

## Microbiological regional profile of infective keratitis

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### ABSTRACT


**Purpose:** To determine epidemiological characteristics, specific pathogenic organisms, risk factors for infective keratitis.

**Methods:** Patients with signs and symptoms of infective keratitis were included. History, examination including visual acuity, slit-lamp bio-microscopy, microbiological examination including Gram staining, KOH mount, culture, sensitivity of corneal scrapings were done.

**Results:** Forty cases were evaluated, thirty (75%) were males, ten (25%) were females. Age ranged from 5 - 70 years, 35% in age group up to 30 years, 50% between 31-60 years, 15% were > 60 years of age. Right eye was involved in 55%, left in 45%. Highest incidence of ulcers occurred in farmers and labourers (65%). History of trauma was present in 80% patients, ocular surface disease in 7.5%. Diabetes was present in 12.5%. 57.5% patients had hypopyon on presentation. Based on culture reports 62.5% had bacterial keratitis, 25% fungal keratitis and 12.5% sterile. 80% bacterial isolates were gram positive and 20% gram negative. *Staphylococcus aureus*(40%) was the commonest organism cultured followed by *Staphylococcus epidermidis*(32%), *Pseudomonas*(12%), *Pneumococcus*(8%) and *Acinetobacter*(8%). *Aspergillus* was isolated in 60% of cases and *Fusarium* in 40%. Gram positive isolates were maximally sensitive to Cefazoline, gram negative isolates to Gentamicin.

**Conclusion:** Males in rural agricultural population in economically productive age group are most vulnerable to infective keratitis. Culture on blood and chocolate agar detects more number of organisms compared to Gram stain alone. *Staphylococcus aureus* was the most common bacterium and *Aspergillus* most common fungus isolated. Cefazoline and Gentamicin combined cover most bacterial isolates.

**Key Words:** Infective Keratitis<sup>2</sup>, epidemiological profile<sup>1</sup>, risk factors<sup>3</sup>

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We decided to conduct a study in order to evaluate the clinical profile of infective keratitis, role of various microbiological investigations in the diagnosis and management of infective keratitis. We determined the factors predisposing to infective keratitis, identified the causative agents prevalent and also analysed the sensitivity of antimicrobial agents commonly being used in developing countries like ours.

### INTRODUCTION

Globally it is estimated that infective keratitis and ocular trauma result in 1.5 to 2 million new cases of corneal blindness annually. Ninety percent of them occur in developing countries, and it has now been recognized as a silent epidemic<sup>[1]</sup>. Corneal blindness is a major health problem in India and infections constitute the most predominant cause. A review of the data on indications for corneal transplantation in the developing world revealed that corneal scar was the most common indication (28.1%), of which keratitis accounted for 50.5%.<sup>2</sup> Successful therapy of a corneal ulcer requires accurate identification of the causative organism and initiation of appropriate anti-microbial therapy. Laboratory investigation is an essential part of the evaluation of any patient with suspected microbial keratitis. To maximize the comfort of the patient and to prevent loss of vision, and in most cases to increase the cost effectiveness of treatment, direct examinations, culture and antibiotic sensitivity is indicated in patients with microbial keratitis.

### MATERIALS AND METHODS

All patients with symptoms and signs suggestive of infective keratitis presenting to the Upgraded Department of Ophthalmology, Government Medical College, Jammu in a period of one year were included in this study and prospectively analysed. All these cases were defined clinically as “corneal ulcers” following observation of loss of corneal epithelium with underlying stromal infiltration and suppuration associated with signs of inflammation, with or without hypopyon as seen on slit lamp bio- microscopic examination. Patients with typical viral ulcers, Mooren’s ulcers, marginal ulcers, interstitial keratitis, sterile neurotropic ulcers and perforated corneal ulcers were excluded from the study. At presentation to the outpatient department, information pertaining to the demographic features, duration of symptoms, risk factors, occupational status and details of previous treatment received was documented for every suspected case of infective keratitis according to a detailed

protocol. All the patients were subjected to general physical examination to rule out possibility of any associated systemic disease.

Visual acuity was recorded at the time of presentation and external ocular examination was done (including sac syringing) to rule out ocular surface disorders and dacryocystitis. Corneal evaluation was done using slit lamp bio microscope and detailed diagrammatic documentation of the ulcer was done which included site, shape, margins, size, infiltration and presence of satellite lesions. Corneal ulcers were graded according to Mohan's classification.

