobacter baumannii*	1	4
Fungal	23	17.04
Fusarium species	9	39.13
• *F. solani*	5	21.74
• *F. oxysporum*	4	17.39
Curvularia species	3	13.04
Aspergillus species	3	13.04
• *A. flavus*	2	8.69
• *A. fumigatus*	1	4.35
Rhizopus species	1	4.35
Penicillium species	1	4.35
Lasiodiplodia theobromae	1	4.35
Chaetomium species	1	4.35
Unidentified dematiaceous fungi	4	17.39

[Table/Fig-4]: List of bacterial and fungal isolates from 48 samples of infective keratitis.

(35/ 91 patients, 38.46%) were presented in the winter season. Fungal keratitis was found to be more common in the winter season, while bacterial keratitis in the summer season [Table/Fig-5].

Of the 87 culture negative cases, 32 were receiving topical antibiotics, 12 were receiving both topical antibiotics and antifungals. About 2.96% of the total patients were on topical steroid and 22.22% were on traditional medicines [Table/Fig-6].Traditional medicines including Ayurvedic preparations, herbal medicines, breast milk were used by 22.22% of the patients in the present study.

Season	No. of cases	Bacterial isolates	Fungal isolates
Winter (2012-13)	35	3	5
Summer (2013)	26	8	2
Monsoon (2013)	30	5	5
Winter (2013-14)	23	3	7
Summer (2014)	21	6	4
Total	135	25	23

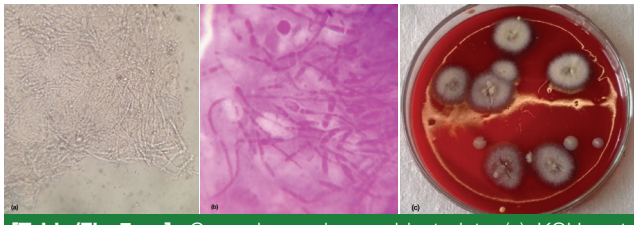
[Table/Fig-5]: Seasonal variation in the occurrence of infective keratitis.

Sensitivity and specificity of corneal scraping smears in the detection of fungal elements in KOH wet mount examination with culture as the gold standard were 82.61% and 91.07% respectively and that of Gram staining was calculated to be 39.13% and 96.43% respectively [Table/Fig-7a-c]. Sensitivity and specificity of Gram staining for bacteria with culture as the gold standard was found to be 56% and 95.45% respectively. Details are summarized in [Table/Fig-8].

Previous treatment received	Culture negative	Bacterial positive	Fungal positive	Total (%)
Topical antibiotic	32	6	13	51 (37.78)
Topical antibiotic and antifungal	12	3	3	18 (13.33)
Topical steroid	0	2	2	4 (2.96)
Traditional medicines	21	6	3	30 (22.22)
No treatment	22	8	2	32 (23.71)
Total	87	25	23	135

[Table/Fig-6]: Treatment received by patients prior to presentation to the hospital.

Number of bacterial isolates susceptible to the tested antibiotics is listed in [Table/Fig-9,10].



[Table/Fig-7a-c]: Corneal scrapings subjected to (a) KOH wet mount; (b) Gram staining; (c) Culture in Blood agar medium: *Fusarium* species grown along the C streak lines.

Investigation	Results	Presence of microbial growth		Sensitivity (%)	Specificity (%)
		Positive	Negative		
Detection of fungal elements in KOH wet mount	Positive	19	10	82.61	91.07
	Negative	4	102		
Detection of fungal hyphae in Gram stained smears	Positive	9	4	39.13	96.43
	Negative	14	108		
Detection of bacteria in Gram stained smears	Positive	14	5	56	95.45
	Negative	11	105		

[Table/Fig-8]: Correlation between direct microscopic (10% KOH wet mount and Gram stained smear) diagnosis and culture based diagnosis (in 135 samples).

Name of the organism	Number of isolates (n=16)	Penicillin	Cefoxitin	Erythro-mycin	Clinda-mycin	Cotri-moxazole	Gentamicin	Ampicillin
S. epidermidis	8	2	8	1	7	6	2	NT
S. aureus	3	0	3	1	2	3	1	NT
Strep. pneumoniae	4	4	NT	3	NT	NT	NT	4
Enterococcus faecalis	1	NT	NT	NT	NT	NT	1	1

[Table/Fig-9]: Number of isolates sensitive to the antibiotics tested in each Gram positive organism.
*(NT – Not tested)

