Bacteriology and antimicrobial susceptibility pattern of external ocular infections in rural tertiary care teaching hospital

Khaja Moinuddin[1,*], Haja Abdul Nazeer[2], Anandi[3], Kalai Vani[4], Bhanu Prakash[5], Murlidhar[6]

[1]Tutor, [2]Associate Professor, [3]Professor, Dept. of Microbiology, [4]Professor, [5]Associate Professor, Dept. of Pharmacology, Vinayaka Mission Medical College, [6]Associate Professor, Dept. of Ophthalmology, Sambaram Institute of Medical Sciences

***Corresponding Author:**
Email: moinnewlook@gmail.com

Abstract

Background: This study was aimed at determining the prevalence of external ocular infections and susceptibility profile of associated bacteria along with special reference to methicillin-resistant Staphylococcus *aureus* (MRSA).

Materials and Methods: During the study period, 227 ocular samples were collected from patients clinically diagnosed with external ocular infections such as conjunctivitis, keratitis, and blepharitis. All samples were processed for direct microscopy, culture and identification by standard methods. Susceptibility testing was done by Kirby-Bauer method as per Clinical and Laboratory Standard Institute (CLSI) guidelines. Methicillin resistance was determined by cefoxitin disc diffusion method. All the analysis was performed using simple percentage method

Results: Out of 227 ocular specimens, 158 specimens yielded significant bacterial growth. The isolation rate was 70.54% in conjunctivitis (91 of 129), 77.05% in keratitis (47 of 61) and 54.05% in blepharitis (20 of 37). Newborn to under two years of age group recorded highest cases 73(46.20%). The predominant bacterial species isolated was staphylococcus *aureus* 39 (27.4%) followed by Pseudomonas *aeruginosa* 35 (22.15%). Overall prevalence of methicillin-resistant Staphylococcus *aureus* was 5 (12.82%). All Gram positive cocci were susceptible to vancomycin including methicillin-resistant Staphylococcus *aureus*. All Gram positive cocci and Gram negative bacilli showed good response towards amikacin and moxifloxacin in a range of 60-100%.Apart from vancomycin, all methicillin-resistant Staphylococcus *aureus* isolates remained susceptible to chloramphenicol.

Conclusion: Gram-positive cocci were the most frequent bacteria isolated from ocular infections and were sensitive to moxifloxacin and vancomycin, while gram negative isolates were more sensitive to amikacin.

Keywords: Bacteriology, External Ocular Infections, Antibiogram

Access this article online	
Quick Response Code:	Website: www.innovativepublication.com
	DOI: 10.5958/2394-5478.2016.00046.7

Introduction

The eye, an organ of the human body, is impermeable to almost all external infectious agents,[1] though the ocular surface invariably is exposed to a wide array of microorganisms.[2] However, pathogenic microorganisms cause ocular disease due to virulence and host's reduced resistance because of the factors like personal hygiene, living conditions, socio-economic status, decreased immune status, etc. The areas of the eye that are frequently infected are the conjunctiva, lid and cornea.[3] External microbial infections of the eye are usually centralized in one place but may frequently be distributed to other tissues. The conjunctiva and eyelid have a normal microbial flora controlled by its own mechanism and by the host. Any change of this normal flora leads to ocular infections.[4] The bacterial etiology and their susceptibility and resistance patterns may vary with geographic location according to the local population.[5] Thus, the current trends in the etiology of bacterial ocular infections and their susceptibilities must be updated to make a rational choice of initial antibiotic therapy.

The most common microorganisms causing ocular infections include Staphylococcus *aureus*, Coagulase negative staphylococci, Streptococcus, Corynebacterium, Bacillus, Nocardia, Pseudomonas *aeruginosa*, Enterobacteriaceae, Nonfermentors, and others.[6]

Staphylococcus. *aureus* is the major ophthalmic bacterial pathogen isolated from various ocular infections.[7] Treatment of Staphylococcus *aureus* infections has become more complicated with emergence of methicillin-Resistant *Staphylococcus aureus*(MRSA) strain in 1961.[8] Despite the fact that MRSA is one of the major topics in clinical microbiological research, very little is known about the prevalence and epidemiology of eye infections due to methicillin-sensitive *S. aureus*(MSSA) or methicillin-resistant Staphylococcus *aureus*(MRSA). Methicillin resistance is conferred on the organism by the presence of a unique mobile genetic element called the staphylococcal cassette chromosome carrying the mecAgene (SCC*mec*). A recent Indian study reports that about 25% of ocular infections are caused by *S. aureus* in South India.[1]

Hence, this study was aimed at determining the bacteriology of external ocular infections and their susceptibility profile along with special reference to MRSA from patients at a rural tertiary hospital.