Grading of Corneal Ulcer<sup>3</sup>:

- Grade 0 - No ulcer
- Grade I - Extent less than 4 mm
- Grade II - Extent upto 5 mm
- Grade III- Extent upto 7 mm
- Grade IV- Extent upto 10 mm
- Grade V - Extent more than 10 mm

On the basis of depth of corneal involvement, the corneal ulcers are grouped as:

1. Corneal depth involved upto 1/3 of corneal thickness.
2. Corneal depth involved from 1/3-2/3 of corneal thickness.
3. Corneal depth involved upto full thickness

Presence of hypopyon and its characteristics were noted.

Grading of hypopyon<sup>3</sup>:

- 0- No hypopyon
- +- < 2mm
- ++- < 5mm (filling upto half of anterior chamber)
- +++ 5mm (filling more than half of anterior chamber)

Following clinical evaluation patients were subjected to microbiological investigations.

Microbiological Investigations:

Corneal scrapings from the margins and base of corneal ulcer were taken after local instillation of 4% lignocaine into the affected eye, under slit lamp with the help of sterile blade No.15. If the patient was taking antibiotics at the time of presentation to the hospital, treatment was stopped and investigations were delayed for 24 hrs. Material obtained from the scrapings was smeared on 2 microscopic slides one for 10% KOH wet mount<sup>4</sup> and the other for Grams stain.<sup>5</sup>

The stained smears were examined for bacteria, yeast, fungal filaments and spores while the KOH preparation was examined for fungal elements. Additional material was inoculated directly onto two tubes of Sabouraud's agar for fungus culture. Material collected on a sterile cotton swab was used to inoculate Sheep blood agar, Chocolate agar and Mac Conkey agar<sup>6</sup>. Media inoculated for aerobic organisms were incubated at 37°C in an atmosphere of 5-10% CO<sub>2</sub>. Colonies growing on the media after incubation for 48 hours

were identified by the standard biochemical tests<sup>7</sup>. Culture on blood agar and chocolate agar were evaluated at 24 and 48 hours, and then discarded if there was no growth. Antibiotic sensitivity tests were done by the disc diffusion technique<sup>8</sup>.

For fungal agents two tubes of Sabouraud's dextrose agar media<sup>9</sup> were incubated at 25°C and 37°C and discarded after 4 weeks if there was no growth. Identification of the growth on the media was made by Lactophenol Cotton Blue Staining<sup>10</sup>. Antifungal sensitivity testing could not be done as facilities were not available in our set up.

## RESULTS

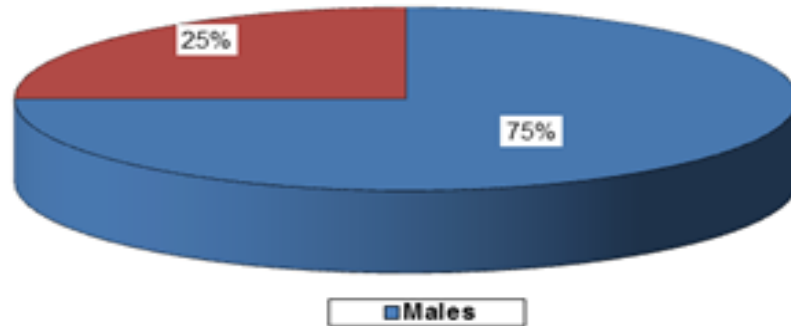
In the present study, a total of 40 patients with infective keratitis were enrolled. All the patients in the study were between 5-70 years of age. The sex and age wise distribution of patients is shown in Fig. 1 and 2 respectively. 26(65%) were from rural areas while 14(35%) were from urban areas which showed that incidence of corneal ulcer was more in the rural population. Out of the forty eyes studied right eye was affected in 55% cases and the left was affected in 45% cases.

