ORIGINAL RESEARCH PAPER

Volume-6 | Issue-12 | December-2017 | ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 93.98

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

METHICILLIN, FLUOROQUINOLONES AND AMINOGLYCOSIDES RESISTANCE IN BACTERIAL KERATITIS

Microbiology

Dr.Pallati Alekhya* Senior Resident, Gandhi Medical College, Secunderabad *Corresponding Author

Dr.Prathibha Associate Professor, Gandhi Medical College, Secunderabad

ABSTRACT

Keratitis is second most common cause of blindness. Prompt treatment has to be started to prevent ocular morbidity. This study was conducted to observe antibacterial susceptibility pattern in Keratitis with regard to Methicillin, Fluoroquinolones and Aminoglycosides. 150 clinically suspected cases of Bacterial keratitis were taken up for the study. Corneal scrapings were inoculated on Blood Agar, Chocolate Agar and MacConkey Agar. Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method. Methicillin resistance was tested by Cefoxitin disc diffusion test. Fluoroquinolones used were Ciprofloxacin, Ofloxacin, Gatifloxacin, Moxifloxacin. Aminoglycosides used were Gentamycin and Tobramycin. Total number of culture positive bacterial isolates was 50. Among the culture positive isolates, 96% were gram positive organisms. 27.6% Methicillin resistant *Staphylococci* were isolated. Among the Fluoroquinolones used, highest resistance of 54% was observed for Ofloxacin. 14% resistance was observed for Tobramycin. *Staphylococcus epidermidis* is a common isolate in keratitis. Fluoroquinolones and Aminoglycosides are most widely used for the treatment of Bacterial keratitis. However, there is an increase in antibacterial resistance towards the above group of antibiotics.

KEYWORDS

Keratitis, Fluoroquinolones, *Staphylococcus epidermidis*, Methicillin

INTRODUCTION

Keratitis is an inflammation of the layers of the cornea. It is most commonly associated with microorganisms that invade into the corneal stroma, resulting in inflammation and ultimately, destruction of these structures[1]. Keratitis can result from infectious agents and non infectious agents[2]. Corneal blindness is a major public health problem worldwide and infectious keratitis is one of the predominant causes[3]. The importance of corneal disease as a major cause of blindness in the world today remains second only to cataract[2]. Infectious keratitis may be caused by bacteria, fungi, viruses or parasites[3]. Bacterial keratitis rarely occurs in the normal eye because of corneal defense to infection. However predisposing factors such as corneal injury, contact lens wear, ocular adnexal dysfunction [including tear deficiencies corneal abnormalities and other exogenous factors, systemic diseases and immunosuppression] may alter the defense mechanisms of the outer eye and permit bacteria to invade the cornea[3]. Infectious keratitis of bacterial origin is the leading cause of ocular morbidity and blindness in India[4]. Timely institution of appropriate therapy must be initiated to control the infections and there by minimize ocular morbidity. Ocular Methicillin resistance *Staphylococci* infections can be aggressive and cause severe ophthalmic disease including blindness[5]. *Staphylococcus epidermidis* has developed the same bacterial resistance as *S.aureus* and has now been termed as methicillin-resistant *S epidermidis* (MRSE). Moreover, reports have shown that MRSE can cause ophthalmic infections and blindness[6]. Quinolones rapidly inhibit DNA synthesis by promoting cleavage of bacterial DNA in the DNA–enzyme complexes of DNA gyrase and type IV topoisomerase, resulting in rapid bacterial death[7]. Bacterial resistance to fluoroquinolones is due to mutation of DNA gyrase genes and poor transport across cell membrane. Aminoglycosides exhibit bactericidal activity by irreversible binding to 30s ribosomal subunit. Resistance is due to drug inactivation by aminoglycoside-modifying enzyme, decreased permeability to gram negative outer membrane and decrease influx of drug[8]. The present study was undertaken at a tertiary care centre, to identify the etiology and to determine the in-vitro antibacterial susceptibility of Methicillin, Fluoroquinolones and Aminoglycosides.

MATERIALS AND METHODS:

A total of 150 clinically suspected cases of bacterial keratitis were included in the study. Patients already on antibacterial therapy are excluded from the study.

Under aseptic conditions after instillation of 4 % lignocaine, corneal scrapings were obtained by using a Bard Parker knife no. 15 to debride material from the base and edges of the ulcerated part of the cornea.

Direct microscopy of corneal scrapings was performed by Gram stain. The scraping material obtained from leading edge and base of the ulcer was inoculated directly on to the surface of solid media such as Blood agar, Mac Conkey agar and chocolate agar in the form of 'C' streaks, and also in to liquid media- Brain heart infusion broth. The inoculated media were incubated at 37°C for 24 hours aerobically. Chocolate Agar plates were incubated at 37°C in the presence of 5 to 10 % CO_2 for 24 to 48 hrs in candle jar.