Material and Methods

This cross sectional study included 227 ocular samples for microbiological evaluation from patients clinically diagnosed with external ocular infections such as conjunctivitis, keratitis, and blepharitis at rural teaching tertiary care hospital for a period of 1 year between November 2014 and November 2015. Patients clinically diagnosed with external ocular infections and those who were willing to give informed consent are included in the study while those with trachoma, peripheral ulcerative keratitis, viral keratitis, allergic and viral conjunctivitis, severe ocular trauma, with recent ocular surgery and positive history of antimicrobial therapy within seven days were excluded.

All the patients were examined clinically using slit-lamp bio-microscope .Specimens for culture and smear were obtained by scraping the eyelid margin using a sterile blade (#15) on a Bard-Parker handle and by swabbing the lid margins with sterile broth-moistened cotton swabs in cases of blepharitis. Similarly, specimens were also obtained from the corneal ulcers by scraping. Conjuctival cultures were obtained by wiping a broth moistened swab across the lower conjunctival cul-de-sac in conjunctivitis cases.

The obtained specimens were inoculated directly onto the blood agar, chocolate agar (5-10% CO2), and Mac Conkey agar. All the inoculated plates were incubated aerobically at 37^0C for 18-24 hours. A part of the collected specimens was subjected to Gram's staining. A positive culture was defined as a growth of the same organismson more than two solid phase media or confluent growth onone solid medium. A standardized protocol was followed for each ocular specimen for evaluation of significant microbiological features.[10]

Antibiotic sensitivity testing

Antimicrobial susceptibility of the isolates was determined against the following antibacterial agents agents (vancomycin-30mcg, amikacin-30mcg, gentamycin-10mcg, ofloxacin-10mcg ciprofloxacin-5mcg, moxifloxacin-5mcg, cefazolin-30mcg, tobramycin-10mcg, chloramphenicol-30mcg) by Kirby Bauer disk diffusion method on Mueller Hinton agar plates according to Clinical and Laboratory Standard Institute (CLSI) guidelines.[11]

Detection of MRSA by Cefoxitin Disc Diffusion Test: The Cefoxitin disc diffusion method was carried out on Mueller-Hinton agar by using a 30 µg cefoxitin disc. An inhibition zone diameter of ≤ 21 mm was reported as methicillin resistant and a diameter of ≥ 22 mm was considered as methicillin sensitive according to CLSI guidelines.[11]

Quality control strains

ATCC control strains [E.*coli* ATCC 25922, Pseudomonas ATCC 27853]
Methicillin sensitive *S. aureus*(MSSA) ATCC 25923
Methicillin resistant *S. aureus*(MRSA) ATCC 43300 – were used as the negative and positive controls, respectively.
Statistical analysis was performed using simple percentage method.

Results

A total of 227 ocular specimens were submitted and processed during the study period. Of these, 158 were culture positive giving an overall isolation rate of 69.60%. The isolation rate was 70.54% in conjunctivitis (91 of 129), 77.05% in keratitis (47 of 61) and 54.05% in blepharitis (20 of 37). Each specimen yielded single organism. The predominant bacterial species isolated was S*taphylococcus aureus* 39(24.68%) followed by *Pseudomonas aeruginosa* 35 (22.15) (Table 1).

