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Original Research Article

Microbial Profile of Bacterial Keratitis in a Tertiary Care Hospital in Southern Odisha

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Abstract

Background: The emergence of bacterial resistance towards topical antimicrobial agents increases the risk of treatment failure with potentially serious consequences in bacterial keratitis.

Aim: To arrive at microbial profile of bacterial keratitis in patients attending ophthalmology out patient department of a tertiary care hospital in Southern Odisha.

Materials and Methods: A Prospective Cross-sectional study was done in a tertiary care center from October 2018- March 2021 in OPD patients. Samples were collected by corneal scrapping and Culture and sensitivity testing done. Results were compiled and analysed.

Results: Out of the 64 cases in our study, 62.5% were male, and common age group was 51-60. The most common predisposing risk factor for keratitis was trauma (43.7%). Most of cases of keratitis were farmers (62.5%) by occupation. 71% of samples were culture positive. The most common bacteria isolated in our study was Staphylococcus aureus, and showed 100% sensitivity to vancomycin and linezolid, 75% to gentamicin and 55% to ciprofloxacin.

Conclusion: Successful management of microbial keratitis requires correct identification of the etiological pathogen and further the appropriate choice of antibiotics, a concept called antibiotic stewardship.

Keywords: Microbial Profile, Bacterial Keratitis, Antibiotic Sensitivity, Corneal Scrapping.

Introduction

Ocular infections still continues to be a major cause of morbidity and blindness in developing countries like India. Any part of the eye can be infected by microbes from the environment. They can form transient flora or invade the tissue and cause infection. [1] Pathogenic microorganisms cause ocular disease due to their virulence and host's reduced resistance. Factors like personal hygiene, living conditions, socio-economic status, decreased immune status etc. play a role. [1,2]

Microbial keratitis is a common sight-threatening ocular infection. The spectrum of microbial keratitis varies with geographical location, local climate and occupational risk factor. Different bacterial agents like Pneumococci, Staphylococci,

Pseudomonas and Nocardia species have been linked with keratitis. [1,3]

On a global level, predisposing risk factors for microbial keratitis vary tremendously with geographical location. Thus, a guideline for initial treatment can be formulated only based on initial smear findings in the clinical sample. Hence, it is imperative to know the local aetiology of keratitis in a particular region for empirical treatment.

Since the discovery of penicillin, many antibiotics have become a major part in any doctor's armamentarium. Discovery of penicillin by Alexander Flemming is considered as one of the major discoveries in the last century in terms of changing the world by making huge impact in reducing morbidity and mortality in human and animals due to infections and the economic gains had been phenomenal. Later many classes of antimicrobial spectrum were introduced in a short time.

Ophthalmological practice also involves use of topical, oral parenteral and intravitreal antibiotics for surgical prophylaxis and ocular infection. However the problems of drug resistance to many drugs slowly emerged and increased steadily jeopardizing the progress we achieved so far. The indiscriminate use of antibiotics has led to the development of resistance to most commonly used antimicrobial medications. [4]

The emergence of bacterial resistance towards topical antimicrobial agents increases the risk of treatment failure with potentially serious consequences. Therefore, the changing spectrum of microorganisms involved in ocular infections and the emergence of acquired microbial resistance dictate the need for continuous surveillance to guide empirical therapy to prevent the advent of the dreaded post antibiotic era.

Aims & Objectives

To arrive at microbial profile of bacterial keratitis in patients attending ophthalmology out patient department of a tertiary care hospital in Southern Odisha. To study age, gender and occupation wise distribution, risk factors and clinical profile of bacterial keratitis patients..

Materials and methods

Study design: Prospective Study.

Study population: All patients having bacterial keratitis attending eye OPD within the study period.

Study period: October 2018- March 2021

Inclusion criteria: All the patients attending eye OPD with provisional diagnosis of bacterial keratitis.

Exclusion criteria: Patients not giving consent for sample collection and suspected fungal or viral keratitis.

Personal information of all patients was recorded. Detailed history of duration of keratitis, medication used, any history of intervention taken for related complaints, particularly antibiotic usage was noted. After patients were subjected to detailed local examination which include best corrected visual acuity (BCVA) with Snellen test for both distant and near, extra ocular movements, torch light , detailed slit lamp examination.