Name of the organism	Number of isolates (n=9)	Genta-micin	Amika-cin	Cipro-floxacin	Ceftazidime	Piperacillin-Tazobactam	Imi-penem	Cefe-pime	Ampi-cillin	Cephe-lexin	Ceftria-xone	Cotri-moxazole
Pseudomonas aeruginosa	4	3	4	3	2	3	4	3	NT	NT	NT	NT
Chromobacterium violaceum	1	1	1	1	0	1	1	1	NT	NT	NT	NT
E. coli	1	0	1	0	NT	1	0	1	0	0	0	0
Klebsiella pneumoniae	1	1	1	1	NT	1	1	1	0	1	1	1
Enterobacter aerogenes	1	1	1	1	NT	1	1	1	0	0	1	1
Acinetobacter baumannii	1	0	0	0	NT	0	1	0	0	0	0	0

[Table/Fig-10]: Number of isolates sensitive to the antibiotics tested in each Gram negative organism.
*(NT – Not tested)

DISCUSSION

In the present study an increased incidence of infective keratitis was seen in the age group of 40-59 years, which is supported by similar study in South Kerala [2]. This may be due to the combined effect of underlying predisposing diseases (either systemic or local) that are more prevalent in older age group. Males were affected 1.9 times more than females (65.93% vs 34.07%) which can be explained by the nature of their work profile. This is in accordance with prior studies by Geethakumari et al., Shafi T et al. and Gopinathan U et al., [2-4].

With respect to occupation, we observed a higher incidence of corneal ulcer among house wives (27.41%) and people involved in manual labour (20.74%) followed by farmers (11.85%). The high incidence in housewives reflects various domestic activities which expose them to corneal injuries. A similar observation was made by Tewari et al., in Ahmedabad [5]. Manual labour is a common occupation in this part of Kerala, which mainly include the works associated with building constructions, which impart a high risk to the workers for corneal injury with foreign bodies like sand, stone pieces, cement and metal pieces.

In the present study corneal injury was found as the risk factor associated with 59.26% (80/135) cases of infective keratitis. Similar findings were obtained in other studies (65.4% in Madurai, 71.5% in Tirunelveli, South India, 82.9% in East India, 72% in North India, 60% in Nepal, 49.96% in Vietnam) [6-11]. We observed a significant association between corneal injury and the culture proven cases of infective keratitis {$p<0.0003$, odds ratio (OR) 4.57, 95% confidence interval (CI) 2.0152-11.09, relative risk (RR) 1.59}, and with mycotic keratitis ($p<0.001$, OR 9.3, 95% CI 2.39-61.33, RR 1.3) which is agreeable with the observations of Bharathi et al., in Tirunelveli and Gopinathan U et al., in Hyderabad [12,13]. Whereas, in a study conducted in the neighboring district of Ernakulam, corneal injury accounted for only 13.3% of infective keratitis [14].Trauma with vegetative matter was observed as the most common type (36.25%), followed by sand (17.5%); broomstick, splinter (13.75%) and finger nail (8.75%).

Our investigation found that 37.07% of the identified cases of infective keratitis had diabetes mellitus as the predisposing systemic condition. Similar findings were observed in other studies [13]. Among the local predisposing conditions, chronic dacryocystitis (8.15%), dry eyes (7.41%), trichiasis (2.22%), and ectropion (2.22%) were observed.

Out of the 135 cases of corneal ulcer, 48 (35.56%) were found to be culture positive. Geethakumari et al., and Paul B et al., reported a lower isolation rate of 21.26% and 28.09% respectively in Trivandrum which is almost agreeable with the present study [2,15]. The low rates of isolation were attributed to the widespread availability of topical medications as reported by Srinivasan et al., and Geethakumari et al., [2,6].

Among the culture positive cases, bacterial isolates constituted 52.08% and the rest were fungal (47.92%). The higher incidence of bacterial isolates when compared to fungi, has been observed by many authors from Madurai, Hyderabad and Ahmedabad [5,6,13].

Out of the 25 bacterial isolates 16 (64%) were found to be Gram positive cocci and the rest were Gram negative bacilli (36%). *Staphylococcus epidermidis* was the predominant bacterial isolate in the present study (8 cases, 32%) followed by *Streptococcus pneumoniae* (4 cases, 16%), *Pseudomonas aeruginosa* (4 cases, 16%) and *Staphylococcus aureus* (3 cases, 12%). Our findings is supported by studies conducted in other parts of the country as well [5,8,16].

The predominant fungal isolate belonged to the genus *Fusarium* constituting 39.13%, followed by Aspergillus species (13.04%) and Curvularia species (13.04%). Among the Fusarium keratitis, *Fusarium solani was identified* in 21.74 % cases.The study also detected *Aspergillus flavus* in 2 cases and *Aspergillus fumigatus* in a single case. Other fungi isolated were Rhizopus species, Penicillium species, *Lasiodiplodia theobrome* and *Chaetomium* species. Similar findings were observed by Gopinathan U et al., Rautaraya et al., Nath R et al., Tilak et al., across the country [4,17-19]. Some of the dematiaceous fungal isolates (4 cases, 17.39%) could not be definitely identified due to lack of spore formation by the fungi in the culture medium (Sabouraud's Dextrose agar, potato dextrose agar). Such difficulty in speciation has been faced by other investigators as well [12,13].