The seasonal variation in the occurrence of corneal ulcer among patients is depicted in Table 1. The incidence of corneal ulcer was highest in farmers and labourers (65%) in comparison to other occupations. Fig. 3 describes the occupation of patients. Most of the patients (85%) reported to the hospital within 1 month of disease onset while only 32.5% of them came within first week of illness. Table 2 showing the potential predisposing risk factors for corneal ulceration. Table 3 describes distribution of patients with systemic risk factors on presentation. Among the systemic factors documented, diabetes mellitus was more frequently noted in keratitis of both bacterial and fungal aetiology accounting for 12.5% of cases. The mode of injury to the eye is depicted in Fig. 4. The treatment history of the patients is shown in Fig. 5, which shows that most of the patients had received treatment elsewhere before reporting to us. The size of ulcers and depth of corneal involvement as measured on slit lamp bio-microscope is shown in Fig. 6 and Fig. 7. The grading of hypopyon is shown in Table 4. Out of 40 cases, gram stain and KOH showed positive results in 32 (80%) cases and (87.5%) of cases showed growth on culture media, the details of the microbiological results is given in Table 5. Staphylococcus aureus was the commonest bacterium cultured (40%) while Aspergillus species. was the commonest fungus identified. The distribution of various bacteria and fungi isolated in cases of bacterial corneal ulcer is depicted in Fig. 8, 9 & 10. In our study most of the bacterial isolates were gram positive cocci (80%) and 20% gram negative bacilli. Most of the gram positive isolates were maximally sensitive to cefazolin and gram negative isolates were to gentamycin/kanamycin. Antibiotic sensitivity of

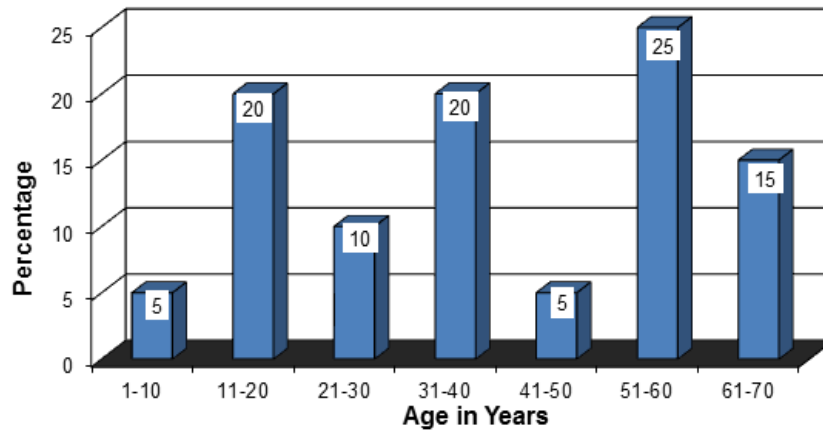
different bacterial isolates from corneal ulcers is shown in Table 6. We can see that ciprofloxacin was still a

broad-spectrum drug covering both gram positive and negative organisms.

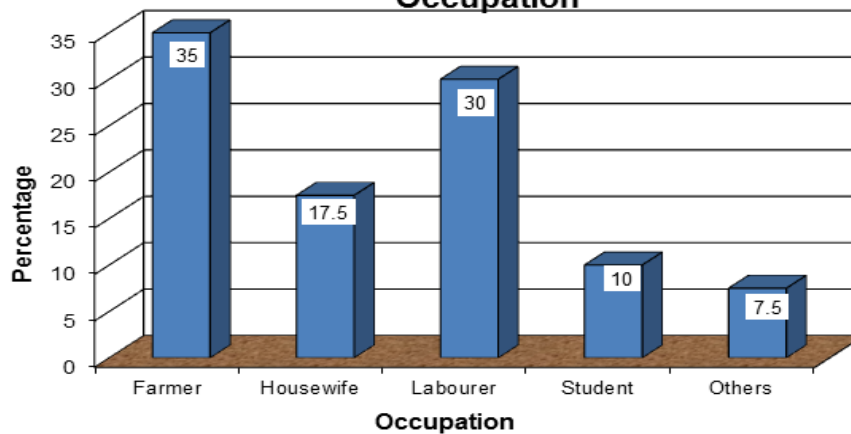
**Fig. I : Distribution of Patients According to Sex**