Table 1: Distribution of bacterial isolates in external ocular infections

Bacteria isolated	Conjunctivitis(%)	Keratitis(%)	Blepharitis(%)	Total
Staphylococcus aureus	22(56.41)	11(28.21)	6(15.38)	39(100)
Pseudomonas aeruginosa	14(40)	19(54.29)	2(5.71)	35(100)
Coagulase negative staphylococci	15(55.55)	7(25.93)	5(18.52)	27(100)
Streptococcus pneumonia	10(65.50)	2(12.50)	4(25)	16(100)
Streptococcus viridians	11(84.62)	2(15.38)	-	13(100)
Eschericia coli	7(58.33)	2(16.67)	3(25)	12(100)
Klebsiella species	5(62.50)	3(37.50)	-	8(100)
Proteus mirabilis	3(100)	-	-	3(100)
Citrobacter	2(66.67)	1(33.33)	-	3(100)
Moraxella	2(100)	-	-	2(100)

The age distribution showed isolation of 73(46.20%) and 41(25.95) in the age group of newborn to under two years and three to under 11 years, respectively.(Table 2)

Table 2: Distribution of bacterial isolates in relation to age

Bacteria isolated	≤2 years	3-11 years	12-17 years	18-39 years	≥ 40 years
Staphylococcus *aureus*	16(21.92)	10(24.39)	7(41.18)	4(30.77)	2(14.29)
Pseudomonas *aeruginosa*	20(27.40)	6(14.63)	3(17.65)	2(15.38)	4(28.57)
Coagulase negative staphylococci	11(15.07)	8(19.51)	2(11.76)	-	6(42.86)
Streptococcus *pneumoniae*	7(9.59)	4(9.76)	1(5.88)	3(23.08)	1(7.14)
Streptococcus *virdans*	10 (13.70)	3(7.32)	-	-	-
Eschericia *coli*	4(5.48)	6(14.63)	1(5.88)	1(7.69)	-
Klebsiella *species*	3(4.11)	3(7.32)	-	2(15.38)	-
Proteus *mirabilis*	2(2.74)	-	-	1(7.69)	-
Citrobacter	-	1(2.44)	1(5.88)	-	1(7.14)
Moraxella	-	-	2(11.76)	-	-
TOTAL	73(100.00)	41(100.00)	17(100.00)	13(100.00)	14(100.00)

There were 39 infections caused by *Staphylococcus aureus,* of which five (12.82%) were MRSA (Table 3).

Table 3: Demograhic profile of MRSA cases

S. No	Age	Gender	Diagnosis	Comorbidities
MRSA 1	58	M	Keratitis	DM
MRSA 2	47	F	Keratitis	AML
MRSA 3	45	F	conjuntivitis	DM
MRSA 4	39	M	Conjuntivitis	DM
MRSA 5	33	M	Keratitis	HIV

Antimicrobial susceptibility pattern of MRSA was summarized in Table 4.

All Gram positive cocci were susceptible to vancomycin including MRSA. Apart from vancomycin, all MRSA isolates remained susceptible to chloramphenicol. In overall higher rate of susceptibility accounted for amikacin 79.74%(126/158) and moxifloxacin 74.68%(118/158) Second most isolated bacteria was Pseudomonas aeruginosa which showed higher degree of susceptibility towards all the aminoglycosides tested while least number of pseudomonas aeruginosa isolates were susceptible to chloramphenicol 10 (28.57%) (Table 4)

Table 4: Antibiogram of bacterial isolates

Bacteria	No. tested	Van	C	Cef	Gen	Ak	Tob	Of	Cip	Mox
Staphylococcus *aureus* (MRSA)	5	5(100)	5(100)	2(40)	3(60)	4(80)	2(40)	1(20)	2(40)	3(60)
Staphylococcus *aureus* (MSSA)	34	34(100)	33(97.06)	19(55.88)	27(79.41)	29(85.29)	28(82.35)	12(47.06)	11(32.35)	28(82.35)
Pseudomonas *aeruginosa*	35	-	10(28.57)	16(45.71)	27(77.14)	30(85.71)	29(82.86)	20(57.14)	12(34.29)	22(62.86)
CONS	27	27(100)	25(92.59)	22(81.48)	10(37.04)	18(66.66)	12(44.44)	11(40.74)	9(33.33)	20(74.07)
Streptococcus *pneumoniae*	16	16(100)	10(62.50)	12(75)	5(31.25)	10(62.50)	11(68.75)	9(56.25)	6(37.50)	11(68.75)
Streptococcus *virdans*	13	13(100)	9(69.23)	3(23.08)	4(30.77)	8(61.54)	10(76.92)	11(84.62)	8(61.54)	9(69.23)
Eschericia *coli*	12	-	6(50)	0(0.00)	3(25)	12(100)	11(91.67)	7(58.33)	7(58.33)	10(83.33)
Klebsiella *species*	8	-	3(37.50)	4(50)	5(62.50)	7(87.50)	7(87.50)	7(87.50)	5(62.50)	7(87.50)
Proteus mirabilis	3	-	0(0)	0(0.00)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)
Citrobacter	3	-	1(33.33)	1(33.33)	1(33.33)	3(100)	2(66.67)	1(33.33)	1(33.33)	3(100)
Moraxella	2	-	1(50)	1(50)	1(50)	2(100)	1(100)	2(100)	2(100)	2(100)