In vitro antibacterial susceptibility testing was performed using Kirby – Bauer disc diffusion method for all the isolates on Muller Hinton agar. *Staphylococcus aureus* ATCC 25923 was used as a control strain. In this study, the susceptibility testing was carried out against Ciprofloxacin (5µg/disc), Ofloxacin (5µg/disc), Gatifloxacin (5µg/disc), Moxifloxacin (5µg/disc), Chloromphenicol (30µg/disc), Ceftazidime (30µg/disc), Gentamycin (10µg/disc), Tobramycin (10µg/disc). The diameter of the zone of inhibition was measured and interpreted according to CLSI guidelines. Methicillin resistance in *Staphylococcus aureus* isolates was detected using Cefoxitin (30µg/disc) disc diffusion test.

RESULTS:

Among the 150 cases of clinically diagnosed bacterial keratitis, 67.33% were male and 32.66% were female. Bacterial keratitis is more common in the age group of 21-30 and 51-60 years with a percentage 23.33% and 24.0% respectively. Among the 150 majority were agriculture workers (44%), followed by Labor (21.33%), House hold (12%), Student (4.6%), Garden workers (2%), Others 15(%). Of the total 150 cases, common predisposing factors were history of corneal trauma (60%), Diabetes Mellitus (4%), Steroid usage (1.3%).

Among 150 cases, culture positive was 33.33%. Out of 50 isolates, 37 were *Staphylococcus epidermidis*, 10 were *Staphylococcus aureus* (Table 1). Percentage of Methicillin resistant *Staphylococci* isolates was 27.6%. Antibiotic susceptibility pattern of the bacterial isolates is shown in table 2

Table 1: Bacterial Isolates

Bacteria Isolate	Number	Percentage (%)
Staphylococcus epidermidis	37	74
Staphylococcus aureus	10	20
Enterococcus fecalis	1	2
Pseudomonas aeruginosa	2	4

Discussion:

Infectious keratitis (Corneal ulcer) is a common disease of the cornea. The severity of corneal infections usually depends on the underlying

Table 2: Antibacterial susceptibility pattern of Bacterial Isolates

Antibiotic	*S.epidermidis*		*S. aureus*		*Enterococcus Fecalis*		*Pseudomonas aeruginosa*	
	S%	R%	S%	R%	S%	R%	S%	R%
Ciprofloxacin	60	14	16	4	2	0	2	2
Ofloxacin	34	40	10	10	0	2	2	2
Gatifloxacin	56	18	8	12	2	0	4	0
Moxifloxacin	68	6	14	6	2	0	2	2
Gentamycin	66	8	18	2	2	0	4	0
Tobramycin	64	10	16	4	2	0	4	0
Chloramphenicol	68	6	6	14	2	0	2	2
Ceftazidime	40	34	14	6	2	0	4	0

condition of the cornea and the virulence of the infecting microbes[9].

In this study, male predominance was seen; this is in line with Geeta kumari et al[10] in 2011 who reported 65.11% in males and in females 34.89%. . In contrast to the present study, Gurdeep singh et al[11] in 2006 reported high percentage in females 54.6% and males 45.3%. Most common age group affected in the present study is 51-60, which is in accordance with the study conducted by Srinivasan et al[12]. In this study, agriculture workers were 66 (44%), laborers 32 (21.33%), house hold 18 (12%), student 7 (4.6%), garden workers 3(2%), others 15(%), an occupational profile similar to Srinivasan M et al[12] in 1997 and Hagan M et al[13] in 1995. In this study, trauma was a major risk factor which is in accordance with the study of Basak et al[14] in 2005 and Tewari A et al[15] in 2012.

In this study, Bacterial isolates from keratitis patients accounted for 33.33 % which correlated well with the study of Bharathi MJ et al [16] which reported 34.98% and Liesesang TJ et al[17] in 1980 reported 35.6%. The most common isolate in the present study was *Staphylococcus epidermidis* (74%) which was similar to the study of Tiago et al[18] in 2011. Methicillin resistant *Staphylococci* isolates were 27.6% in the present study. Alekhya et al[19] reported 21.5% Methicillin resistance in all ocular samples from the same centre in the previous year. There is increase in incidence of Methicillin resistance. Highest Methicillin resistance was reported by Sharma et al[20] in 2004 from Scotland. Among the Fluoroquinolones, highest resistance was seen to Ofloxacin (54%) followed by Gatifloxacin (30%). Measures have to be taken to prevent its further incidence. Resistance to Gentamycin was 10% and Tobramycin was 14%. Gatifloxacin and Tobramycin are newer drugs which has also developed resistance. Talukder et al[21] reported 43% resistance to Gatifloxacin, 74% to Ciprofloxacin, 73.3% to Moxifloxacin and 22.3% to Tobramycin.