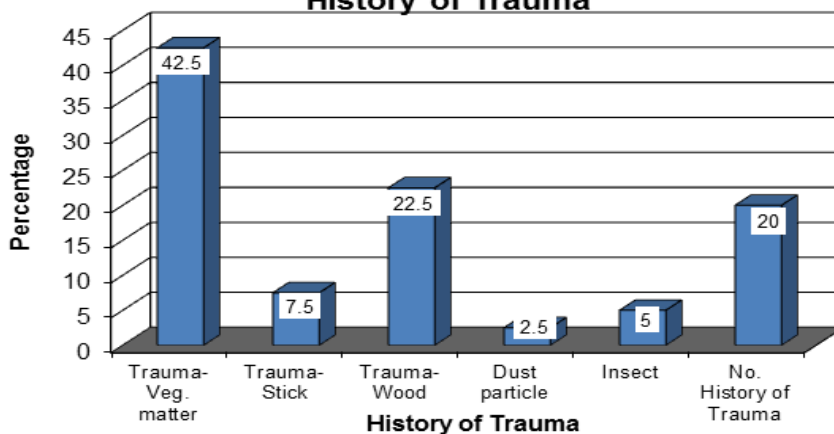
**Fig. II : Age Distribution of Patients**



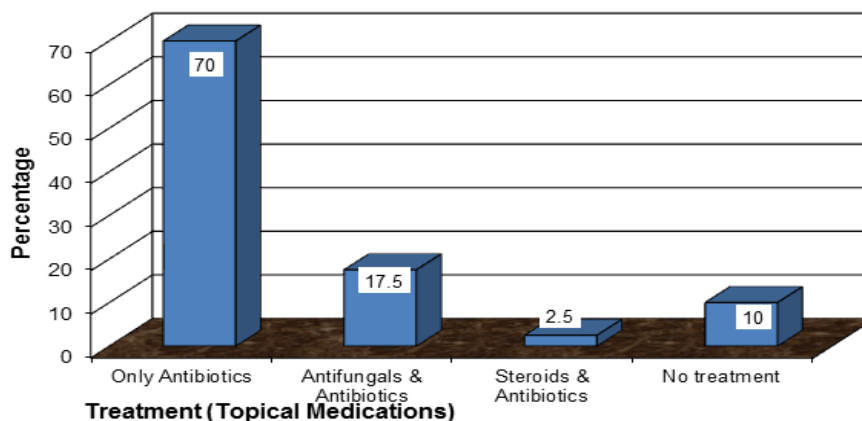
**Fig. III : Distribution of Patients According to Occupation**



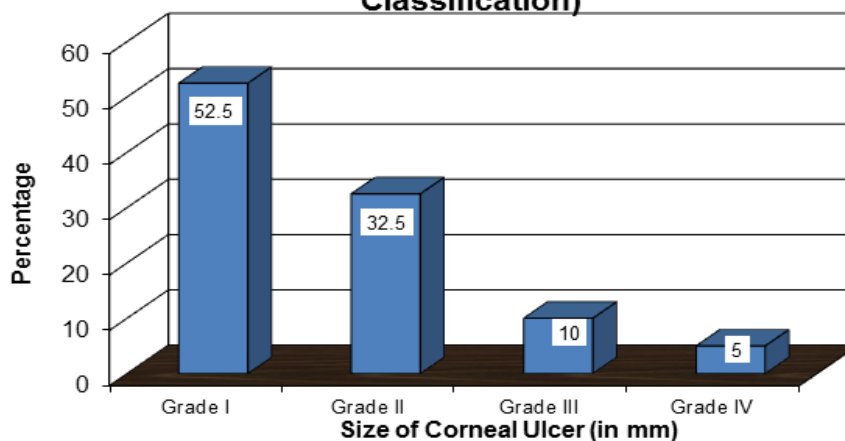
**Fig. IV : Distribution of Patients According to History of Trauma**



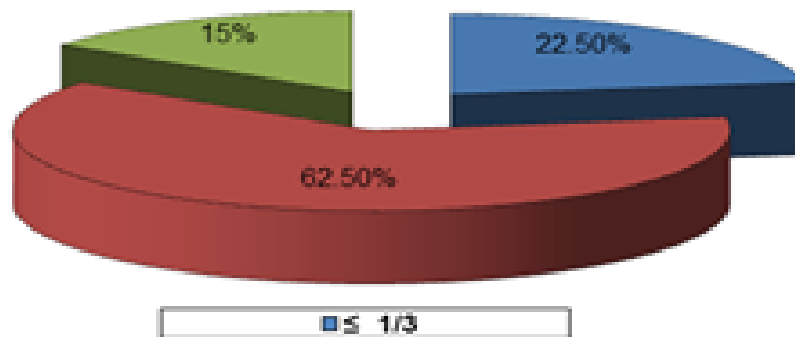
**Fig. V : Distribution of Patients According to Treatment Received Before Evaluation**