CONS: Coagulase negative staphylococci, **MSSA** : methicillin sensitive S. aureus; **MRSA** : methicillin resistant S. aureus **VAN-**Vancomycin, **AK**–Amikacin, **GEN** – Gentamicin, **OF** – Ofloxacin, **C** – Chloramphenicol, **CIP** – Ciprofloxacin, **CEF** –Cefazolin, **TOB**-Tobramycin, **MOX**-Moxifloxacin

Discussion

In this study, the overall culture-positivity was 69.60%. The ability to isolate the causative organism depends on a variety of factors including the amount of inocula,[12] the site from which specimen was taken, the media used for culture (whether enriched media are used or not).[13] The most common isolate observed in this study was *S.aureus* (24.68%; 39 of 158) This finding is in agreement with the findings from similar studies conducted by Ramesh, et al [1] and Bharathi, et al[14] in which S.aureus accounted for 25% (195 of 776) and 26%(697of 2611) respectively. However, in another study[15] the predominant isolates were Coagulase-negative staphylococci followed by S. *aureus*. In this present study, *S. aureus* was the most common isolate in conjunctivitis and blepheritis. This is in agreement with the findings from study conducted by Ubani,[3] *P. aeruginosa* was found to be the predominant isolate in cases of microbial keratitis which accounted 54.29% of the overall bacterial isolates of bacterial keratitis. This finding was supported by similar studies conducted by Bataineh, et al[16] and Biradar, et al.[17] Pseudomonas keratitis has been attributed to the action of proteases and glycocalyx that allow the organisms to adhere to the host cells forming micro colonies that resist phagocytosis.[18] However, other studies[19,20] reported *S. pneumonia* as the most common isolate in microbial keratitis.

This study showed fewer isolates of enteric bacteria (10.76%) when compared to similar study conducted by Anagaw et al.[15] This low number of enterobacteriaceae may be due to reduction in hand-faecal contamination and/ or increased access to potable water sources in this region.

The majority of the bacterial isolates (46.20%), were from patients in the age range of less than two years of life. Susceptibility to infection is increased in babies because they are at a greater risk due to their low immunity.[21] In addition to this, the air plays an important role in the transfer of bacteria.[22]

S.*aureus* is the commonest cause of ocular infections and other nosocomial infections. S.*aureus* was once susceptible to penicillin but widely resistant organisms soon emerged. The introduction of methicillin initially solved the problem, but later, strains which were resistant to methicillin developed. Thus, an increased number of resistant strains have been seen worldwide.[23]

In this study, 39 external ocular infections were caused by Staphylococcus *aureus*, of which 5(12.82%) of the isolates were MRSA. An earlier study, also from Japan, put this figure at 25% but included all ocular infections and not just external ocular infections.[24] This illustrates the marked variation inthe prevalence of MRSA ocular infections geographically and at different time points.

