International Journal of Integrative Medical Sciences,
Int J Intg Med Sci 2018, Vol 5(4):607-12. ISSN 2394 - 4137
DOI: https://dx.doi.org/10.16965/ijims.2018.107

Original Research Article

ISOLATION, IDENTIFICATION OF BACTERIAL AGENT CAUSING KERATITIS, ANTIBIOTIC SENSITIVITY TESTING

Venkatalaxmi Rajamanickam *[1], Aruna Sunder [2], C Prathiba [3].

*[1] Assistant Professor in Microbiology, Dr. Patnam Mahender Reddy Institute of Medical sciences and hospital, Chevella, Rangareddy, Telangana, India.

[2] Retd. Professor in Microbiology, Osmania medical college, Koti, Hyderabad, Telangana.

[3] Associate professor in Microbiology, Gandhi Medical College, Secundrabad, Telangana.

ABSTRACT

Corneal infections are known to be the second most significant cause of monocular blindness rated after unoperated cataract in some developing countries. The present study was carried out in the Department of Microbiology Sarojini Davi Eye Hospital, a tertiary care centre. Hyderabad for a period of six months with 150 clinically diagnosed keratitis cases were studied for microbial involvement. Corneal trauma with vegetative matter was identified as the major predisposing factor followed by co-existing ocular conditions. 51 cases yielded pure bacterial isolates. Culture sterile was 42 cases. Among the bacterial isolates, Staphylococcus epidermidis were the predominant organism isolated followed by Staphylococcus aureus. Streptococcus spp was isolates in 7 cases and Enterococci fecalis was isolated in 5 cases. Pseudomonas aeruginosa was isolated in 4 cases. No MRSA was isolated. Majority of the isolates were sensitive to Moxifloxacin followed by Tobramycin.

KEY WORDS: Corneal infections, Staphylococcus epidermidis, Enterococci fecalis, MRSA, Moxifloxacin.

Address for correspondence: Dr. Venkatalaxmi Rajamanickam, Assistant Professor in Microbiology, Dr. Patnam Mahender Reddy Institute of Medical sciences and hospital, Chevella, Rangareddy, Telangana, India. **E-Mail**: drlaxmi04@gmail.com

Online Access and Article Informtaion

Quick Response code

DOI: 10.16965/ijims.2018.107

International Journal of Integrative Medical Sciences

www.imedsciences.com

Received: 11-04-2018 **Accepted:** 22-04-2018

Reviewed: 11-04-2018 **Published:** 30-04-2018

Source of Funding: Self **Conflicts of interest:** None

BACKGROUND

Keratitis is the term applied for inflammation of the cornea [1]. Corneal infections are known to be the second most significant cause of monocular blindness rated after unoperated cataract in some developing countries in the tropics [2-4]. Corneal infection is a leading cause of ocular morbidity and blindness worldwide. Corneal ulceration is a major cause of monocular blindness in developing countries. Almost any microorganism can invade the corneal stroma if the normal defence mechanism i.e. lids, tear film and corneal epithelium are compromised [5].

Microbial keratitis is a common potentially sight threatening ocular infection that may be caused by bacteria, fungi, viruses or parasites. Bacterial keratitis rarely occurs in the normal eye because of the human cornea's natural resistance 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 [6]. Infectious keratitis of bacterial origin is the leading cause of ocular morbidity and blindness in India [7].

Bacterial corneal ulceration is an ocular emergency due to often rapid progression of this corneal infection with the threat of vision loss

and potential corneal perforation. Timely institution of appropriate therapy must be initiated to control the infections and there by minimize ocular morbidity. If they are not treated promptly, it may lead to sight threatening condition. The present study of microbial Keratitis antibacterial susceptibility pattern was undertaken to identify the aetiology and to determine the invitro antibacterial susceptibility of bacterial pathogens to commonly used antibacterial agents.

MATERIALS AND METHODS

The present prospective study of microbial Keratitis antibacterial and antifungal susceptibility pattern was undertaken from march 2015 to Aug 2015 at a tertiary care centre, Sarojini Devi Eye Hospital Hyderabad, Telangana with a total of 150 outpatient patients of all age groups, of either sex, who were clinically diagnosed as keratitis by the ophthalmologist were included in the study. Patients already on antibacterial and antifungal therapy are excluded from the study. After a detailed ocular examination by an Ophthalmologist, corneal scraping was collected under aseptic conditions from each ulcer by an Ophthalmologist after instillation of 4% lignocaine without preservative using a sterile Bard Parker blade No.15. The procedure was performed under operating microscope. Corneal scrapings were placed on 2 slides to prepare 10% KOH wet mount and Gram staining / Giemsa staining. In cases of suspected actinomycetes keratits Kinyoun's acid fast staining was performed. The scraping material obtained from leading edge and base of the ulcer was initially inoculated directly on to the surface of solid media such as Blood agar MacConkey agar, and chocolate agar Sabouraud dextrose agar and also on to liquid media such as Brain heart infusion broth. The inoculated media was incubated at 37°C for 24 hours aerobically. CA plates were incubated at 37°c in the presence of 5 to 10 % CO_2 for 24 to 48 hrs. SDA were incubated in BOD incubator at 25°c.

