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Microbial Keratitis – a One Year Experience from a University Hospital of Lucknow

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ABSTRACT

Aim of study: *To study the etiological profile and risk factors for microbial keratitis in patients presenting to Ophthalmology Department in King George's Medical University, Lucknow.*

Material and Methods: *A total of 120 corneal scrapings were performed in patients presenting withcorneal ulcers between August 2016 to July 2017. Gram staining , KOH mount and culture on blood agar and Sabouraud's dextrose agar was done for all the scrapings received to identify bacterial and fungal pathogens.*

Results: *Microbial etiology was established in 41/120 (34.16%) scrapings. The most common risk factor for corneal ulcer was found to be trauma (68/120) and trauma due to vegetative matter was the commonest cause of traumatic injury (31/68). Out of the 41 positive cultures, 34.14% (14/41) were bacterial while 65.85% (27/41) were fungal in etiology. The most common bacterial isolate was 50% (7/14) were Coagulase negative Staphylococcus spp while the most common fungal isolate was Aspergillus spp 25.93% (7/27). Antimicrobial susceptibility testing was done for both bacterial and fungal isolates. None of the fungal isolate were resistant to voriconazole and majority of the bacterial isolates were also found to be susceptible to major drug groups.*

Conclusion: *Trauma due to vegetative matter is the most common predisposing factor for microbial keratitis in our setting. Fungal infections was commoner than bacterial. The "regional" findings play an important role in public health implications to understand the etiology better and to initiate appropriate treatment to save the eye of the patient.*

INTRODUCTION

Keratitis is an inflammation of the cornea produced by infectious organisms or non infectious agents or stimuli [1]. Microbial keratitis is a potentially sight threatening infectious corneal inflammation which that can be caused by bacteria, virus, fungi or parasite [2]. In some developing tropical countries corneal infections are the second most common cause of monocular blindness after UN operated cataract.

Corneal ulceration has been labeled as "silent epidemic" in developing countries [3]. It forms an important cause of ocular morbidity due to inaccessibility and unavailability of ocular investigations everywhere and often delays in presentation on the part of patient himself.

Etiological and epidemiological pattern of corneal ulcers vary with patient's population, health of cornea, geographical location, seasonal variation and also with time [4].Thus it is important to carry out local studies periodically to be aware of the local disease trend and sensitivity pattern.

MATERIAL AND METHODS

The study was conducted on 120 patients with corneal ulcers presenting to cornea clinic in Department of Ophthalmology from August 2016 to July 2017. Consent was taken from the patients/guardians prior to enrollment in the study. Patients of all groups were included in the study.

Data was collected in a brief pre-designed format. It included patient's name, age, sex, occupation, duration of symptoms, history of (H/O) ocular trauma, contact lens wear, bathing in pond water, any associated ocular illness, systemic illness, therapy received prior to presentation and clinical examination. Corneal scrapings were performed under strict aseptic conditions by an ophthalmologist after instillation of 4% lignocaine

without preservative using sterile Bard-Parker needle (No. 15) under slit lamp magnification [5, 6]. The material was taken from leading edge and base of the ulcer. It was placed in a C shaped streak on the 5% Sheep Blood Agar (SBA) plate and two Sabouraud's Dextrose Agar (SDA) plates. Any growth on C streak was considered to be significant. Another sample was taken for slide Gram staining and 10% KOH mount [7].

One set of SBA and SDA plate were incubated at 37 °C and the other SDA plate was incubated at 25°C.SBA plate was considered sterile if no growth was observed within 48 hours and SDA plate was considered sterile if no growth was found in 28 days.

The specific identification of bacterial colonies was done by Gram staining and standard tests using standard laboratory protocols and fungal colonies were identified by their colony characteristics on SDA and morphological appearance of hyphae and spores in lacto phenol cotton blue mount.

Antifungal susceptibility testing was done for yeast and molds by broth microdilution method using drugs itraconazole and voriconazole with concentrations between 0.313 to 16mg/lt.M 100 CLSI 2016 guidelines were referred for results.