In our study, the most common presentation of ocular MRSA infections was keratitis (three cases) followed by conjunctivitis (two cases). No MRSA was isolated from lid disorders. The type of infections described in previous studies were broadly the same, with conjunctivitis and keratitis the most common manifestations.[25] MRSA is believed to cause a more severe disease than MSSA,[26] But, our results did not show that MRSA caused more severe ocular diseases than MSSA, this agrees with Freidlin's study, which reported MRSA and MSSA caused similar eye disease.[27]

Resistance and sensitivity based on *in vitro* testing may not reflect true clinical resistance and response to an antibiotic because of the host factors and penetration of the drug. In this study, all Gram positive cocci were 100% sensitive to vancomycin including MRSA, although vancomycin retains extremely high efficacy against MRSA. S. *aureus* with reduced susceptibility to vancomycin was identified.[28] All MRSA strains were also susceptible to chloramphenicol. According to Fukuda et al's study, chloramphenicol was clinically effective in 81% of cases with MRSA conjunctivitis and this may gain favour as a first-line choice of antibiotics.[29]

In this study, amikacin and moxifloxacin showed good coverage against Gram positive cocci and Gram negative bacilli. Recent studies have also shown the excellent Gram-positive coverage of moxifloxacin in ocular infections.[30] Most of the ocular pathogens showed decreased susceptibility to ciprofloxacin and ofloxacin. The relationship between antibiotic use and resistance is complex. Improper selection of antibiotics, inadequate dosing and poor compliance to therapy may play an important role in increasing antibiotic resistance.

In conclusion, the prevalence of bacterial isolates in external ocular infections was high in this study. Gram-positive cocci were the most frequent bacteria isolated from ocular infections and were sensitive to moxifloxacin and vancomycin, while Gram negative isolates were more sensitive to amikacin. It also stresses the need to pursue detailed studies of this kind in rural areas which may lead to better understanding of the aetiology of external ocular infections and the magnitude of antibiotic resistance.

References

1. Ramesh S, Ramakrishnan R, Jayahar Bharathi M, Amuthan M, Viswanathan S. Prevalence of bacterial pathogens causing ocular infections in South India. Indian PatholMicrobiol.2010;53:281–286.
2. Akpek EK, Gottsch JD. Immune defense at the ocular surface. Eye (Lond). 2003;17:949–956.
3. Ubani, U.A, "Bacteriology of external Ocular Infections in Aba, South Eastern Nigeria," ClinExpOptom2009;92(6).482-9.
4. Delhi N: Parsons' diseases of the eye. 20th edition. Edited by Sihota R, Tandon R;2007;423–469.

