



**BACTERIAL AND FUNGAL PROFILE OF CORNEAL ULCERS OF PATIENTS
ATTENDING INTEGRAL INSTITUTE OF MEDICAL SCIENCES &
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Microbiology

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ABSTRACT

A total of fifty corneal ulcer samples were collected & processed for bacterial & fungal study. The study showed that 80% (40 out of 50) samples were positive for growth of either fungus or bacteria, there was no growth seen in 10 samples (healthy individuals). Among 40 positive samples, 13 (32.5%) samples showed positive bacterial isolates whereas 27 (67.5%) samples were positive for fungal keratitis. Corneal ulceration was encountered in all age groups in this study with preponderance among 30-70 years of age group, The prevalence was higher in males than females attributing to 60% of total samples. Male preponderance in bacterial keratitis was 53.8% whereas in fungal keratitis came out to be 62.9% respectively. Among bacterial isolates, gram positive bacteria were more predominant than gram negative bacteria & showing incidence of 83.6% and 23.07% respectively. The commonest bacterial isolates were *Staphylococcus aureus* (53.8%) and *Pseudomonas aeruginosa* (23.07%). Vancomycin, teicoplanin and linezolid showed 100% sensitivity for gram positive bacterial isolates followed by gentamycin (71%) & *P. aeruginosa* isolates showed 100% sensitivity with ceftazidime/clavulanic acid, cefepime, polymixin B and colistin sulphate whereas 66.66% sensitivity was found in gentamycin and tazobactam, aztreonam, meropenem and low sensitivity of 33.33% was seen with piperacillin. All strains of *P.aeruginosa* were found resistant to levofloxacin and ciprofloxacin.

Fungal isolates of *Fusarium spp.* 33.33% , *Aspergillus flavus* 14.81% and *Aspergillus niger* 14.81% were grown in our study. Organic matter was found to be most common predisposing factor in microbial keratitis & unilateral eye was mostly affected in our study.

KEYWORDS

corneal ulcer, Keratitis, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Fusarium*, *Aspergillus*, *Antimicrobial Sensitivity*

INTRODUCTION

Infectious keratitis is the leading cause of corneal blindness in developing countries such as India and South Asia. Corneal ulceration results in 1.5- 2 million new cases of corneal blindness per year and a major public health problem according to World Health Organization (WHO) reports. Fungus is the most common etiological agent which accounts for 30-40% of cases whereas, bacteria account for 13-48% of all cases of suppurative keratitis.

The condition is marked by moderate to intense pain, impaired eyesight, photophobia, red eye and a gritty sensation and often results in corneal scarring and opacification ultimately leading to blindness. Retinal diseases (40-54%) are important cause of blindness in developing nations while it is cataract (44-60%) and corneal diseases (8-25%) for developing countries, (1)

Corneal ulceration occurs due to the host cellular and immunologic responses to the offending agent which may be bacterial, viral, fungal or protozoan organism (2).

MATERIAL & METHODS

Cotton swabs moistened with brain heart infusion broth were used to culture the material from conjunctivae and lid margins of both the eyes. Corneal specimen was taken from leading edge as well as the base of an active ulcer with a sterile surgical blade number 15. Whenever possible multiple scrapings were taken(3).

Samples were processed in the Microbiology laboratory for bacteriological and mycological investigation. Over all 50 samples were taken for the combined study and statistical analysis was done by chi-square test.

Each scraping was inoculated on to blood agar in a row of C- streak and then the material was placed on the two slides, one for gram stain & other for KOH mount. The material was plated on to chocolate agar, MacConkey agar & Sabouraud's dextrose agar. The media were incubated aerobically as per standard protocol. (3, 8)

Aerobic bacterial culture was incubated at 37°C and examined at 24 hours and was further examined daily for at least 7 days before considering it negative. Brain heart infusion broth showing no growth after 3 days was considered negative.