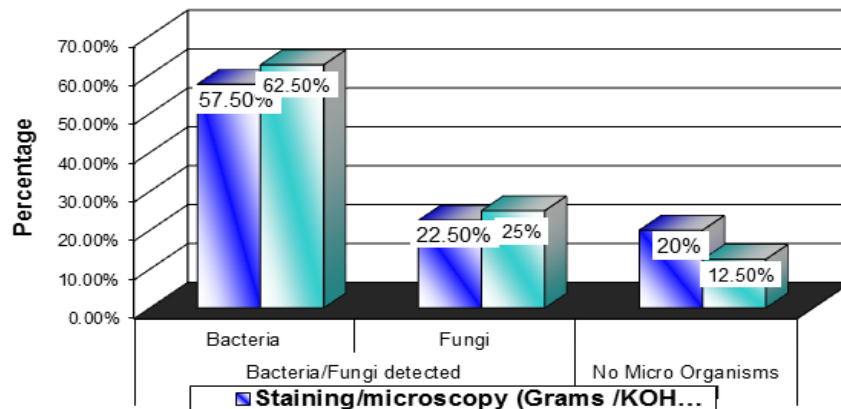
**Fig. VI : Grading of Corneal Ulcers (Mohan's Classification)**



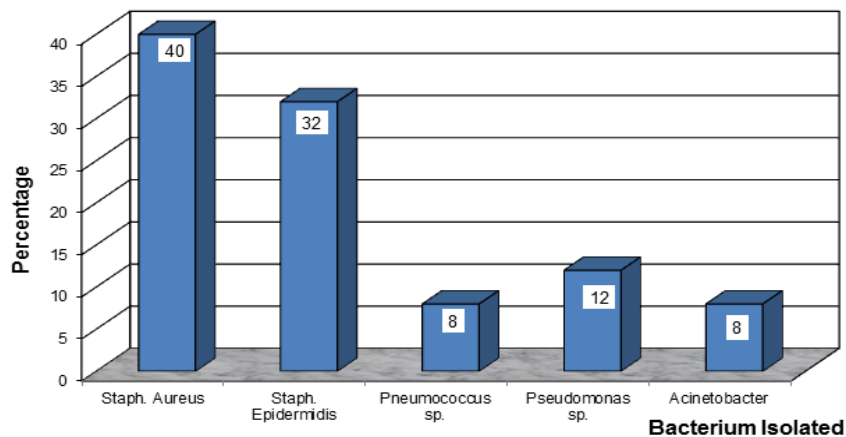
**Fig. VII : Distribution of Patients According to Depth of Corneal Involvement**



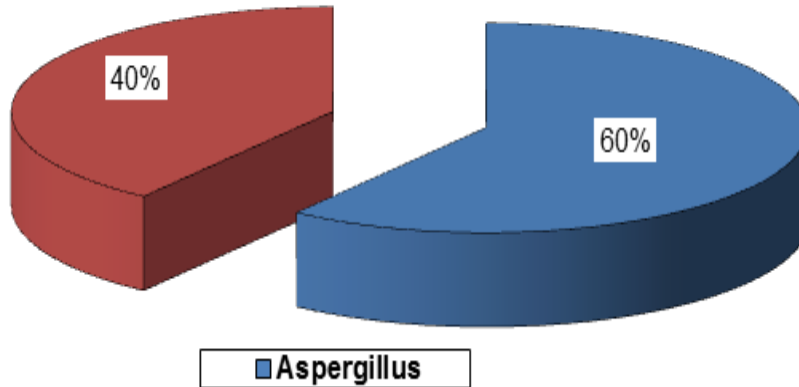
**Fig. VIII : Detection of Bacterial and Fungal Ulcers based on Microbiological Investigations**



**Fig.IX : Distribution of Various Bacteria in Culture Positive Cases**



**Fig. X : Incidence of Various Fungal Species (in Culture Positive Cases)**



**Table 1: Seasonal Variation in Incidence of Corneal Ulcers**

	Number of Cases	Percentage (%)
April – September (warm and humid climate)	27	67.5
October – March (winters)	13	32.5
<b>Total</b>	<b>40</b>	<b>100</b>