Antibacterial testing was done using disk diffusion method using β-lactams (penicillins), aminoglycosides (amikacin, gentamicin), macrolide (erythromycin), fluoroquinolones (levofloxacin), lincosamide (clindamycin), glycopeptide (vancomycin), oxazolidinones (linezolid) and sulphonamide (cotrimoxazole) for Gram positive organisms and β-lactase (penicillin's, cephalosporin's), amino glycoside (amikacin, gentamicin), floroquinolone (levofloxacin), carbapenem (impanel) and polymixin (colitis and polymixin B) for Gram negative isolate's 100 CLSI 2016 guidelines were referred for results.

Results

Of 120 clinically suspected corneal ulcers-Males showed higher preponderance in the study (68.33%; n=82) as compared to females. The most common age group of presentation was 41-65 years with majority of the patients belonging to rural background (85.00%; n=102). Most of the patients were engaged in outdoor occupations (57.50%; n=69), maximum being farmers (73.9%; 51/69).

Housewives were common in indoor population (41.17%; 21/51). History of ocular trauma was elicited in 56.67% (n=68) of patients and injury due to vegetative material (45.59%; n=31) was the leading cause of ocular trauma.

Fungal isolates were recovered from 22.50% (n=27) samples whereas bacterial isolates from 11.67% (n=14) samples.

Aspergillus spp (25.93%; n=7) was the commonest isolated fungal isolate followed by *Fusarium* spp (18.52%; n=6) and *Alternaria* spp (18.52%; n=5).

Coagulase Negative Staphylococcal spp (CONS) predominated the bacterial isolates (50%;n=7) while *Pseudomonas*spp (0.83%;n=1) was the least common bacterial isolate.(Table 1 and 2).

Table1. *Bacterial pathogens isolated from 14 culture positive bacterial keratitis cases*

	No. of patients	Percentage (%) (n=14)
Sterile	106	88.3%
Bacterial Positive	14	11.6%
CONS	7	50.00
S. aureus	4	28.57
S. pneumoniae	2	14.29
Pseudomonas spp	1	7.14

Table2. *Fungal pathogens isolated from 27 culture positive fungal keratitis cases*

	No. of patients	Percentage (%) (n=27)
Sterile	93	77.5%
Fungal Positive	27	22.5%
*Acremonium*spp	1	3.70
*Alternaria*spp	5	18.52
A. fumigatus	3	11.11
A. flavus	4	14.81
Aureobasidium pullulans	1	3.70
Candida albicans	2	7.41
*Cladosporium*spp	1	3.70
*Curvularia*spp	4	14.81
Fusarium spp	6	18.52.22

Taking culture as the Gold standard, the sensitivity and specificity of 10%KOH is 81.5% and 78.5% respectively and the sensitivity and specificity of Gram stain 29.6% and 93.5% respectively for the fungal isolates.

The sensitivity and specificity of Gram staining for bacterial isolates is 100% and 70.8% respectively.(Table 4,5 and 6).

None of the fungal isolates showed resistance to voriconazole. All Aureobasidium pullulans, Alternariaspp, Candida albicans, Cladosporiumspp and 25% Aspergillus flavus were sensitive to itraconazole. (Table-3).

Table3. *Antifungal susceptibility pattern of the fungal isolates*

		A. flavus (n=4)	*A. fumigatus (n=3)*	*Curvularias pp (n=4)*	*Fusarium spp (n=6)*	*Aureobasidium pullulans (n=1)*	*Alternarias pp (n=5)*	*Candida albicans (n=2)*	*Cladosporium spp (n=1)*	*Acremoniumspp (n=1)*
Itracoazole	S	1 (25%)	0	0	0	1(100%)	5(100%)	2(100%)	1(100%)	0
	I	2(50%)	2 (66.6%)	2(50%)	0	0	0	0	0	1(100%)
	R	1(25%)	1(33.3%)	2(50%)	6 (100%)	0	0	0	0	0
Voriconazole	S	4(100%)	3(100%)	4(100%)	6(100%)	1(100%)	5(100%)	2(100%)	1(100%)	1(100%)
	I	0	0	0	0	0	0	0	0	0
	R	0	0	0	0	0	0	0	0	0

Majority of the bacterial isolates were sensitive to all groups of antibiotics tested for. There was only 1 Methicillin Resistant Staphylococcus aureus (MRSA) and there were 3 MR-CONS (Table 4 and 5). Both the *Streptococcus pneumoniae*were sensitive to benzylpenicillin , so further antibiotic testing was not done.