5. Chalita MR, Hofling-Lima AL, Paranhos A Jr, Schor P, Belfort R Jr. Shifting trends in *in-vitro* antibiotic susceptibilities for commonocular isolates during a period of 15 years. Am J Ophthalmol2004;137:43-51.
6. Kunimoto DY, Das T, Sharma S, et al. Microbiologic spectrumand susceptibility of isolates: part 1. Postoperative endophthalmitis. Endophthalmitis Research Group. Am J Ophthalmol1999;128:240–242.
7. Behlau I, Gilmore MS. Microbial Biofilms in 4. Ophthalmology and Infectious Disease. *Arch Ophthalmol* 2008; 126: 1572-1581.
8. Rehm SJ, Tice A. 8. *Staphylococcus aureus*: Methicillin-Susceptible *S. aureus* to Methicillin-Resistant *S. aureus* and Vancomycin-Resistant *S. aureus*. *Clin Infect Dis* 2010;51:S176-S82.
9. Byrne, K.A, Burd, E., Tabbara, K., Hyndiuk, R, Diagnostic Microbiology and Cytology of the Eye, Butterworth-Heinemann, Boston,1995;40-2.
10. Wilhelmus KR, Liesegang TJ, Osato MS, Jones DB, Specter SC, editors. Cumulative Techniques and Procedures in Clinical Microbiology: Laboratory diagnosis of ocular infections. Washington DC1994; American Society for Microbiology.
11. Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing.21st information supplement M100- S21.Wayne,PA, USA: Clinical and laboratory standards institute,2011.
12. Bharathi MJ, Ramakrishnan R, Meenakshi R, Mittal S, Shivakumar C, Srinivasan M. Microbial diagnosis of infective keratitis: Comparative evaluation of direct microscopy and culture results.Br J Ophthalmol 2006;90:1271-6
13. Moeller CT, Branco BC, Yu MC, Farah ME, Santos MA, Hofling-Lima AL. Evaluation of normal ocular bacterial flora with two different culture media. Can J Ophthalmol 2005;40:448-53.
14. Bharathi, M.J., Ramakrishnan, R., Shivakumar, C., Meenakshi, R., Lionalraj, D, "Etiology and antibacterial susceptibility pattern of community-acquired bacterial ocular infections in a tertiary eye care hospital in south India," Indian J Ophthalmol, 2010;58(6).497-507.
15. Anagaw B, Biadglegne F, Belyhun Y, Anagaw B, Mulu A: Bacteriology ofocular infections and antibiotic susceptibility pattern in Gondar University Hospital, North west Ethiopia. Ethiop Med J 2011;49:117–123.
16. Bataineh, H., Hammory, Q., Khatatba, A, "Bacterial Keratitis: Risk Factors and Causative Agents," Sudan JMS, 2008;3(1).6-10.
17. Biradar, S., Chandrashekhar, D.K., Gangane, R., Chandrakanth, C., Biradar, K.G., Vinod Kumar, C.S, "Spectrum of microbial keratitis and antimicrobial susceptibility at tertiary care teaching hospital in north Karnataka," Int J Pharm Biomed Res,2012;3(2).117-20.
18. Ramphal R, McNiece MT, Polack FM. Adherence of *Pseudomonas aeruginosa*to the injured cornea: A step in the pathogenesis ofcorneal infections. Ann Ophthalmol 1981;13:421-5.
19. Matoba A., O` Brien T, Wilhemus K., Jones D. Infectious crystalline keratopathy due to streptococcus pneumoniae. Ophthalmology 1994;101:1000-1004.
20. Bharathi, M.J., Ramakrishnan, R., Meenakshi, R., Padmavathy, S., Shivakumar, C., Srinivasan, M, "Microbial Keratitis in South India: Influence of Risk Factors, Climate, and Geographical Variation," Ophthalmic Epidemiol,2007;14(2).61-69.
21. Prescott LM, Harley JP, Klein DA: Microbiology. 5th edition. Madison, New York: Mc Graw Hill Education Publisher 2002;667–763.
22. IJsselmuiden CB, Faden RR. Research and informed consent in Africa -- another look. N Engl J Med 1992;326:830-834
23. Nazeer HA, Shaik KM, Kolasani BP. Aerobic bacteriology of wound infections with special reference to MRSA. J Clin Exp Res 2014;2:74-79.
24. Tazawa H, Ooishi M. MRSA ocular infections. Nippon Rinsho1992;50:1122–1126(in Japanese).
25. Shanmuganathan V, Armstrong M, Buller A, Tullo A. External ocular infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) Eye. 2005;19:284–291.
26. Cosgrove SE, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible Staphylococcus aureus bacteremia: a meta-analysis. Clin Infect Dis 2003;36:53–59.
27. Freidlin J, Acharya N, Lietman TM, Cevallos V, Whitcher JP, et al. Spectrum of eye disease caused by methicillin-resistant Staphylococcus aureus. American Journal of Ophthalmology2007;144:313–315.
28. Hawser SP, Bouchillon SK, Hoban DJ, Dowzicky M, Babinchak T. Rising incidence of Staphylococcus aureus with reduced susceptibility to vancomycin and susceptibility to antibiotics: a global analysis 2004–2009. Int J Antimicrob Agents,2011;37:219–224.
29. Fukuda M, Ohashi H, Matsumoto C, Mishima S, Shimomura Y. Methicillin-resistant Staphylococcus aureusand methicillin-resistant coagulase-negative Staphylococcus ocular surface infection. Cornea 2002;21(Suppl. 2):86–89.
30. Kowalski RP, Dhaliwal DK, Karenchak LM, Romanowski EG, Mah FS, Ritterband DC, *et al*. Gatifloxacin and moxifloxacin: An*in-vitro* susceptibility comparison to levofloxacin, ciprofloxacin, and ofloxacin using bacterial keratitis isolates. Am J Ophthalmol2003;136:500-5.

How to cite this article: Khaja M, Nazeer HA, Anandi, Vani Kalai, Prakash B, Murlidhar. Bacteriology and antimicrobial susceptibility pattern of external ocular infections in rural tertiary care teaching hospital. Indian J Microbiol Res 2016;3(2):203-208.