



Original Research Article

Microbiological profile in keratoconjunctivitis

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ABSTRACT

Introduction: Microbial keratitis is a common, potentially vision-threatening ocular infection that may be caused by bacteria, fungi, viruses or parasites. Treating infective suppurative corneal ulcers as an ophthalmic emergency and quick administration of appropriate anti-microbial therapy is the need of the hour for saving the eye of the patient and preventing the catastrophe of life-long blindness

Materials and Methods: This study was undertaken in the department of ophthalmology F.H. Medical College, Agra & department of microbiology. 392 patients with a clinical diagnosis of infective corneal ulcer were enrolled for the study. A complete demographic profile, associated risk factors, and microbial etiology were studied.

Results: In our study, patients with infective keratitis were enrolled. We found 266 positive for bacterial, fungal and mixed culture. 65.8% were male and 34.2% were female. In which 38% culture was positive for bacteria, 14.3 % was fungal positive and 5.1% was mixed isolates were found. In bacteria we found *S. epidermidis* (40%) was the most common and in fungal *Aspergillus* (60.7%) was common

Conclusion: Fungal corneal ulcers were the most common type found in our study. Timely detection and appropriate management are recommended to prevent prolonged ocular morbidity and blindness.

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1. Introduction

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.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 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}

Under normal condition, the eye enjoys a high degree of protection from infectious agents that are present in highly contaminated environment. Numerous factors such as flushing mechanism provided by tears, bactericidal action of

lysozyme, phagocytosis and mechanical barriers of an intact mucous membrane all play important role in protecting the eye.³

Conditions predisposing the eyes to infection such as breaking of mechanical barriers due to trauma, use of corticosteroids, antibiotics, lowered body resistance and other conditions decrease the defense mechanism of eye, thus causing great risk to ocular structures.⁴

The conjunctival sac is practically never free from microorganisms and harbors a wide range of bacteria in health and disease, varying in number, nature and pathogenicity, depending upon various factors like climate, season, personal habits and hygiene, local customs and use of medications. Although most microorganisms normally present in the conjunctival sac are fortunately non-pathogenic, yet some of them are morphologically identical to pathogenic types. Pathogenic group, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pneumococcus* are

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commonest. In the potentially pathogenic is *Staphylococcus epidermidis* and *Corynebacterium xerosis*.⁹

Microbial population in the normal flora of lid and conjunctiva can protect the eye by retarding colonization of pathogenic organisms. If the normal flora is altered by prolonged use of antibiotics or the tissue resistance is decreases by use of steroids, pathogenic and opportunistic fungi and bacteria proliferate, creating severe problems.

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. Early diagnosis and prompt treatment with appropriate antimicrobials is the hallmark of management of keratoconjunctivitis

2. Material and Methods

The study was conducted on 392 patients attending department of Ophthalmology, FH Medical College Agra. The samples were collected from October 2016 to June 2018.

All the patients were thoroughly interrogated and examined by touch and slit lamp. Data related to symptoms, predisposing factors, history of corneal trauma, traumatic agents, associated ocular conditions, other systemic diseases, therapy received prior to presentation, visual acuity at the time of presentation and all clinical findings were collected.

2.1. Technique of obtaining conjunctival specimen-

The conjunctival swab was taken from inferior fornix of conjunctiva by sterilized moist cotton swab sticks containing only adequate amount of cotton or by commercially available swab sticks or best by the Calcium Alginate tipped applicator. Swab sticks were rubbed back & forth several times over greater part of lower tarsal conjunctiva, care taken not to touch the swab stick with lid border & eye lashes.

2.2. Technique of Obtaining Corneal Specimen-

After a detailed ocular examination, using standard techniques, corneal scrapings were taken under aseptic conditions using a commercially available swab sticks or by the Calcium Alginate tipped applicator or by the sterile Bard-Parker blade (No. 15). The procedure was performed after instillation of 4% lignocaine without preservative.