Table4. *Antibiotic susceptibility results for Gram positive isolates*

DRUGS	***Staphylococcus aureus* (n=4)**			**CONS (n=7)**		
	S	I	R	S	I	R
Erythromycin	100%	0	0	42.8%	0	57.2%
Clindamycin	100%	0	0	42.8%	0	57.2%
Vancomycin	100%	0	0	100%	0	0
Amikacin	100%	0	0	100%	0	0
Gentamicin	100%	0	0	100%	0	0
Levofloxacin	75%	25%	0	100%	0	0
Penicillin	75%	0	25%	57.2%	0	42.8%
Cefoxitin	75%	0	25%	57.2%	0	42.8%
Cotrimoxazole	100%	0	0	100%	0	0
Linezolid	100%	0	0	100%	0	0

Table5. *Antibiotic Susceptibility results for Gram negative isolates*

DRUGS	***Pseudomonas spp*(n=1)**		
	S	**I**	**R**
Ceftazidime	100%	0	0
Piperacillin/Tazobactum	100%	0	0
Cefipime	100%	0	0
Imipenem	100%	0	0
Colistin	100%	0	0
Polymixin B	100%	0	0
Amikacin	100%	0	0
Gentamicin	100%	0	0
Levofloxacin	100%	0	0

DISCUSSION

The prevalence of microbial keratitis in our study was found to be 43.3%. This is much less when compared to study by Aarti *et al* which was 59.3%, Bharathi *et al* which was 69.59% or other Indian studies [4, 8]. All the patients had unilateral eye involvement. Males were more commonly affected (68.33%).

Similar preponderance has been seen in studies by T Gogoi *et al* and Bashir *et al*, where 63.30% and 65% of males were affected respectively [9, 10].

The ratio of Female: Male is 1: 2.16. This is more attributed to outdoor work done by males. Our study reports people belonging to 40-65 years of age group (46.67%) to be more affected which is similar to Chhangte *et al* where 53.9% cases were of the same age group [5].

Gupta *et al* in New Delhi [11] and Chander J *et al* in North India [12] respectively found 20-40 year old and 20-49 (56.67%) year old age group was more commonly affected. Mahran *et al* [13] also reported 30-40 years age group to be more commonly affected as seen in 22.53% cases. However the age group ranged between 21-65 years is the one commonly involved in various professions hence most prone to various traumas.

Majority of the patients were from rural setup (85%). Major bulk of Indian population lives in rural areas and they are more involved in manual labor thus they have more chances of

injuries and infections as compared to desk job people in urban setup. Trauma as a whole accounted for 56.67% of all the cases thus being the commonest predisposing risk factor. Similar findings of trauma have been seen in Paraguay with 48% cases [14], Eastern Nepal 53%[15], South India with 65% [16] and Eastern India with 83% [17] of all the keratitis cases. Majority of the traumatized patients were outdoor workers (70.59%) [18].

Sitoula RP *et a l*also showed trauma to be commonest risk factor in their study [19]. 47.50% patients enrolled in this study had encountered corneal trauma mainly from vegetative matter and less commonly from dust, insect bite or any other cause. 15% (n=18) of the patients had systemic illness in form of blood pressure, diabetes, rheumatoid arthritis, facial palsy, seizure, thyroid, asthma or heart surgery and 15% (n=18) patients presented with ocular injuries like cataract, glaucoma, vitreous prolapse or any operative procedure. None of our patient had history of using contact lenses.

Out of 120 corneal scrapings performed 65.83% (n=79) of the cultures were sterile and 34.17% showed growth. Total microbial etiology of 34.17% is less as compared to other studies by Tewari *et al* [4] and Bharathi *et al* [20] with recovery of 59.3% and 69.59% respectively but is in accordance with study by Gupta *et al* where 38.3% cases were culture positive [11]. 11.67 %(n= 14) of the cultures showed bacterial growth, 22.50% (n= 27) cultures showed fungal growth. Only 11.67% of the cultures were positive for bacterial isolates. It is lower when compared with other studies like Bharathi *et al* (34.98%), Tewari *et al* (38%), Kumar *et al* (26.5%) and Basak *et al* (28.8%) [4,20,21,22]. This reduction in bacterial corneal ulcers at the referral centres can be credited to better treatment outcome at peripheral centers since the introduction of topical floroquinolones in the late 1990s [23].

