Section: Ophthalmology

Original Research Article

STUDY THE MICROBIOLOGICAL PROFILE AND ITS ANTIBIOTIC SENSITIVITY IN CLINICALLY SUSPECTED CASES BACTERIAL CORNEAL ULCERS

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Abstract

Background: Corneal blindness resulting from microbial keratitis has been recognised as an emerging cause of visual disability by World Health Organisation. The objective is to study the Microbiological profile and its antibiotic sensitivity in clinically suspected cases bacterial corneal ulcers. **Materials and Methods:** A total of 23 cases of clinically suspected bacterial corneal ulcer of all ages and either sex, who presented in Department of Ophthalmology, Dr Rajendra Prasad Govt Medical College, Kangra at Tanda (Himachal Pradesh) during a period of one year were included in this study. All patients met the inclusion criteria as per the protocol). **Result:** Among gram positive isolates, most common bacterial isolate was Methicillin sensitive Staphylococcus aureus (MSSA) (4. 36.4'%) and amongst gram negative isolates, the most common bacterial isolate was Pseudomonas aeruginosa (2.66.7%)(1magc 14). Enrichment culture on BHI was more sensitive than direct culture. This method has also reduced the number or corneal scrapings to be taken from a patient. Thus. causing, less damage to the compromised cornea. **Conclusion:** Staphylococcus aurous is most common isolates. Routine Microbiological examination of the patients with corneal ulcer is necessary so as to analyse and compare the changing trends in the microbial etiology and their susceptibility pattern. All the aerobic gram positive cocci were sensitive to vancomycin, tobramycin 100% each, to be followed by amikacin and Gentamicin.

INTRODUCTION

Bacterial keratitis is considered a leading cause of monocular blindness in developing countries. In developed countries, the increasing popularity of contact lens wear has contributed to its rising incidence. Given the potential blinding complications of severe bacterial keratitis, these infections are a significant public health issue.[1]

Bacterial keratitis produces a wide spectrum of clinical signs and symptoms ranging from small peripheral superficial keratitis to deep corneal stromal ulceration. Clinical features include symptoms of pain, photophobia, blurred vision, mucopurulent or purulent discharge, chemosis and eyelid swelling (in severe cases).[1]

Early diagnosis and prompt adequate therapy is essential to eradicate the infectious agents, to prevent tissue damage and to minimise scarring or melting.[2]

A host of bacterial organisms can cause infectious keratitis. These organism are commonly grouped by their staining pattern with gram stain and their response to oxygen that is gram positive versus gram negative bacteria and aerobic versus anaerobic respectively.[1]

The most common bacterial pathogens are the following:

1. Staphylococcus aurous: Gram positive and coagulase positive commensal of nares, skin and conjunctiva.
2. Streptococcus: Common gram positive commensal of throat and vagina.
3. Streptococcus pneumoniae (pncumococcus)• Grom positive commensal of upper respiratory tract.

4. Pseudomonas aeruginosa, a ubiquitous Gram negative bacillus (rod), Commensal or gastrointestinal tract. is responsible for over 600/4 of contact lens- related keratitis.

The protocol for management or bacterial keratitis ideally involves collection of corneal scraping material for smear and culture and starting empirical intensive antimicrobial therapy that is either monothcrapy with a broad spectrum antibiotic or a combination of two fortified antibiotics to cover both gram negative and gram positive organisms.

Treatment is modified according to host clinical response and laboratory susceptibility data concerning the organisms along with decisions regarding adjunctive therapy.

There are a few shortcomings of combined fortified regimen. These are variable and shorter shelf life of the fortified preparations. Also, fortification requires special mixing of drugs by pharmacist which increases the risk of contamination. Moreover, fortified drugs need to be prepared frequently owing to its variable shelf life which adds to the cost. Frequent dosing of multiple antibiotics simultaneously may result in increased toxicity and damage to the ocular surface epithelium. Moreover, reflex production of tears due to the increased tonicity of fortified drops leads to dilution and eventually leading to decreased tissue penetration. 2 Using two drugs as a combination may enhance ocular toxicity and may prevent re-epithelisation. Besides this, if both the drugs are administered together, there is potential risk of first drug being washed away. Furthermore, poor patients from rural areas, often are uneducated and have poor access to tertiary care hospitals or pharmacy. They may not be able to store the fortified medication at a cool temperature to maintain its shelf life. Patient's compliance is also difficult to maintain with more medications and confusing regimens.[3]

This study is aimed to study the Microbiological profile and its antibiotic sensitivity in clinically suspected cases bacterial corneal ulcers.

On a wider perspective, this information will also guide us while formulating recommendations for preferred practice patterns and preventive measures of suppurative keratitis in the population at risk.