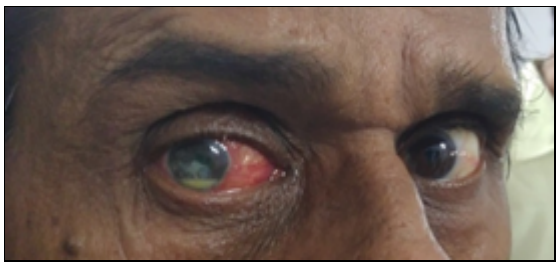

Figure 1

Corneal scraping was performed under strict aseptic conditions by an ophthalmologist using a sterile number 15 Bard-Parker blade. [5] Subsequently, material was obtained from scraping of the leading edge and base of each ulcer. Processing of samples was done by inoculating in Brain heart infusion broth and then subculture was done after 24 hours. Subculture was done on Blood agar, Mac Conkey's agar and incubated at 370 c for 24 -48 hours aerobically.

Following day, the colonies were observed using a hand lens and staining was done from all the inoculated plates. Microbial cultures were considered positive only if growth of the same organism was demonstrated on two or more solid media; or there was semiconfluent growth at the site of inoculation on one solid medium associated with the identification of the organism of appropriate morphology and staining characteristics on Gram stained corneal smears. [6]

Gram positive cocci were further identified by enzymatic tests like catalase and coagulase, differentiation disks like bacitracin, optochin and inoculating on bile esculin agar. [7] Gram-negative bacilli were identified by detecting motility, enzymatic tests like catalase, oxidase tests and biochemicals like sugar fermentation (Glucose, Lactose, Sucrose and Mannitol), Oxidation Fermentation, Urea hydrolysis, Nitrate reduction, citrate utilisation, Triple sugar iron agar, H_2S production and Methyl red and Voges Proskauer and for indole production. [8]

All the keratitis samples were examined with 10% KOH wet mount for observation of fungal hyphae. In case Fungal hyphae were present, sample was not send for bacterial culture, and excluded from study. Antimicrobial susceptibility testing of all the aerobic bacterial isolates was performed by Kirby-Bauer disc diffusion method and interpreted using Clinical and Laboratory Standards Institute (CLSI). [9]

Figure 2

For the gram positive bacteria commercially available discs such as Linezolid (Lz) 30µg, Vancomycin (Va) 30µg, Cefoxitin (Cx)30µg, Gentamycin (Gen)10µg, Ciprofloxacin (CIP)5µg, Moxifloxacin (MO)5µg, were used.

For gram negative bacilli commercially available discs Ciprofloxacin (CIP)5µg, Moxifloxacin (MO) 5µg, Tobramycin (TOB) 10µg, Ceftazidime (CAZ) 30µg), Piperacillin tazobactam (PIT) (100/10µg) discs were used.

For staphylococcus aureus a zone diameter of <21mm in Cefoxitin 30ug discs were interpreted of Methicillin Resistance. [10]

Results

Table 1: Age wise distribution

Age groups (years)	Number	Percentage
0-10	5	7.5%
11-20	3	4.5%
21-30	5	7.5%
31-40	9	46.6%
41-50	13	19.7%
51-60	16	24.7%
61-70	11	16.1%
71-80	2	4.5%
81-90	1	1%
Total	64	100%

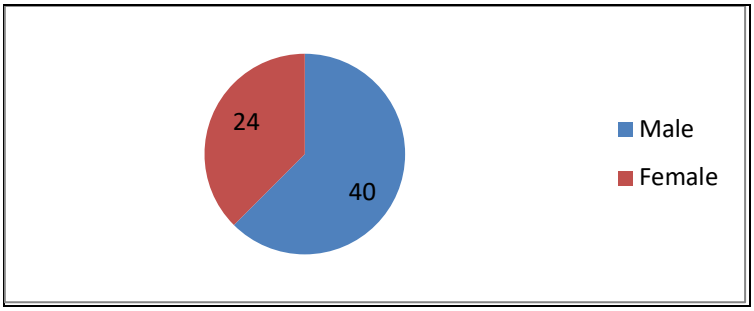

Figure 3: Sex wise distribution

Male 40 (62.5%) Female 24 (37.5%)