Amongst 11.67% of the bacterial cultures positive, 50% (n=7) showed growth of *Coagulase negative Staphylococcus spp*, 28.57% (n=4) of *Staphylococcus aureus* (CONS), 1.67 %(n=2) of *Streptococcus pneumoniae* and 7.14% (n=1) of *Pseudomonas spp*.The isolation rate of *Coagulase negative Staphylococcus spp* (60%) is almost similar to study by Tewari *et al* [4]. The most common bacterial infection in Nepal and South India was *Streptococcus pneumoniae* and in Ghana and Bangladesh it was *Pseudomonas spp*.

Gram positive bacilli were not observed in the present study. This differs from various studies which have demonstrated a 4.0-12.5% incidence [17, 22, and 24]. We isolated only one Gram negative isolate-*Pseudomonas spp.* This is in contrast to other studies where isolation of Gram negative organisms has been as high as 39.7% by Tewari *et al* [4].

Fungal positivity was seen in22.50% of the cultures. This isolation rate is lower when compared to studies done by Sitoula RP *et al* (70%), Leck *et al* (44.1%), Srinivasan *et al* (51.9%) and Basak *et al* (59.3%) but is almost similar to Bharathi *et al* (32.26%) [5]. Out of the 22.50% positive fungal cultures, *Aspergillusspp* as the commonest fungal isolate in 25.93% (n=7) cases of which 14.81% (n= 4) were *Aspergillus flavus* and 11.11% (n=3) were *Aspergillus fumigatus.*

This was followed by *Fusarium spp* which were seen in 22.22% cases (n=6), *Alternariaspp* in 18.52% (n=5), *Curvulariaspp* in 14.81% (n=4), *Candida albicans* in 7.41% (n=2) and 3.7% (n=1) cases showed *Acremoniumspp*, *Aureobasidium pullulans* and *Cladosporium spp*. *Aspergillus spp* has been found to be a common fungal isolate in West Bengal (Basak *et al*), Nepal (Upadhyay*et al*) and Bangladesh (William *et al*) as well [3]. In contrast, the prevalence of fungal corneal ulcers in developed countries was as low as 20% in Florida [25] to 3% in UK [26]. However *Fusarium spp* was the commonest cause of fungal keratitis in South India, Western India and few studies from abroad [8,21,27,28]. *Aureobasidiumpullulans* was an unusual pathogen isolated in the study.

Amidst the scrapings which showed fungal growth on culture media (n=27), 22 of them were positive for KOH mount on microscopy and 5 were KOH negative on microscopy. Amongst 93 of culture negative isolates 20 were positive in KOH mount. Remaining 73 isolates were both KOH negative and culture negative. The sensitivity and specificity of KOH in this study is 81.5% and 78.5% respectively. The sensitivity is almost similar but specificity is lower in this study when compared with Hegan *et al* which was 80% and 93% respectively [74]. For fungal identification through Gram staining, amongst 27 SDA positive cultures only 8 showed fungal hyphae on Gram staining. Out of 93 culture negative isolates only 6 were positive on direct microscopy. Thus sensitivity and specificity 29.6% and 93.5% respectively. Out of 14 blood cultures positive for bacterial

growth, all the isolates were positive on Gram stain. Out of 106 blood cultures negative only 31 were positive for bacterial isolates on microscopy. Thus the sensitivity and specificity of Gram staining for bacterial isolates was found to be 100% and 70.8% respectively.

The combined sensitivity and specificity of Gram stain for both bacteria and fungus is found to be 55.6% and 52.7% respectively. Combining the above findings we can state that the sensitivity of Gram stain (100%) for bacterial detection was similar to the findings of Bharathi *et al* (100%) but higher than that reported by Shrama *et al* (36%), Asbell and Stenson (67%) and Dunlop *et al* (62%) [30]. The sensitivity of 10% KOH (81.5%) was markedly higher than the sensitivity of Gram stain (29.6%) in detection of fungus. In short Gram stain plays a pivot role in establishing diagnosis of bacterial keratitis whereas 10%KOH plays an equally crucial role in establishing the diagnosis of fungal keratitis, *Nocardia* keratitis and *Acanthamoeba* keratitis [30]. Voriconazole showed susceptibility to all fungal isolates. This is consistant with the findings of Saha *et al* and Lalitha *et al* [31, 32].