About 37.78% of patients of the present study had visited a general practitioner/ophthalmologist and had applied topical antibiotics. 13.33% have received both topical antibiotic and antifungal agents. Despite receiving appropriate prior treatment by half of the culture proven infective keratitis patients, the success of etiological diagnosis may be due to the lower dosage as proposed by Gopinathan et al., [13]. The present finding is agreeable with the study in Madurai [6]. Prior topical corticosteroid usage was seen in 2.96% of the patients in the present study.

In the present study, the maximum number of cases was found during the dry, chilly and windy winter season (December, January and February) of Thrissur and Palakkad districts of Kerala. 38.46% of the cases in the year 2012-2013 were presented in the winter season, which was followed by monsoon season (June to November). Fungal isolates obtained were almost 1.6-2.3 times more than bacterial isolates during winter seasons, whereas bacterial isolates were almost 1.5-4 times more than fungal isolates in summer season. Increased incidence of fungal keratitis in winter and monsoon seasons was observed by Gopinathan U et al., in Hyderabad [4]. An increased incidence of fungal keratitis during the months when agricultural activity was greater (June to September) was reported by Bharathi et al., [12].

Direct microscopic examination of corneal scrapings is found to be an important technique for the etiological diagnosis of infective keratitis. A total of 25 bacterial keratitis cases were identified of which, 14 cases (56%) showed both microscopic (Gram staining) and culture evidences of bacterial infection and in all the 14 cases, the organism detected on direct microscopy tallied well with the culture obtained. The sensitivity and specificity of Gram staining was 56% and 95.45% respectively, which was agreeable with the study by Gopinathan et al., in Hyderabad [13].

Gram smear examination of scraping material is not found to be a sensitive method to detect fungal keratitis. 23 cases of fungal keratitis was detected by culture. Of these, Gram smear examination of the corneal scrapings revealed fungal elements only in 9 cases. Sensitivity of Gram staining in fungal keratitis was low, 39.13%. Anasuyadevi et al., reported a similar value (28.21%) in Bangalore and Jampala S et al., (37.5%) in Kerala [14, 20]. Chander J et al., reported a sensitivity of 56% [21]. Specificity of Gram stain in fungal keratitis in the present study was 96.43%. This is comparable with the study in Delhi by Chowdhary and Singh (97%) [22].

KOH mount examination of corneal scrapings is found to be very useful in the early diagnosis of mycotic keratitis. Sensitivity and specificity of KOH wet mount examination in the present study was 82.61% and 91.07%. The sensitivity and specificity in various studies across the country shows a variable pattern; Bangalore (82% & 77%), Hyderabad (81% & 84%) and Delhi (62% & 83.8%) [20,22,23]. Sensitivity of 81% was reported from Warangal and 64.7% from Chandigarh [21,24].

LIMITATIONS

Our study had some limitations as well. Anaerobic cultures were not performed in the specimens during the period of the study. Molecular methods for the identification and speciation of fungal isolates were not conducted.

CONCLUSION

Understanding the geographical pattern of the pathogenic organisms responsible and the identification of risk factors, helps to create a broad strategy for the diagnosis and management of corneal ulcers. Males are more prone to corneal ulcers than females as they are more involved in outdoor activities. Trauma is the leading cause for the corneal ulcers. The incidence of the bacterial and fungal keratitis is almost the same in this geographical area. A characteristic pattern of seasonal trend and the etiology of microbial keratitis in this area is of great value in the understanding of its epidemiology. The incidence of fungal ulcers may be related to the agricultural activities and the environmental conditions. Direct microscopy can aid the clinicians in the management of infective keratitis to start the prompt treatment, particularly in mycotic keratitis. The microbiological and epidemiological profile obtained in the current study can guide the ophthalmologists towards initiating specific treatment for this clinical emergency.

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AUTHOR(S):

1. Dr. Reena Anie Jose
2. Dr. Vijayakumar S
3. Dr. Rajini KC
4. Dr. Prithi Nair K
5. Dr. Reena John

PARTICULARS OF CONTRIBUTORS:

1. Tutor, Department of Microbiology, Believers Church Medical College Hospital, Thiruvalla, Kerala, India.
2. Additional Professor, Department of Microbiology, Government Medical College, Konni, Kerala, India.
3. Professor and Head, Department of Ophthalmology, Government Medical College, Thrissur, Kerala, India.
4. Professor and Head, Department of Microbiology, Government Medical College, Thrissur, Kerala, India.
5. Additional Professor, Department of Microbiology, Government Medical College, Thrissur, Kerala, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Reena Anie Jose,
Believers Church Medical College Hospital,
St. Thomas Nagar, Kuttapuzha PO-689103,
Thiruvalla, Kerala, India.
E-mail: reenakocherry@gmail.com

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