Table 2: Risk factors for keratitis

Risk factor	Number of cases	Percentage
Trauma	34	53.1%
H/O of steroid intake	4	6.25%
Prior Surgery	6	9.3%
H/O Diabetes mellitus	14	21.8%
Pre-existing ocular disease	3	4.6%
Contact lens wear	3	4.6%
Total	64	100%

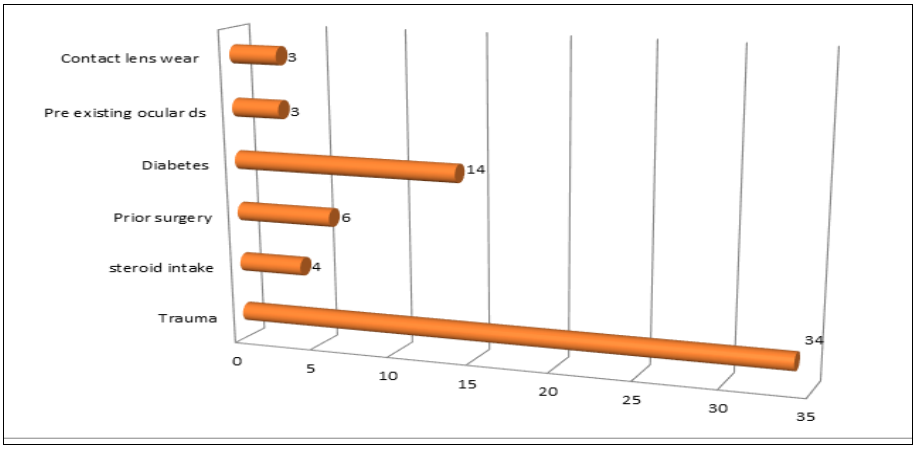

Figure 4: Risk factors for keratitis

Table 3: Occupation wise distribution

Ocupation	Number	%
Farmers	40	62.5%
Students	10	15.6%
Housewife	8	12.5%
Labourer	6	9.3%
Total	64	100%