Fig. 1: Illustration for collection of conjunctival swab



Fig. 2: Commercially available cotton swab sticks for scrapping conjunctival corneal swab

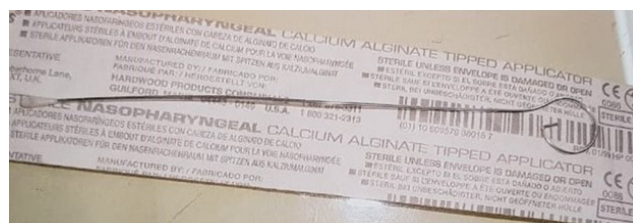


Fig. 3: Calcium alginate tipped applicator for scrapping conjunctival and corneal swab

2.3. Laboratory Procedures

The material was smeared on two slides - One for Gram staining and other as 10% potassium hydroxide (KOH) wet mount. For culture and sensitivity, the material was also directly inoculated by multiple C-shaped streaks, on blood agar, chocolate agar, nutrient agar and two tubes of Sabouraud dextrose agar (SDA) with chloramphenicol (50 mg/ml). The laboratory diagnosis was performed using standard protocols. All the inoculated media, i.e., blood agar, MacConkey agar, and nutrient agar were inoculated at 37°C and were evaluated at 24 and 48 h. They were subsequently discarded at 48 h if no growth was observed. The inoculated SDA media for fungi was incubated at 25°C and 37°C and examined daily. It was discarded after 3 weeks if no growth was present. Identification of growth on SDA was done by lactophenol cotton blue stain, by pigment production and by the morphological appearance of hyphae and spores.¹²

3. Results

This study was undertaken in the department of ophthalmology FH. Medical College, Agra & department of microbiology, F H Medical College, Agra.

In accordance with material & methods, In the present study a total of 392 patients of presumed microbial Keratoconjunctivitis were studied for bacterial & fungal flora. The criteria of diagnosis were based on clinical findings, microscopic examinations and culture findings as described in the material and methods.

In our study, 226(57.7%) patients were found to be culture positive. While the remaining 166 patients failed to give a positive yield on culture examination. Among the culture positive patients, 150(38.3%) patients were positive for bacteria, while 56(14.3%) patients gave a positive yield for fungus and 20(5.1%) observed with mixed growth (Diagram 1). *S.epidermidis* was identified in 60 (40) patients, *S.aureus* was 36(24%), *Strepto. pneumonia* 23(15.3), *pseudomonas* was 23(15%), *streptococcus viridace* were 9(6%), while *kelbsiella* were observed 5(3.3%) (Table 1) .

Among the 54 patients testing positive for fungus, *Aspergillus* was highest (60.7 %), *candida* incidence was (26%) *panicillium* (7.14%). *Mucor* (3.6) and *fusarium* (1.8%) comparatively low.

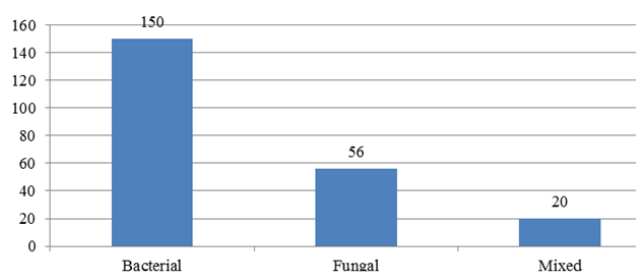


Diagram 1: Type of isolates in the study group (n=266)

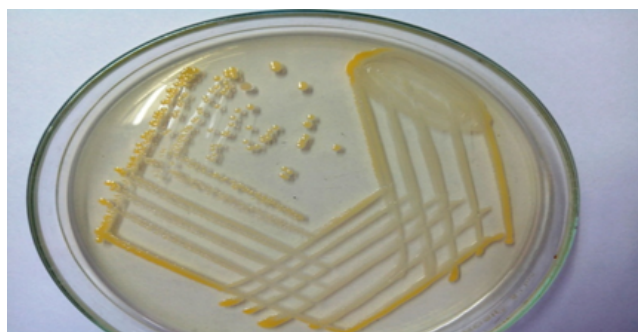


Fig. 4:

Distribution of patients of microbial keratitis according to sex. In the study group of total 392 presumed cases of microbial keratoconjunctivitis, 258(65.8%) were males

& 134(34.2%) were females. The incidence of bacterial (38.8%) & fungal (15.5%) was slightly more in male pt's while incidence of mixed infection (6%) & sterile culture s lightly more in female patients.