Material was inoculated also on Sabouraud dextrose agar with

chloramphenicol followed by incubation at 25°C, and examined daily. The material was discarded after 3 weeks if no growth was seen (4,8). After the bacterial culture, isolate was obtained in pure subculture & confirmed by biochemical tests. (5)

The antimicrobial susceptibility testing was done by Kirby Bauer's disk diffusion method on Mueller Hinton Agar and interpreted as per Clinical Laboratory Standard Institution guidelines (CLSI. 2018) and antibiotics discs were used according to bacterial isolate (6).

RESULTS

Total 50 samples were enrolled for this study with 30 samples of males (60%) & 20 of females(40%). Total samples affected by bacteria were 13 out of which 7 were males and 6 were females. In 7 males, all 7 were affected by Gram positive bacteria and none were affected by Gram negative. Out of 6 females three each i.e 50% accounted for Gram positive & Gram negative bacteria.(Table 1)

Table 1: Bacterial keratitis.

Total Samples N=50	Male N=30 (60%)	Female N=20(40%)
Bacterial N=13	7 (23.33%)	6 (30%)
Other	23 (76.68%)	14 (70%)

Fungal etiology was elucidated in 17 males & 10 females i.e 56.66% & 50% respectively. (Table 2).

Table 2: Fungal keratitis- Gender distribution.

Total Samples N=50	Male N=30 (60%)	Female N=20 (40%)
Fungus N=27	17(56.66%)	10(50%)
Others	13(43.33%)	10(50%)

Among forty positive cases, 14 resulted by vegetative matter contamination, 18 by crops, 3 by contact lenses, 2 each due to trauma with cow tail & one due to soil contamination. Out of 30 males 19 i.e. 63.33% were having infection in left eye whereas right eye was found infected in 11 cases i.e. 36.66% & similarly out of 20 females 10 i.e. 50% were having infection in left eye while right eye was infected in 10 i.e. 50% of cases.

Out of total of 13 (32.5%) bacterial culture positive cases distribution of percentage positivity in bacteria in that order was *Staph aureus* 7 (53.84%), *S. epidermidis* 1(7.6%), *Diphtheroids* 2 (15.38%) & *Pseudomonas aureginosa* 3 (23.07%) respectively whereas percentage positivity among fungal isolates in a total of 27 (67.5%) cases was

Fusarium spp. 9 (33.33%), *Aspergillus flavus* 4 (14.81%), *Aspergillus niger* 4 (14.81%), *Aspergillus fumigatus* 2 (7.4%), *Candida albicans* 3 (11.11%), *Mucor spp.* (14.81%) and *Trichophyton rubrum* 1 (3.7%). Table 3 Fig 1

Table 3: Etiology wise distribution of bacterial and fungal keratitis

Bacteria N=13	Micro-organisms	Frequency	Percentage
	<i>S.aureus</i>	7	53.84%
	<i>S.epidermidis</i>	1	7.6%
	<i>Diphtheroides</i>	2	15.38%
	<i>P.aeruginosa</i>	3	23.07%
Fungus N=27			
	<i>Fusarium spp.</i>	9	33.33%
	<i>Aspergillus flavus</i>	4	14.81%
	<i>Aspergillus niger</i>	4	14.81%
	<i>Aspergillus fumigatus</i>	2	7.4%
	<i>Candida albicans</i>	3	11.11%
	<i>Mucor.</i>	4	14.81%
	<i>Trichophyton rubrum</i>	1	3.7%

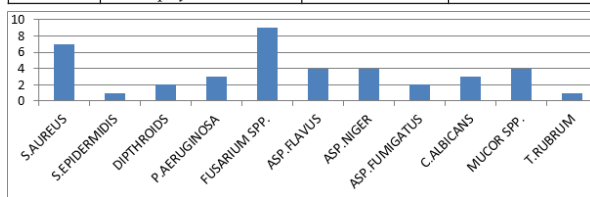


Fig 1

A broad range of antibiotics were implied on the culture positive isolates. Vancomycin, teicoplanin and linezolid showed 100% sensitivity on bacterial isolates followed by gentamycin (71%).