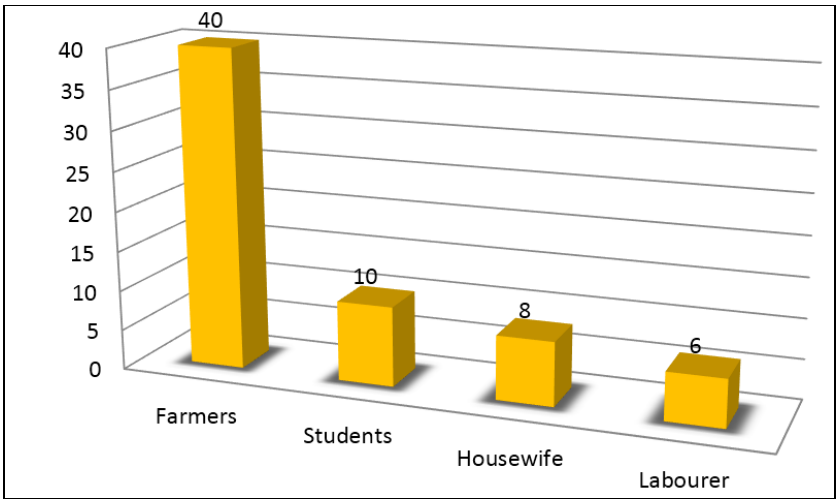

Figure 5: Occupation wise distribution

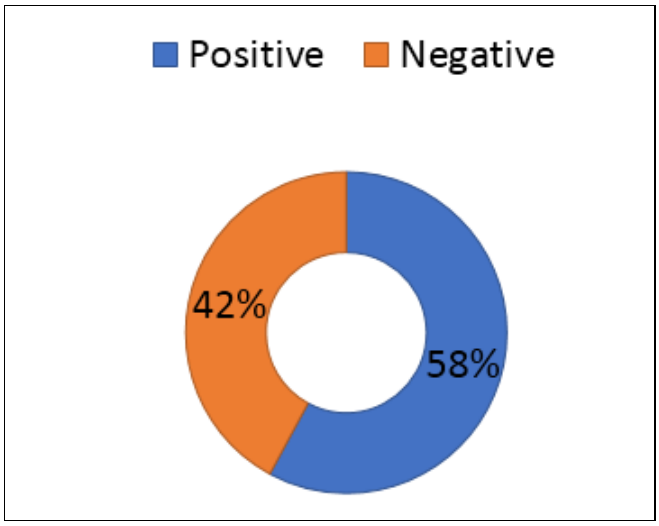

Figure 6: Bacterial isolates based on Gram's stain

Table 4: Bacterial isolates

Bacterial species	Number (Percentage)
S. aureus	20(43%)
Coagulase negative Staph.	-
Enterococcus	4(8.6%)
Clostridium perfringens	-
Klebsiella spp.	5(10.8%)
Citrobacter spp.	4(8.6%)
Enterobacter	2(4.3%)
P. aeruginosa	4(8.6%)
P. vulgaris	1(2.1%)

P. mirabilis	1(2.1%)
A. baumanii	3(6.5%)
A. lwoffii	2(4.3%)

Table 5: Sensitivity pattern of gram positive bacteria

Organism	Linezolid	Vancomycin	Cefoxitin	Moxifloxacin	Gentamycin	Ciprofloxacin
***Staph. aureus* (n=20)**	20(100%)	20(100%)	12(60%)	12(60%)	15(75%)	11(55%)
Enterococcus (n=4)	4(100%)	4(100%)	---	2(50%)	---	1(25%)

Table 6: Sensitivity pattern of major gram negative bacteria

Organism	**Ceftazidime**	**Piperacillin tazobactam**	**Gentamycin**	**Ciprofloxacin**	**Tobramycin**	**Moxifloxacin**
Klebsiella spp.	3(60%)	3(60%)	4(80%)	2(40%)	3(60%)	1(20%)
Citrobacter sp	3(75%)	*3(75%)*	*2(50%)*	*2(50%)*	*1(25%)*	*2(50%)*
P.aeruginosa	*3(*75%)	*3(75%)*	*3(75%)*	*1(25%)*	*2(50%)*	*2(50%)*

The predominant affected age group in our study was 51-60 years.(Table 1) In the present study overall, males are predominantly affected with keratitis (Figure 1), which may be because of involvement of males in outdoor activities and thus prone for injury.

The most common predisposing risk factor for Keratitis in our study was trauma, followed by Diabetes mellitus.(Table 2) In this study in cases of keratitis, the most common occupation was farmers.(Table 3) Farming might be exposing them to trauma due to vegetable matters.

Out of 64 samples collected,46(71%) were culture positive, and were analysed in this study. 58% of isolates were gram positive bacteria ,while 42% were gram negative.(Figure 4). In our study, most common bacteria causing ocular infections is S. aureus accounting for 20 isolates (43%).(Table 4)

Though second most common group, enerobacteria is nowhere near as prominent as Staph.aureus. This low number of Enterobacteriaceae may be due to reduction in hand-faecal contamination and/ or increased access to potable water sources in the study area.

P. aeruginosa, a predominant cause of microbial keratitis and the predisposing risk factors are history of trauma and use of contact lens. It produces exotoxin A, causing tissue necrosis leading to corneal ulceration which accounted for 8.3% of bacterial isolates in our study.

In our study among gram positive bacteria , all the isolates were 100% sensitive to Linezolid and Vancomycin, (Table 5). Among gram negative bacteria, tested antibiotics like fluroquinolones and aminoglycosides showed varying resistance (Table 6)

Discussion

The predominant affected age group in our study was 51-60 years similar to study done by Suja .C et al. [11] where the affected age group belonged to more than 60 years, which may be due to poor cell mediated immunity, malnutrition and comorbidities.