3.1. *Staphylococcus aureus* on nutrient Agar

The incidence of microbes was also studied in persons belongs to the different age groups to note any difference in the prevalence of the bacterial flora. 41 children belonging to age group below 10 year s were studied for their conjunctival flora and in 31.7%, the culture was found to be positive i.e. in 68.3% culture was negative (sterile). In 11-30 years found in 30-60 years age group, it was 68.2%. In 61- onwards the incidence of bacteria causing Keratoconjunctivitis was higher in 31-60 years age group and lower in 0-10 years age group (Table 3).

Occurrence of the microorganism in the different seasons of the year. Maximum incidence was found in the month of March – June 42.8%, While in the July – February months it was minimum 32.7%. The probable reason for higher incidence in the month of March-June can be the climatic conditions prevailing in these seasons which might be favorable for bacterial growth and multiplication. The incidence in July – February months were lower because cold climate which is not favorable for growth of micro organisms. In our findings it was found that in mixed group which contained different bacteria and fungus were more common in all months. In the literature no clue is available for comparison purposes.

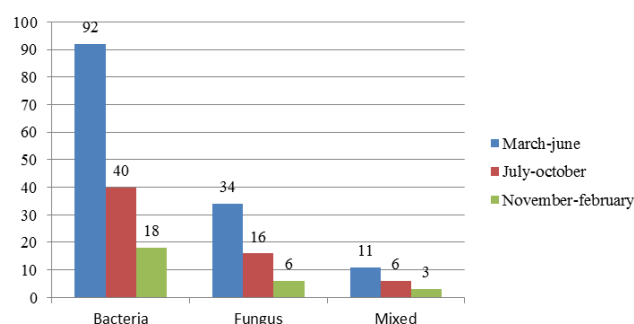


Diagram 2: Seasonal distribution of cases in study group

Socioeconomic status plays an important role in the causation as well as management of patients with keratoconjunctivitis. Access to medical facilities as well as affordability of treatment becomes significant in the final visual outcome. In our study 392 cases studied, 114 cases were farmers, 94 cases were ordinary house females, 86 laborers, 32 cases were involved in business of various kinds, 36 cases were students and 30 had other occupations.

Table 1: Etiological distribution of microbial keratoconjunctivitis in study group

Bacteria		
Etiology	No. of culture positive case	%
S. epidermidis	60	40
S. aureus	36	24
S.pneumoniae	23	15.3
Pseudomonas	17	11.3
Streptococcus viridencence	9	6
Klebsiella	5	3.3
Total	150	66.6
Fungus		
Eiology	No. of culture positive	%
Aspergillus	34	60.7
Candida	15	26.8
Penicillum	4	7.14
Mucor	2	3.6
Fusarium	1	1.8
Total	56	14.3

Table 2: Incidence of Micro-organism in Keratoconjunctivitis related to sex

Sex	Total		Bacterial		Fungus		Mixed	
	No.	%	No.	%	No.	%	No.	%
Male	258	65.8	100	38.8	40	15.5	12	4.6
Female	134	34.2	50	37.3	16	12.	8	6.0
Total	392	100	150	38.3	56	14.3	20	5.1

Table 3: Distribution of etiological agent of kertoconjunctivitis related to age

Age	Total		Bacterial		Fungus		Mixed	
	No.	%	No.	%	No.	%	No.	%
0-10	41	10.5	8	19.5	4	9.8	1	2.4
11-30	102	26.0	40	39.2	16	15.7	4	3.9
31-60	176	45.0	84	47.7	28	16.0	8	4.7
> 60	73	18.5	18	24.7	8	11.0	7	9.6
Total	392	100	150	38.3	56	19.3	20	5.1

Table 4: Distribution of Patients according to Occupation

Occupation	Total	
	No. of Cases	Percentage
Farmer	114	29.0
Laborers	86	21.9
House Wife	94	24.0
Business Man	32	8.2
Student	36	9.2
Others	30	7.7
Total	392	100.0

4. Discussion

In the belief, that an accurate knowledge of the natural history of the microbial flora in Keratoconjunctivitis, is one of the most effective methods, to prevent the wastage and misuse of the precious local antibiotic and antifungal agents, and of great help in the management of ocular infection and their prevention.

With the similar idea, it was considered to be of utmost importance to carry out a detailed study of the bacterial and Mycotic flora in the Keratoconjunctivitis. It comprised of a survey of the incidence of bacterial and Mycotic conjunctival flora in persons of different age and sex, belonging to different residual area i.e. urban and rural. The incidence was also studied in persons of different socio-economic strata and carried out throughout the period of 2 full years to observe any seasonal variation in the bacterial and Mycotic flora of the Keratoconjunctivitis.