Conclusion:

Rational use of antibiotics and antibiotic policy would help to overcome the emergence of ocular antibiotic resistance

References:

1. Zubair ansari, Darlene miller, Anat galor, Current thoughts in fungal keratitis: diagnosis and treatment. NIH public access author manuscript. 2013, September, 1; 7(3): 209–218.
2. Sarah A, Collier, Michael P, Gronostaj, Amanda k. Macgurn, Jennifer R, Cope, Kate L, Awsumb, Jonathan S, Yoder, Michael J, Beach. Estimated burden of keratitis- United States, 2010. Centre for disease control and prevention. 2014, November, 63(45);10271030.
3. Bharathi M J, Ramankrishnan R, Vasu S, Meenashi R, shivakumar C, Palaniappan R. Epidemiology of bacterial keratitis in a referral centre in South India. Indian Journal of Medical Microbiology. 2003, October, (4), 21.239-245.
4. Nayak N, Satpathy G, Vajpayee RB. Phenotypic and plasmid pattern analysis of Staphylococcus epidermidis in bacterial keratitis .Indian J Ophthalmol 2007; 55:9-13.
5. 5.Matthew D. Walvick, Amer Khan. Ophthalmic methicillin-resistant Staphylococcus aureus infections: Sensitivity and resistance profiles of 65 isolates in central California. Advances in Bioscience and Biotechnology, 2013, 4, 263-265.
6. Sotozono C, Fukuda M, Ohishi M, et al.Vancomycin Ophthalmic Ointment 1% for methicillin resistant Staphylococcus aureus or methicillin-resistant Staphylococcus epidermidis infections: a case series. BMJ Open 2013;3:e001206. doi:10.1136/bmjopen-2012- 001206.
7. R Yag˘ cı, Y Oflu, A Dinc¸el, E Kaya, S Yag˘ cı, B Bayar, S Duman and A Bozkurt. Penetration of second-, third-, and fourth-generation topical fluoroquinolones into aqueous and vitreous humour in a rabbit endophthalmitis model. Eye (2007) 21, 990–994 (2007), doi:10.1038/sj.eye.6702414.
8. Apurba Sankar Sastry, Sandhya Bhat k. Essentials of Medical Microbiology, Jaypee brothers Medical publishers (P)Ltd, p.no-80-81.
9. Gebauer A, McGhee CNJ, Crawford GJ. Severe microbial keratitis in temperate and tropical western Australia, Eye.1996;10:575-586.
10. Geethakumari PV, Remya R, Girijadevi PS, Reena A; Bacterial keratitis and fungal keratitis in South Kerala: A comparative study. Kerala Journal of Ophthalmology, 2011; XXIII(1): 43-46.
11. Gurdeep singh ,Manikandan Palanisamy ,Bhaskar Madhavan, Revathi Rajaraman .Multivariate Analysis of Childhood Microbial Keratitis in south India Ann Acad Med Singapore, 2006;35:185-9.
12. Srinivasan M, Christine A Gonzales, Celine George, Vicky Cevallos, Jeena M Mascarenhas, AsokanB, John Wilkins, Gilbert Smolin, John P Whitcher. Epidemiology and aetiological diagnosis of corneal ulceration in Madurai, south India. British Journal of Ophthalmology. 1997, June, 81:965–971.
13. Hagan M, Wright E, Newman M, et al. Causes of suppurative keratitis in Ghana. Br J Ophthalmol 1995;79:1024–8.
14. Basak SK, Basak S, Mohanta A, Bhowmick A. Epidemiological and Microbiological Diagnosis of Suppurative Keratitis in Gangetic West Bengal, Eastern India. Indian J Ophthalmol 2005;53:17-22.
15. Tewari A, Sood N, Vegad MM, Mehta DC. Epidemiological and microbiological profile of infective keratitis in Ahmedabad.Indian J Ophthalmol 2012;60:267-272.
16. Bharathi MJ ,Ramakrishnan R , Vasu S ,Meenakshi. Aetiological diagnosis of microbial keratitis in South India –A study of 1618 cases .Indian J Med Microbiol 2002;20:19-24.
17. Liesegang TJ, Forster RK. Spectrum of microbial keratitis in South Florida. Am J Ophthalmol 1980;90:38–47.
18. Tiago M.Yamanaka, Sr.,Paulo J.Bispo, Antonio C.Piganatari, Maria C. yu, Michel E.Farah, Ana L.Hofling – Lima; Selection of Fluoroquinolone resistant coagulase negative Staphylococcus after Moxifloxacin eyedrop usage. Invest.Ophthalmol. Vis.Sic.2011;52(14);1493.
19. Pallati Alekhya, C. Aruna sunder, Prathibha, Incidence of Methicillin resistance in Staphylococcus species and their antibiotic susceptibility pattern, in ocular samples at a tertiary care eye hospital, Hyderabad, Sch. J. App. Med. Sci., 2016; 4(12D):4457-4461, DOI: 10.21276/sjams.2016.4.12.50.
20. Sharma S. Antibiotic resistance in ocular bacterial pathogens. Indian journal of medical microbiology. 2011 Jul 1; 29(3):218.
21. Talukder AK, Sultana Z, Jahan I, Khanam M, Rahman M, Rahman MF, Rahman MB. Antibiotic resistance: New challenge in the management of bacterial eye infections. Mymensingh Med J.2017 Jan;26(1):29-36.