In the present study overall, males are predominantly affected which is similar to various studies. [11,12] because of involvement of males in outdoor activities and thus prone for injury. However, females were commonly affected in Keratitis in studies done by Al Yousouf N et.al [13].

The most common predisposing risk factor for Keratitis in our study was trauma, which differs from study done by Green et.al [14] who have reported contact lens 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. However, Pre-existing ocular diseases and topical steroid usage was the predominant risk factor in study done by Varaprasathan G et.al. [15]

In this study in cases of keratitis, the most common occupation was farmers (62.5%) similar to study done by Srinivasan et.al [16] and Reddy et.al. [17]

In our study 46(71%) showed culture positivity similar to study done by Assudani et al [18] demonstrating a culture positivity of 69.6%. The ability to isolate the causative organism depends on a number of factors which include the amount of inoculum, site from which specimen was taken and the media used for culture. The culture negativity may be because of the fact that previous treatment was not an exclusion criterion, and the cases treated with hidden antibiotics and unknown medications should be considered as possible causes of negative culture results.

58% of isolates were gram positive and rest 42% were gram negative, which is similar to study done by Reddy et al [17]. They reported majority of the isolates were Gram positive (70.5 %) and the rest 29.4 % were Gram negative bacteria.

Staphylococcus aureus (43%) was the most common bacteria in our study causing corneal ulcer similar to study done by Gupta. S et.al. [19] Enterococcus spp. (8.6%) was also seen in our study among gram positive isolates. Among the gram negatives isolates in the present study, Klebsiella spp (10.8%) was predominant. In our study, among cases of Keratitis Citrobacter spp 4(8.6%) Enterobacter spp. 2 (4.3%)., 1 isolate each (2.1%) of Proteus vulgaris, Proteus mirabilis ,3 isolates (6.5%)Acinetobacter baumanii and 2 isolates (4.3%)Acinetobacter lwoffii was also isolated similar to study by Bashir et.al. [20]

P. aeruginosa,a predominant cause of microbial keratitis and the predisposing risk factors are history of trauma and use of contact lens. It produces exotoxin A, causing tissue necrosis leading to corneal ulceration which accounted for 8.3% of bacterial isolates in our study that matches with the findings of Kaliamurthy et al [21] who reported 9.7% of Pseudomonas isolates but varies with study G. Singh et al. [22] where it was 28.1%.

In our study among gram positive bacteria, all the isolates were 100% sensitive to Linezolid and Vancomycin. Staphylococcus aureus showed relatively low resistance rate to gentamicin (25%) suggesting that aminoglycosides are still reasonably good treatment options for bacterial infections.

Staphylococcus aureus is the common cause of ocular infections and other nosocomial infections. It was once susceptible to penicillin but widely resistant organisms have emerged. The introduction of methicillin initially solved the problembut later, strains resistant to methicillin also developed. Marked variation in the prevalence of MRSA ocular infections are seen geographically and at different time points. Thirteen (65%) isolates were sensitive to Cefoxitin (MSSA) and rest seven(35%) were resistant to Cefoxitin (MRSA).

All the gram-negative isolates showed maximum sensitivity to Gentamycin and Piperacillin tazobactam and were least sensitive to ciprofloxacin and moxifloxacin. This means, Moxifloxacin, one of the most commonly prescribed in

our Opd, may not cover for Gram negative bacteria.

Conclusion

The acute infections of the eye are caused by a vivid range of microbial pathogens encompassing bacteria, virus, fungus etc. Bacterial keratitis is one such infection, if left untreated, can lead to damage in structure of eye, with possible complications such as blindness. Resistant pathogenic organisms are increasingly encountered due to widespread use of anti microbial agents without proper microbial diagnosis. So ocular microbiology and the newer technologies paveway to better understanding of ocular diseases and their proper diagnosis and treatment. Thus successful management of microbial keratitis requires correct identification of the etiological pathogen and further the appropriate choice of antibiotics, a concept called antibiotic stewardship.

Patient Consent

Written informed consent was taken from all patients for their participation in the study. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity.