We studied total 392 cases of Keratoconjunctivitis for their bacterial and Mycotic flora. Out of which, 226 (57.7%) eyes were found to contain micro organism. Out of total 226 patients found to be culture positive, Bacteria alone were found in 150(66.4%) patients, Fungal alone in 56(24.8%) patients & mixed bacterial & fungal in 20(8.8%) patients.

This incidence of microbial agents is in close agreement that of Gopinathan U, et al (2009) who found that Out of 5897 suspected cases of microbial keratitis, 3563 (60.4%) were culture-proven (bacterial – 1849, 51.9%; fungal – 1360, 38.2%; Acanthamoeba – 86, 2.4%; mixed – 268, 7.5%).

The incidence of microbes was also studied in persons belongs to the different age groups to note any difference in the prevalence of the bacterial flora. 41 children belonging to age group below 10 years were studied for their conjunctival flora and in 31.7%, the culture was found to be positive i.e. in 68.3% culture was negative (sterile). In 11-30 years found in 30-60 years age group, it was 68.2%. In 61- onwards the incidence of bacteria causing Keratoconjunctivitis was higher in 31-60 years age group and lower in 0-10 years age group.

A reflection of improved ante-partum and post partum care and prophylaxis use of antibiotics among the very young may be one reason for the low incidence of infection in 0-10 year's age group. High sterile cultures in these age groups may be due to the fact that high incidence of allergic conjunctivitis are present in these patients.

In our study the incidence of staphylococcus in causing the Keratoconjunctivitis was, it was found 77%. In accordance with our findings are the findings of Jain et al. (1970) who in a series of 46 cases of corneal ulcers reported a positive culture rate of 93.46%, coagulase-negative Staphylococcus epidermidis being the most common organism involved followed by Staphylococcus aureus.

The other things to be noted were that micro-organism tends to occur more frequently in villagers as compared

to town dwellers. In the rural population the incidence of organisms were found to be 65.3%, while in the urban population it was 48.6%. Our observations of the difference in positive cultures in rural and urban cases is in accordance with the findings of M. Jayahar Bharthi et al. (2003) who demonstrated that there were 564 (54.07%) rural residents and 479 (45.93%) urban residents in their study. The possible reasons for higher incidence of organisms in rural cases can be unhygienic conditions of the villagers and their exposure to dry and dusty atmosphere. In our study the incidence of mixed group infections which include both bacteria and fungus was more in rural population (6%), while it was found only (3.9%) in urban dwellers.

The probable reasons for the higher incidence of the lower socio-economic strata can be poverty, ignorance, lack of hygiene, and lack of education etc. Moreover, malnutrition leading to poor body resistance can also be found a higher incidence of bacteria in the conjunctivae of people belonging to low socio-economic group as compared to middle and upper groups.

The maximum incidence was found in the month of March – June 42.8%, While in the July – February months it was minimum 32.7%. The probable reason for higher incidence in the month of March-June can be the climatic conditions prevailing in these seasons which might be favorable for bacterial growth and multiplication. The incidence in July – February months were lower because cold climate which is not favorable for growth of micro organisms. The incidence of isolated bacterial in various season of the year has been studied in our series of cases. We found that the incidence of staphylococcus was maximum in March – October and minimum in November – February. In agreement without findings are the findings of various previous workers who have reported that Staphylococci were more prevalent in hot climates.

Infective keratoconjunctivitis is a major cause loss of vision. This is more so in developing countries like India, where the vast majority of people are socially and economically backward, dwelling in rural areas and pursuing agriculture and other manual work as their main source of livelihood. These individuals are at an increased occupational risk of ocular trauma. In addition, the problem is compounded by frequent late presentation of the patient due to lack of awareness, and inaccessibility to specialist ophthalmic care. The occurrence of corneal ulcers peaks during the harvesting season. Treating infective suppurative corneal ulcers as an ophthalmic emergency and quick administration of appropriate anti-microbial therapy is the need of the hour for saving the eye of the patient and preventing the catastrophe of life-long blindness..

5. Source of Funding

None.

6. Conflict of Interest

None.

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