**Table 2: Distribution of Patients according to Risk Factors Present**

Risk Factor	No. of Cases	Percentage (%)
Trauma	32	80
Ocular Surface Disease	3	7.5
Lid Disorders	2	5
Dacryocystitis	1	2.5
Unknown	2	5
<b>Total</b>	<b>40</b>	<b>100</b>

**Table 3: Distribution of Patients with Systemic Risk Factors**

Risk Factor	No. of Patients	Percentage (%)
Diabetes	5	12.5
Tuberculosis	2	5
Lymphoma	1	2.5

**Table 4: Grading of Hypopyon (Mohan’s Classification)**

Grade	Number of Cases	Percentage (%)
0	17	42.5
+ (≤ 2mm)	11	27.5
++ (≤ 5mm)	8	20
+++ (> 5mm)	4	10
<b>Total</b>	<b>40</b>	<b>100</b>

**Table 5: Detection of Bacterial and Fungal Ulcers Based on Microbiological Investigations**

Method of detection used	Bacteria/Fungi detected		No Micro Organisms isolated		Total
	Bacteria	Fungi			
Staining/microscopy (Grams / KOH Mount)	23 (57.5%)	9 (22.5%)	8 (20%)		40 (100%)
Culture	25 (62.5%)	10 (25%)	5 (12.5%)		40 (100%)

**Table 6: Antibiotic Sensitivity of Different Bacterial Isolates from Corneal Ulcers**

Susceptibility of Organisms Isolated to Antibiotic (%)						
Organism Isolated	Ampi/Pencillin	Ciprofloxacin	Tetracycline	Kanamycin	Gentamycin	Cefazolin
Staph. epidermidis	25	75	37.5	75	62.5	87.50
Staph. aureus	20	70	40	70	70	90
Pneumococcus sp.	50	100	50	100	100	100
Pseudomonas sp.	0	66.67	33.33	66.67	100	33.33
Acinetobacter	0	50	0	50	100	50

## DISCUSSION

Accurate diagnosis is critical to management of corneal ulcers. Incorrect diagnosis can lead to rapid progression of ulcers, thereby threatening integrity of eye and resulting in visual loss. The present study was conducted to study the profile of infective keratitis and the role of microbiological investigations in its diagnosis and management.

Similar to our study a significant male preponderance and higher incidence in young active patients from rural background has been reported by most previous studies<sup>[11,12,13]</sup>. This is due to greater involvement of males especially younger age group in outdoor activities thus predisposing to trauma and corneal infection. Those from rural set up have high chance of exposure to agricultural products and vegetative matter.<sup>13,14</sup> Warm and humid climate favours the growth of offending organism and hence most patients have frequently reported infective keratitis in the months of April to July.<sup>15</sup>

Ecological factors favour growth on vegetative matter and agricultural products. Farmers and labourers are in constant contact with these products and thus prone to corneal ulcers. This may be the reason for the highest incidence of corneal ulcers in farmers and labourers as compared to various other occupations.<sup>13,14</sup>

In our study, the timely reporting of diseased within first month of infection can be ascribed to increased disease awareness and better transportation services in India, which is much better than reported percentages in other developing countries like Nepal. Similar observations have been made by **Srinivasan et al**<sup>13</sup>.

Similar predisposing factors for corneal ulcer have been reported by **Srinivasan**<sup>13</sup>, **Prasad**<sup>[15]</sup>, **Upadhyay et al**<sup>[16]</sup> and **Basak et al**<sup>17</sup> Our findings contrast with those of **Shaefer et al**<sup>18</sup> and **Green et al**<sup>19</sup> who have reported

contact lens wear as the most common risk factor for development of keratitis. This difference can be explained as the study was carried out in a developed country, where more people use contact lenses and history of occupational trauma is uncommon due to increased awareness and occupational safety measures.

In our study majority(90%) of patients had received antimicrobial agents topically prior to presentation similar to study conducted by **Naumann et al**<sup>12</sup>. Unlike studies of **Koul et al**<sup>10</sup> who have reported usage of steroids prior to presentation in 60% patients, only 2.5% cases in our study gave a definite history of usage of steroids as initial management. Topical therapy with antibiotics and/or steroids predisposes to mycotic keratitis. The inflammatory response is suppressed by corticosteroids. The incidence of use of topical steroids initially as treatment is remarkably low in our study possibly due to better understanding of pathology of corneal ulcers by the treating physicians.