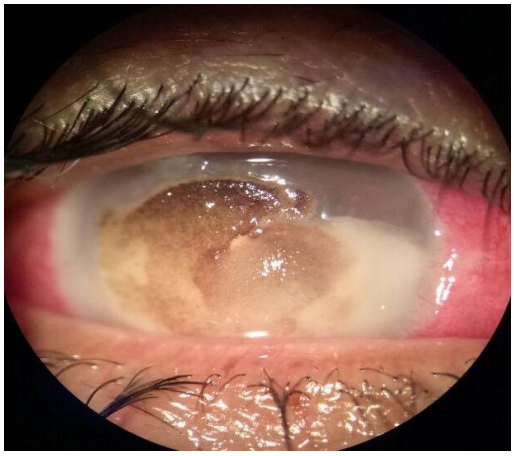

Image1. *Corneal ulcer under slit lamp biomicroscope*

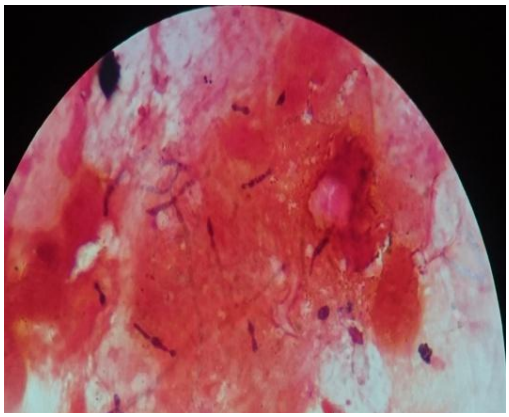

Image2. *Fungal hyphae on Gram stain as seen under microscope (100X)*

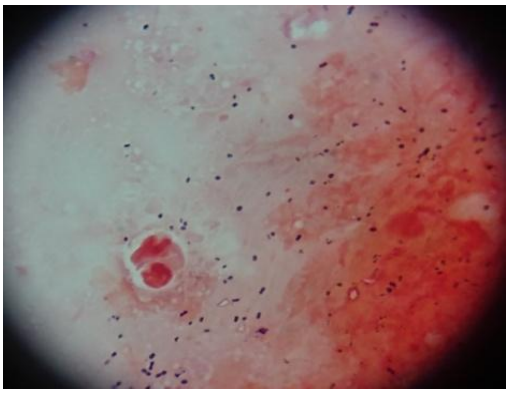

Image3. *Gram positive cocci in pair as seen under microscope (100X)*

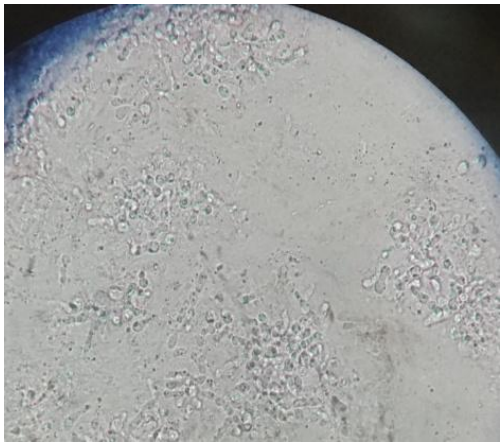

Image4. *Fungal hyphae on 10% KOH mount as seen under microscope (40X)*

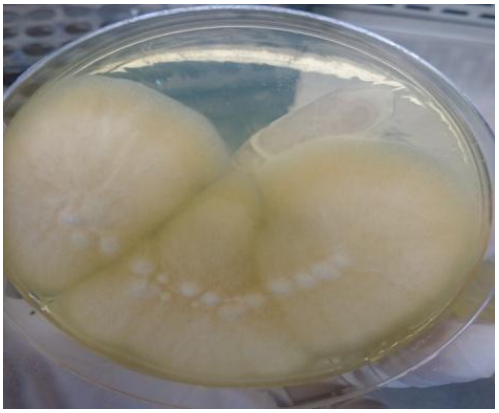

Image5. *Fungal growth on SDA culture plate*

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