Microbial culture is considered positive if:

1. There is semi-confluent growth at the site of inoculation on solid Medium.

2. Same organism was grown from repeated specimens.

3. It is consistent with clinical signs.

4. Smear results were consistent with culture.

Identification of bacterial pathogens: Morphology on Gram stain, cultural characteristics on different media and biochemical properties using standard laboratory criteria.

Staphylococcus aureus – gram positive cocci in clusters on gram stain, positive coagulase test, positive mannitol fermentation and phenolphthalein phosphate test.

Coagulase negative staphylococci – coagulase negative and mannitol non fermenting. Enterococci – oval gram positive cocci in pairs, heat resistant test positive (60°c for 30 minutes), growth in 6.5 % Nacl and Bile esculin test.

Pseudomonas aeruginosa – gram negative bacilli, catalase positive, oxidase positive, motile non fermenting with positive dihydrolase test and bluish green pigmentation on nutrient agar plate.

Antibiotic susceptibility testing: In vitro susceptibility by Kirby – Bauer disc diffusion method for all the isolates on Muller Hinton agar and interpreted using CLSI guidelines [8] Staphylococcus aureus ATCC 25923 was used as a control strain. In the present study, the susceptibility testing was carried out against the following antibiotics.

Specimen processing

All the antibiotic discs were procured commerically from Hi-Media Laboratories Pvt. Ltd. Mumbai. The diameter of the zone of inhibition was measured and interpreted according to CLSI guidelines.

Detection of methicillin resistance in Staphylococcus aureus isolates: All the isolates were subjected to cefoxitin disc diffusion test using a 30 microgram cefoxitin disc. A 0.5 Mc Farland suspension of the isolate was made and lawn culture was done on Muller Hinton agar plate. Plates were incubated at 37 ^{0} C for 18 hours and zone diameter of d" 21mm was reported as resistant and e" 22 mm was considered as sensitive. Quality control strains – methicillin sensitive S.aureus (MSSA) ATCC 25923 and methicillin resistant S.aureus (MRSA) ATCC 43300 was used as negative and positive controls respectively.

Days	Bacteria	Mycology
Day 1	Inoculated on BA, MA, CA, BHI broth & incubated for 24 hrs at 37oc. Chocolate agar incubated in candle jar 48 hrs	Inoculated on SDA
Day 2	Observed for growth	
	No growth	1. Looked for growth, if creamy white colonies were present germ tube test performed. If no growth, then reincubated.
	Growth 1.Colony characters observed 2.Subcultured 3.Smear for Gram's stain 4.Hanging drop for motility 5. Biochemical tests. 6.Antibiotic sensitivity done	2. Velvety or powdery dark greenish colonies were present, observe for colony characters.
Day 3	Biochemical tests and sensitivity pattern were read using standard laboratory criteria	1. If growth was present, colony characters observed. 2. LCB made and morphology studied. 3. Slide culture done. 4. Discard after 4 weeks if no growth.

Antimicrobial agents

Antimicrobial agents	Symbol	Disc con.µg/disc
Ciprofloxacin	CIP	5
Ofloxacin	OF	5
Gatifloxacin	GAT	5
Moxifloxacin	MO	5
Chloramphenicol	CIP	30
Ceftazidime	CAZ	30
Gentamicin	GEN	10
Tobramycin	TOB	10

Fig. 1: Heat resistant test.