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References

1. Michelle Callegan, Meredith Gregory-Ksander, Mark Willcox, Susan Lightman, Ocular Inflammation and Infection, International Journal of Inflammation, 2012; 1-4.
2. Hemavathi, Pooja Sharma, Poornima Shenoy, Profile of Microbial Isolates in Ophthalmic infections and Antibiotic Susceptibility of the Bacterial Isolates: A Study in an Eye Care Hospital, Bangalore, Journal of Clinical and Diagnostic Research. 2014 Jan; 8(1): 23-25
3. Mebrahtu Teweldemedhin et al, Bacterial profile of ocular infections: A systematic review, BMC Ophthalmology. December 2017; 126-31.
4. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. P T. 2015;40(4):277-283.
5. Nema H V, Diagnostic procedures in ophthalmology-3rd edition, Kaylee brothers, 2014; 264-92.
6. Wilhelmus KR, LIesegang, Osato MS, Jones DB. Cumitech, Laboratory Diagnosis of Ocular Infections, Washington DC: American Society for Microbiology; 1994.
7. Leck AK, Thomas PA, Hagan M, Kaliamurthy J, Ackuaku E, John M, et al. Aetiology of suppurative corneal ulcers in Ghana and south India, and epidemiology of fungal keratitis. Br J Ophthalmol 2002; 86:1211-15
8. Marmion BP, Simmons A, Mackie and McCartney Practical Medical Microbiology. 14th Edition,Chapter 41, Churchill Livingstone 1996:95-100
9. https://asm.org/getattachment/2594ce26-bd44-47f6-8287-0657aa9185ad/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Protocol-pdf.pdf
10. Panda RK, Mahapatra A, Mallick B, Chayani N. Evaluation of Genotypic and Phenotypic Methods for Detection of Methicillin Resistant Staphylococcus aureus in a Tertiary Care Hospital of Eastern Odisha.J Clin of Diagn Res. 2016; 10(2): DC19-DC21.
11. Suja C, Jasmine. Vinshia and Uma Mageswari S.S, Bacterial and Fungal profile of Ocular infections in a tertiary care hospital, Int. Curr. Microbi iol. App. Sci., 2019; 8(02): 2081-2089.

12. Miller D, Galor A. Current thoughts in fungal keratitis: Diagnosis and treatment. Curr Fungal Infect Rep. 2013;7(3):209-218.
13. 13.Al Yousaf N, Microbial Keratitis in Bahrain, Clinical and Microbiological study, Middle East J Ophthal, 2007, April, 122-29
14. Green M, Apel A, Stapleton F. Risk factors and causative organisms in Microbial Keratitis, Cornea, 2008, 12, 24-29.
15. Varaprasathan G Miller K Lietman T. Trends in the etiology of infectious corneal ulcers at the F. I. Proctor Foundation. Cornea. 2004; 23:360–364.
16. Srinivasan et al, Incidence of corneal ulceration in Madurai district, South India, Ophthalmic Epidemiol. 1996 Dec.
17. Reddy P Siva, Satyendran O M, Satapathy M, Kumar H Vijaya, Reddy P, Mycotic keratitis, 1972; 20(3):101-1085.
18. Assudani HJ, Pandya JM, Sarvan RR, Sapre AA, Gupta AR, Mehta SJ. Etiological diagnosis of microbial keratitis in a tertiary care hospital in Gujarat. Natl J Med Res. 2013; 3(1): 60-62.
19. Reena Gupta, Chekitaan Singh, Bella Mahajan, AK Khurana, Microbiological regional profile of infective keratitis, Indian Journal of Clinical and Experimental Ophthalmology, October – December 2015;1(4):264-272.
20. Bashir G, Shah A, Thokar MA, Rashid S, Shakeel S, Bacterial and fungal profile of corneal ulcers--a prospective study, Indian J Pathol Microbiol. 2005 Apr;48(2):273-7.
21. Kaliamurthy J, Ackuaku E, John M, et al. Aetiology of suppurative corneal ulcers in Ghana and south India, and epidemiology of fungal keratitis. Br J Ophthalmol 2002; 86:1211-15.
22. G Singh, Bhatia, Corneal ulcers in northern India- A correlated histological and microbiological study, Indian J Ophthalmol, 1974;1-7.