Majority of bacterial isolates growth on culture as compared to fungal isolates has been similarly reported by **Srinivasan et al**<sup>13</sup>. In our study, we observed that culture was positive in 35 cases (87.5%) of the 40 cases of presumed infective keratitis. This is comparable to report of various author<sup>13,14,20</sup>. In our study 12.5% cases were sterile. The culture negativity in our study may be due to the reasons that many of these patients received prior treatment of infectious keratitis as the protocol of culture techniques followed in our study and procedure of sample inoculation directly in the clinic leaves virtually no scope for role of laboratory related reasons for low yield in culture.

Direct microscopic examination of corneal scrapings provides rapid diagnosis and forms the basis for initiating initial antimicrobial therapy which may be modified later according to culture reports. An accurate

smear diagnosis therefore becomes important in achieving optimum treatment outcome. We observed that potassium hydroxide wet mount revealed presence of hyphal structure in 90% of culture proven mycotic ulcers. Our findings are comparable with those of **Sharma et al**<sup>20</sup>. In their study fungi were identified by KOH method in 100% of the culture proven cases of mycotic ulcers.

In our study *Aspergillus* species is the commonest organism isolated in 60% followed by *Fusarium* sp. in 40% of the cases. This is in accordance with the reports of other authors<sup>12,21,22</sup>. A high prevalence of fungal keratitis caused by filamentous fungi in our study may be due to the presence of hot and humid climate in India while **Laspina et al**<sup>23</sup> found that *Acremonium* species was the most commonly identified fungi (40%) followed by *Fusarium* species (15%). This can be attributed to difference in geographic location and environmental factors between India and Paraguay.

**Upadhyay et al**<sup>16</sup> have similarly reported a higher culture positivity rate of 80% in their study. They have reported higher positive detection rates than our study. As discussed earlier reason for relatively lower yield in our study cannot be because of lab related reasons but can be explained by patient related causes such as use of antimicrobials prior to presentation.

As is well known *Staph. aureus* is a common commensal of nares and *Staph. epidermidis* is of the eye. These organisms can easily invade corneal tissue when compromised by trauma or antimicrobial use. Many of the studies have reported similar bacterial isolates as the causative organism<sup>18,20</sup>. **Basak et al**<sup>17</sup> reported *Staph. aureus* as the most common bacterial isolate (42.6%), and *Aspergillus* as the most common fungal isolates which is in agreement with the present study. However, they have reported a higher incidence of fungal ulcers in their study which can be attributed to farming as a primary occupation in the area studied (Gangetic West Bengal). **Green et al**<sup>19</sup> have reported that *Pseudomonas* was the most common isolate in culture (17%), coagulase negative staphylococcus. in (9%), staphylococcus. aureus in (8%) and fungi in (3%) of cases. A higher incidence of *Pseudomonas* infection in contrast to our study can be attributed to a higher incidence of contact lens wear in this study which was also the most common risk factor mentioned for the development of keratitis.

Our antibiotic sensitivity results were quite comparable to studies done by **Sharma et al**<sup>20</sup> where cefazolin was the most effective drug against 83% of isolates followed by ciprofloxacin, then gentamycin and then norfloxacin. **Goldstein et al** in their five year review have shown a rapid increase in *Staph. aureus* resistance to ciprofloxacin with 5.8% resistance in 1993, 9.4% in 1994, 11.4% in 1995, 26.5% in 1996 and 35% in 1997. Our study also agrees with the findings of **Sharma et al**<sup>20</sup> and **Goldstein et al**<sup>24</sup> that fluoroquinolones have a broad spectrum of action, but reveals a rate of

insensitivity i.e. too high to justify its use as monotherapy. **Shaefer et al**<sup>18</sup> in their study in which *Staph. sp.* was the most common isolate have shown 1 to 15% resistant strains to fluoroquinolones, 13-22% to aminoglycosides and 37% to cefazoline. A lower percentage of resistant strains to fluoroquinolones in their study can be attributed to use of more potent ofloxacin and lomefloxacin medications in their study instead of ciprofloxacin which was used in our study.

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