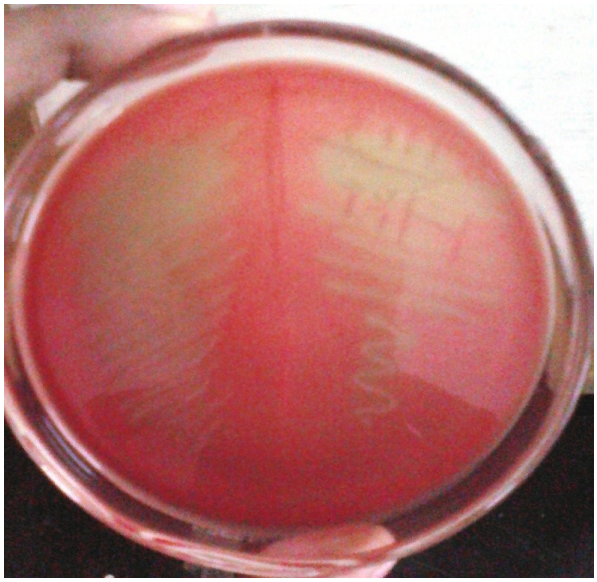
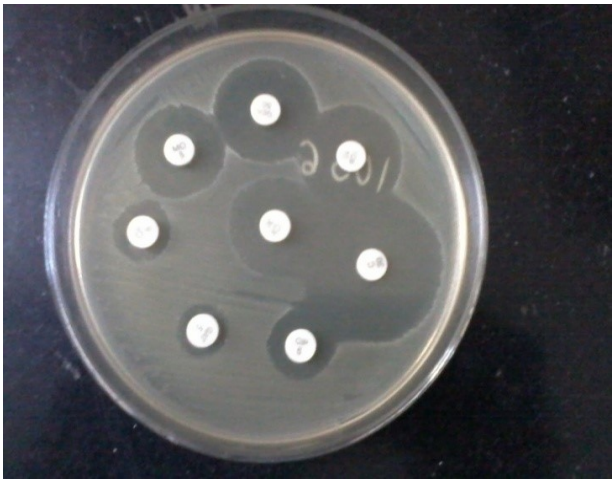


Fig. 2: Enterococci showing Bile aesculin test positive.

Fig. 3: Antibiotic sensitivity testing on Muller Hinton agar.



RESULTS

Table 1: Predisposing factors associated with keratitis.

Predisposing factor	Number of cases	Percentage %
Corneal trauma	124	82.66%
Co existing ocular conditions	17	11.33%
Post surgery	9	6%

Table 2: Distribution of Culture Positive and Negative Case.

No of cases	No of culture positive cases		No of culture negative cases	
150	108	72%	42	28%

Table 3: Incidence of various microbial isolates.

Type of Isolate	Number	Percentage (%)
Pure Bacterial	51	34
Pure Fungal	33	22
Mixed (Bacterial & Fungal)	24	16
Culture Sterile	42	28

Table 4: Distribution of pure bacterial isolates.

Bacteria Isolate	Number	Percentage (%)
S.epidermidis	24	47.1
S.aureus	11	21.6
Streptococcus spp	7	13.7
Enterococcus fecalis	5	9.8
Pseudomonas aeruginosa	4	7.8

Table 6: Table showing MSSA.

Staphylococcus aureus	MRSA	MSSA	TOTAL
	0%	13 (100%)	13 (100%)

Table 5: Antibacterial susceptibility pattern of various bacterial isolates.

Antibiotic agent	S. epidermidis		S. aureus		Streptococcus		Enterococcus		Pseudomonas spp	
	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%
Ciprofloxacin	58.07	41.9	25	75	100	0	50	50	0	100
Ofloxacin	46.8	53.2	25	75	100	0	50	50	100	0
Gatifloxacin	50	50	13	87.5	100	0	50	50	0	100
Moxifloxacin	88.7	11.3	100	0	100	0	100	0	0	100
Chloramphenicol	56.5	43.5	75	25	50	50	50	50	100	0

DISCUSSION

Suppurative keratitis and its complications constitute important causes of ocular morbidity often leading to blindness if early management is not instituted. A proper clinical history coupled with detailed clinical examination would be beneficial to identify the predisposing factors for corneal perforation in microbial keratitis. In the present study included 150 cases of clinically diagnosed keratitis, male (66%) subjects were more affected than female (34%) patients which is in agreement with the study done by Tityal et al. [9] The age range of 41–60 years was more affected consistent with the results of Cameron et al [10] in Sydney and Das et al [11] in Kolkata. This could be attributed to the agricultural workers, labourers and domestic workers men especially in the agricultural workers, labourers and domestic workers and most of the cases were residents from rural area 54.67% and 45.33% were from urban area. In contrast, in the study done in China, women were more affected and most of them were over the age of 60 [12]. This could be due to higher employability of women particularly in the agricultural sector in China.