MATERIALS AND METHODS

This Restricted randomized trial with Allocation concealment was conducted among all patients with clinically suspected bacterial corneal ulcer who visited Department of Ophthalmology and Department of Microbiology at Dr. RPGMC, Kangra at Tanda. A total of 23 patients of clinically suspected bacterial corneal ulcer during a period of one year that was from June 2015- June 2016 with following inclusion and exclusion criteria were included. This study was conducted after getting approval of Institutional ethics committee. .

Inclusion Criterion

All cases of bacterial corneal ulcers of all ages and either gender diagnosed on clinical examination were included.

Exclusion Criterion

1. Patients suspected of fungal corneal ulcer or if culture report showed fungal growth.
2. Patients suspected of viral corneal ulcer
3. Neuroparalytic keratitis.
4. Interstitial keratitis.
5. Complications such as corneal perforation, descemetocoele at time of presentation.
6. Size of ulcer less than 2mm.
7. Patient's refusal

Methods: Patients were subjected to meticulous history taking, documenting socio-demographic information including duration of symptoms, previous treatment, predisposing ocular conditions and associated risk factors. A complete clinical evaluation of patients were done.

CJ Laboratory work up to determine the causative organism meticulous and aseptic collection of corneal scrapings for microbiological evaluation is of critical importance. The corneal ulcer was scraped for microscopy, culture and drug sensitivity for all cases. Procedure was explained to the patient and informed consent was taken prior to start of study from each patient. Corneal scraping was performed under magnification of slit lamp biomicroscope as per the standard protocol.[3]

Specimen Collection: The media and sterile tube with swab stick, for culture were collected from laboratory just before taking sample. Sterile gloves and an aseptic technique for handling specimens were used in all cases. After topical anaesthesia with xylocaine 4%, the sample was collected with the help of Bard Parker 15 no. sterile blade from the base and the leading edges of the ulcer and by debriding the surface layer, which allowed collection of organisms from the deeper layer. Four scrapes were taken. First for gram stain, second for 10% KOH wet mount and third one along with the blade was dropped in 0.5 mL Brain Heart Infusion (BHI) broth and the fourth one was directly inoculated on Blood Agar plate (in a "C" shaped streak).49Scrapes were placed onto a pre-marked and labelled area of the clean glass slides. One for Gram staining and other for 10% KOH wet mount. A separate swab from conjunctiva! sac of the uninvolved eye for culture was also taken after moistening the swab stick with sterile normal saline from a freshly open vac. The slides along with the inoculated BHI broth and Blood agar medium were transported to the microbiological laboratory to be plated onto the appropriate culture media.

Direct Examination: Direct Examination of 10% KOH wet mount and Gram stain for demonstration of fungal elements and bacteria was done.

Gram staining: Smear was first prepared with normal saline on a clean glass slide. Slide was covered with crystal violet and was kept for 1 minute. Then it was washed with tap water and was

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allowed to remain for 1 minute. The smear was then decolourised with acetone for 2-3 seconds and again was washed with tap water. It was then counter stained with safranin for 1 minute. Finally, it was washed with tap water, was allowed to dry and examined microscopically under 100x magnification.

KOH staining: Corneal scraping was taken on a clean glass slide. Then 2-3 drops of 10% KOH was added to it. A cover slip was placed over this so that no air bubble is trapped. Preparation was passed over flame once or twice to hasten dissolution of protein debris. Then, it was examined under 40x of microscope for presence of hyphae, conidia or yeast cells.

Culture: After overnight incubation at 37°C, from BHI broth, subcultures were made onto Blood agar, Chocolate agar, Mac-Conkey agar and Saboraud's Dextrose Agar (SDA) tubes with and without antibiotics. The inoculated Blood agar plates (direct and after subculture), Chocolate agar and Mac-Conkey agar plates were kept at 37°C and were evaluated at 24 hours, 48 hours and discarded after 72 hours if no growth was seen. The SDA tubes were incubated at 25 ° C and 37° C separately for a period of 4 weeks.

RESULTS

Mean age of the subjects was 57.09±18.46 years with a range between 3-80 years of age. Mean age of subjects in Group A was 53.0±22.6 years and in Group B was 60.23±14.73years. Majority of subjects in each group were from 51-80 years of age.

In Group A, equal number of patients had right and left eye involvement whereas in Group B, more number of patients had involvement of left eye than right eye.

Male subjects comprised 65.2% of the study group. Females comprised 34.8% in the study group.

ln group A, 80% (8) of subjects were males and 20% were females, In Group B. Males comprised 53.8% and females compared 46.2% of subjects.[6-8]

Out of all the subjects, 91.3% of subjects belonged to rural areas whereas 8.7% of subjects belonged to urban areas. In Group A, 90% were from rural areas and 10% belonged to urban areas. In Group B, 92.3% subjects belonged to rural area and 7.7% subjects belonged to urban areas.

Majority of subjects were from rural area in both the groups.

Most of the subjects in the study group were illiterate. Of total subjects, 30% of subjects were literate and 70% were illiterate. In Group A, 20% of subjects were literate and 80% were illiterate. In Group B, 38.5% of subjects were literate and 61.5% were illiterate.