A group of 14 antibiotics were applied on to the *S. aureus* bacteria isolated from 7 samples in this study & it showed 100% sensitivity to Vancomycin, teicoplanin, and linezolid followed by gentamycin showing 71% sensitivity and resistance was found to a marked extent with erythromycin, clindamycin, doxycycline, tetracycline, amikacin, and tobramycin. Ciprofloxacin and levofloxacin were completely ineffective with 100% resistance.

P.aeruginosa isolates showed 100% sensitivity with ceftazidime/ clavulanic acid, cefepime, polymixin B and colistin sulphate whereas gentamycin and tazobactam, aztreonam, meropenem and doripenem showed sensitivity of 66.66 to Tobramycin, Ticracillin with clavulanate and imipenem & low sensitivity of 33.33% was seen with piperacillin. All the strains of *P.aeruginosa* were found resistant to levofloxacin and ciprofloxacin.

Fig 2 & 3 depict antibacterial sensitivity pattern of two common bacterial isolates such as *S. aureus* & *P.aeruginosa*.

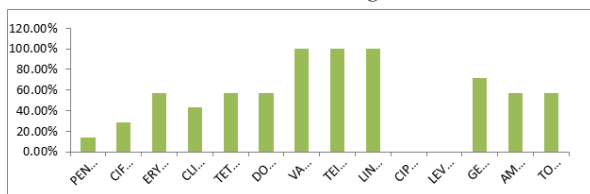


Fig 2-Antimicrobial Sensitivity of Staphylococcus aureus.

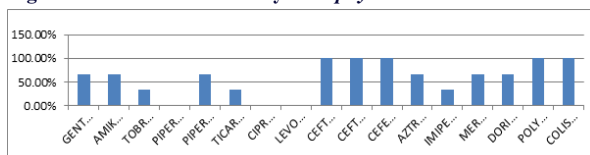


Fig 3 Antimicrobial Sensitivity of Pseudomonas aeruginosa

DISCUSSION

When our study was compared with other studies, a study from Andhra Pradesh, south India, out of 100 corneal ulcer patients which were reported 58% were males and 42% females. Corneal ulcer

showed prevalence in the economically active age group of 21-50 yrs. Culture positivity was obtained in 73(73%) cases. Out of 73 cases, a history of recent corneal injury was obtained in 72.6% of patients who had corneal injury due to vegetative matter (43.39%). Of the 24 bacterial isolates 16 (66.67%) were gram positive and 8(33.33%) gram negative. *P. aeruginosa* (29.17%) and *S. aureus* (25%) were the predominant isolates followed by *S. epidermidis* (20.83%). Of the 52 fungal isolates, yeasts constituted only 3(5.76%) & 2 isolates of moulds remained unidentified. *Fusarium spp.* (36.53) was predominant isolate followed by *Aspergillus spp.* (7)

In another study from North India at SKIMS, out of 80 corneal ulcer patients, 42(52.5%) had significant growth of microorganisms while 38(47.5%) showed no growth while pure fungal growth was seen in 10(12.5%) cases. In 32 bacterial isolates, 22 were gram positive cocci and 10 gram negative bacilli. *S. pneumoniae* was the most commonly isolated bacterium in this study, accounting for 31.25% of all bacterial isolates, Other gram positive organism isolated were *S. epidermidis* (18.75%) followed by *S.aureus* (9.37%) and *Micrococcus* (3.12). *P. aeruginosa* was the most frequently isolated gram negative bacterium accounting for 12.5% of all bacterial cultures. This was followed by *Klebsiella aerogenes* 9.37% and *Enterobacter areogens* i.e.13.12%. In 10 fungal isolates 4(40%) were *Aspergillus fumigatus* and 2 each(20%) were *fusarium spp* and of *Curvularia spp* (8)