The most common associated risk factors in our study were trauma [Table 1] followed commonly caused by vegetative matter followed by sand/ stone/dirt [13-16], paddy or its stalk [17], jute followed by steroid [18,1], and also vitamin A deficiency and acquired external ocular disease as predisposing factors for microbial keratitis [19,20]. In the present study out of 150 corneal scraping 108 (68%) were culture positive, and bacteria were recovered more frequently than

CONCLUSION

fungi (51 vs 33 eyes, respectively) [Tables no. 2 and 3]. Srinivasan et al [13] isolated numbers of bacterial (47.1%) agents causing infectious keratitis. Katara et al [21] also reported a culture positivity of 40%, 14% of samples had bacterial etiology.

Bacteria account for 65–90% of corneal infections with Staphylococcus aureus, S. pneumonia and Pseudomonas aeruginosa accounting for more than 80% of bacterial keratitis [22]. In the present study the majority of the bacterial infections were caused by staphylococcus epidermidis (47%) was the most common isolate, [Table no.4] in the Laspina F et al [15] 79%, Gopinathan U et al [23] 42.3%, Tewari A et al [1] 43 %, Gurdeep singh et al [18], but Hagan M et al [24] reported that coagulase negative staphylococci 22.7% were the predominant pathogen of the gram positive cocci. This Staphylococcus species was the predominant cause of bacterial keratitis and coagulase negative staphylococcus was the leading cause [25]. Currently, indigenous bacteria such as coagulase negative staphylococcus are increasingly being isolated in bacterial keratitis and have become the bacterial pathogen most responsible for infectious keratitis in this hospital. Considering the fact that staphylococcus epidermidis forms the commonest commensal of the extraocular surfaces, it is highly probable that the organisms invade corneal tissue when compromised by anti-microbial and/or corticosteroid therapy or trauma.

The standard protocol for treatment of bacterial keratitis in our patients was topical instillation of antibiotics. As there are no standard CLSI guidelines yet for topical ocular antibiotics, proper interpretation of the drug sensitivity testing is not possible; antibiotic sensitivity pattern coupled with clinical improvement is needed to assess the efficacy of a particular antibiotic. Staphylococcus aureus isolates among the total bacterial isolates (including pure and mixed isolates) of keratitis cases in our study accounted to 17.33%.

Regarding the antimicrobial susceptibility pattern, All Gram positive bacteria were sensitive to moxifloxacin. 89.33% sensitivity was seen in S. epidermidis isolates to Ciprofloxacin, Chloramphenicol, and Gatifloxacin. All Streptococcus strains were sensitive to all antibiotics except Chloramphenicol. S. aureus strains were sensitive to moxifloxacin (100%) and Chloramphenicol (75%). Among the Gram negative isolates, Pseudomonas aeruginosa exhibited good sensitivity to Ofloxacin (100%), and Chloramphenicol (100%). and Enterobacter spp exhibited good sensitivity to Moxifloxacin (100%) [Table no. 5]. All of them were subjected to MRSA screening using Cefoxitin disc diffusion test as it is in accordance with the PCR for mec gene (26). No MRSA were isolated in this study [Tab.6].

CONCLUSIONS

In conclusion, it is imperative to know the local aetiology ‘of keratitis in a particular region. Climate and the environment in which the person lives influence the type of infection that develops. Thus the spectrum of the microbial keratitis varies with geographical location, influenced by the local climate and occupational risk factor. Awareness of changes in aetiology and antimicrobial resistance are critical in managing keratitis cases. The study of microbial aetiology of keratitis would greatly help the practicing ophthalmologist in the management of keratitis.

REFERENCES

[1]. Tewari A, Sood N, Vegad MM, Mehta DC. Epidemiological and microbiological profile of infective keratitis in Ahmedabad.Indian J Ophthalmol. 2012~60:267--72.

[2]. Upadhyay MP, Karmacharya PC, Koirala S, Tuladhar NR, Bryan LE, Smolin G, et al. Epidemiologic characteristics, predisposing factors, and aetiologic diagnosis of corneal ulceration in Nepal. Am J Ophthalmo. 1991;111:92-9.

[3]. Gonzales CA, Srinivasan M, Whitcher jP, Smolin G. Incidence of corneal ulceration in Madurai District, south India. Ophthalmic Epidemiol. 1996;3:159-66.

[4]. Whitcher JP, Srinivasan M, Upadhayay MP. Corneal blindness: a global perspective. Bull World Health Organ. 2001; 79:214-21.

[5]. 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.

[6]. Bharathi MJ, Ramakrishnan R, Vasu S, Meenakshi R. Epidemiology of bacterial keratitis in a referral centre in South India . Indian J Med Microbiol. 2003; 21: 239-45.

[7]. Nayak N, Satpathy G, Vajpayee RB. Phenotypic and plasmid pattern analysis of Staphylococcus epidermidis in bacterial keratitis Indian J Ophthalmol. 2007; 55:9-13.

[8]. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty Fourth Informational Supplements. CLSI document M100-S24.Wayne, PA: Clinical and Laboratory Standards Institute;2014.

[9]. Titiyal JS, Negi S, Anand A, Tandon R, Sharma N, Vajpayee B. Risk factors for perforation in microbial corneal ulcers in north India. Br J Ophthalmol. 2006;90:686 9.

[10]. Cameron NL, Pham JN, Paul BR, Sydney B, Glenn H, Diane RL, et al. Bacteria commonly isolated from Keratitis specimen retain antibiotic susceptibility to Fluoroquinolones and Gentamicin plus Cephalothin. Clin Exp Ophthalmol. 2006;34:44 50.

[11]. Das S, Konar J. Bacteriological profile of corneal ulcer with references to Antibiotic susceptibility in a tertiary care hospital in West Bengal. IOSR J Dent Med Sci. 2013;11:72 5.

[12]. Cao J, Yang Y, Yang W, Wu R, Xio X, Yuan J, et al. Prevalence of infectious keratitis in Central China. BMC Ophthalmol. 2014;14:43.

[13]. Srinivasan M, Gonzales CA, George C, Cevallos V, Mascarenhas JM, Asokan B, et al. Epidemiology and aetiological diagnosis of corneal ulceration in Madurai, south India. Br J Ophthalmol. 1997;81(11) :965-71.

[14]. Bharathi M J, Ramakrishnan R, Vasu S, Meenakshi R, Palaniappan R. Epidemiological characteristics and laboratory diagnosis of fungal keratitis. A three-year study. Indian J Ophthalmol. 2003;51:315-21.

[15]. Laspina F, Samudio M, Cibils D, Ta CN, Fariña N, Sanabria R, et al. Epidemiological characteristics of microbiological results on patients with infectious corneal ulcers: a 13-year survey in Paraguay. Graefes Arch Clin Exp Ophthalmol. 2004; 242(3):204–9.

[16]. Khanal B, Deb M, Panda A, Sethi HS. Laboratory diagnosis in ulcerative keratitis. Ophthalmic Res. 2005; 37(3):123–7. Eastern Nepal.

[17]. 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.

[18]. 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..

[19]. Kunimoto DY, Sharma S, Garg P, Gopinathan U, Miller D, Rao GN. Corneal ulceration in the elderly in Hyderabad, south India. Br J Ophthalmol. 2000;84:54–9.

[20]. Ormerod LD, Hertzmark E, Gomez DS, Stabiner RG, Schanzlin DJ, Smith RE. Epidemiology of microbial keratitis in southern California. A multivariate analysis. Ophthalmology. 1987;94:1322–33.

[21]. Katara RS, Patel ND, Sinha M. A Clinical Microbiological Study of Corneal Ulcer Patients at Western Gujarat, India. Acta Med Iran 2013;51:399 403.

[22]. Forbes BA, Sahm DF, Weissfeld AS. Infections of the eyes, ears and sinuses. In: Forbes BA, Sahm DF, Weissfeld AS, editors. Bailey and Scott's Diagnostic Microbiology. 12th ed. St. Louis: Mosby; 2007. p. 832 7.

[23]. Gopinathan U, Sharma S, Garg P, Rao GN; Review of epidemiological features, microbiological diagnosis and treatment outcome of microbial keratitis: Experience of over a decade. Indian J Ophthalmology, 2009; 57(4): 273-9.

[24]. Hagan M, Wright E, Newman M, Dolin P, Johnson G. Causes of suppurative keratitis in Ghana. Br J Ophthalmol. 1995;79(11):1024-8.

[25]. Sharma S. Ocular infections. Research in India. Indian J Med M ICROBIOL. 2010;28:91-4.

[26]. Anand KB, Agarwal P, Kumar S, Kapila K. Comparison of cefoxitin disc diffusion test,oxacillin screen agar, and PCR for mec A gene for detection of MRSA. Indian J Med Microbiol. 2009;27:27-9.

How to cite this article: Venkatalaxmi Rajamanickam, Aruna Sunder, C Prathiba. ISOLATION, IDENTIFICATION OF BACTERIAL AGENT CAUSING KERATITIS, ANTIBIOTIC SENSITIVITY TESTING. Int J Intg Med Sci 2018;5(4):607-612. **DOI:** 10.16965/ijims.2018.107