One more study conducted at Bangalore, Karnataka India showed that out of 312 patients, 117 (37.5%) were positive for smear and culture. Of 117 patients, the age range of 41-60 years was the higher affected group & bacteria were found in 52 cases as etiological agent i.e.44.5% and fungi accounted for 58 (49.5%) cases. Risk factors seen were trauma in 54 (46%) cases followed by diabetes mellitus in 31 (26.5%), contact lens usage in 22 (19%) and corticosteroid therapy in 4 (3.5%) cases. The common fungus isolated was *Fusarium* in 36 (31%) followed by *Aspergillus spp.* in 13 (11%) cases. Common bacterial isolates were *S. aureus* in 21 cases (18%) followed by *P.aeruginosa* in 10 cases & *Streptococcus pneumoniae* in 9 cases, *Klebsiella pneumonia* was found in 4 cases, All Gram positive cocci were susceptible to vancomycin followed by gatifloxacin, chloramphenicol and moxifloxacin respectively whereas all gram negative bacilli were susceptible to gatifloxacin followed by moxifloxacin and ofloxacin.(1)

In a study conducted in Kerala India, out of 200 patients, 122(61%) were males and 78(39%) were females. Co-existing ocular diseases predisposing to microbial keratitis were identified in 24 (12%) patients, compared to another predisposing risk factors in 177 (88.5%) patients. A history of corneal injury was recorded in 157 (78.5%) patients, of which 83 (53%) had corneal injury with vegetative matter and 12 (7.6%) had injury due to fingernails. The surprising finding was that out of the 200 cases 10 (5%) had taken treatment from quacks/village healers to remove foreign body from eyes. Prior treatment with topical medication was noted in 116 (58%) of the patients of which 72 (62.06%) were on topical antibiotics, 15 (12.93%) were on antifungals, and 29 (25%) were on corticosteroids

In this study fungal isolates were present in 54% of specimens. All these patients had a significant history of trauma. The commonest isolates were *Fusarium species* (33.3%) and *Aspergillus flavus* (18.5%), followed by *Aspergillus fumigatus*(7.4%) and *Aspergillus niger* (14.8%)(7). *Mucor species* and *Trichophyton rubrum* were also found in 14.8% and 3.7% cases respectively. Among yeasts, *Candida albicans* was the only isolated species having incidence of 11.1% in this study. (2)

REFERENCES

- Chittur Y. Ranjini, MD; Vishnu V. Waddepally, MDJ Ophthalmic Vis Res 2016; 11 (4): 363-367
- Anil Kumar1,&, Snehal Pandya2 , Ghanshyam Kavathia3 , Sejul Antala4 , Molly Madan5 , Tanuja Javdekar6 Pan African Medical Journal. 2011; 10:48
- Liesegang TJ, Forester RK. Spectrum of microbial keratitis in South Florida. Am J Ophthalmol 1980;
- Ormerod LD, Hertzmark E, Gomez DS, Stabiner RG, Schanzlin,DJ, Smith RE. Epidemiology of microbial keratitis in southern Calofornia. Ophthalmol 1987.
- Mackie & McCartney. Practical Medical Microbiology, 14th ed; Churchill living stone; 68-70, 9
- CLSI Guidelines (2018) Performance Standards for Antimicrobial Susceptibility Testing, 28th Edition (M100S).
- Dr. Ratnakumari G , Jayalakshmi L , Sirisha T , Rajeswara Rao V , Jyothi Padmaja Etiological Diagnosis and Epidemiological Characteristics of Microbial Keratitis at Regional Eye Hospital, Visakhapatnam Scholars Academic Journal of Biosciences, 2015;3(7):627-632
- Gulnaz Basheer, Azra shah, Manzoor A Thokar, Sabia Rashid, Saman Shakeel Bacterial & fungal profile of corneal ulcers-prospective study Indian J Pathol Microbiol 2005; 48(2):273-277.