Among all subjects in the study, majority were agriculturists (52.2%,12). In Group A, 60% of subjects were agriculturists, 20% were labourers followed by homemakers and others which accounted for 10% each. In Group B, 46.2% of subjects were agriculturists followed by 23% homemakers, labourers and others accounted for 15,4% each, In Group A, 20% of subjects had history of foreign body removal from local traditional healer and had instilled some plant juice/honey in the affected eye. In Group B, 23.1% of subjects had similar history.

One subject had diabetes and other had pulmonary tuberculosis in Group A and leukemia and anaphylaxis was seen in Group B subjects.

Majority of patients in both the groups were on topical antibiotics and topical steroids before presentation.

Gram Staining

Table 1: Results on Gram staining.

Grain staining	Group A(n=I 0)	Group B(n-13)
Grain positive organisms	2(20%)	3(23.1%)
Gram negative organisms	1(10%)	0(0'/4)
No microorganisms seen	7(70%)	10(76.9%)

Out of 23 commensal scrapes taken. 6 (26. l%) isolates on grain staining were identified. Out of these 6. 5(21.7%) were gram positive isolates (2 in Group A and 3 in Group B each) and 1(4.3%) was gram negative isolate (in Group A).

Pyogenic Culture: Out of 23 samples processed. I4 (60.9%} were culture positive. Out of these 14. Majority (11, 78.6%) were gram positive isolates and 3(21.4%) were gram negative isolates. Among gram positive isolates. Most common bactcrial isolate was, Methicillin sensitive staph aureus (MSSA, 36.4%) and amongst gram negative isolates. the most common bacterial isolate was Pseudomonas aeruginosa (2,66.7%).

Direct Culture on blood agar

Table 2: Bacterial isolates on blood agar (Direct culture method)

Bactcrial isolates	Group A(n=IO)	Group B (n:13)
Methicillin sensitive staph aureus (MSSA)	0(0%)	2 (28.6%)
Pseudomonas aeruginosa	2 (50%)	0(0%)
Streptococcus pneumoniae	I (25%)	2 (28.6%)
Streptococcus viridans	0(0%)	2 (28.6%)
CONS	0(0%)	I (14.3%)

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Citrobacter koserii	1(25%)	0 (0'/4)
Tomi	4(100'/4)	7(100'/4)

Out of 23 (l00%) samples processed, 11 (47.8%) were culture positive by direct culture on blood agar. Out of these 11(100%) culture positive. 4(36.4%) belonged to Group A and 7(63.6%) belonged 10 Group B.

In Group A. the organisms which were isolated included, Pseudomonas aeruginosa (2,SO%). Streptococcus pneumoniae (I, 25%) and Citrobacter koserii; (I. 25%).

ln Group B, 2 isohucs csch of Methicillin sensitive staph aureus (MSSA), Streptococcus pneumoniae, Streptococcus viridans, were isolated. Accounting for 28.6% each, followed by Coagulase negative Staphylococcus (CONS)(I, 14.3%).

Figure 1: Pie chart showing bacterial isolates on direct culture in Group A

B. Enrichment culture on Brain heart infusion broth (BHI)

Table 3: Bacterial isolates on Brain Heart Infusion broth (BUI)

Bacterial isolates	Group A(n-10)	Group B(n-13)
Methicillin sensitive staph aureus (MSSA)	2 (28.6%)	2 (28.6%)
Pseudomonas aeruginosa	2 (28.6%)	0(0%)
Streptococcus pneumoniae	I (14.3%)	2(28.6%)
Streptococcus vlridans	0(0%)	2(28.6%)
Coagulase negative Staphylococcus CONS)	1(14,3%)	1(14.3%)
Citrobacter koserii	1(14.3%)	0(0%)
Total		
	7(100%)	7(100%)

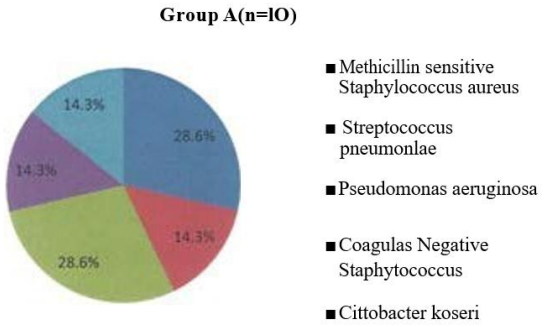

Figure 1: Pie chart showing bacterial isolates on BHI in Group A

Out of 23(100%) samples processed 14(60.9%) were culture positive by direct culture on BHI. Out of these 14 (100%) culture positive, 7(50%) belonged 10 Group A and 7(50%) belonged to Group B.

In Group A, predominant bac1crial isolates were Pseudomonas aeruginosa (2) and Methicillin sensitive staph aureus (MSSA)(2). accounting for 28.6% each, followed by Streptococcus pneumoniae (I, 14.3%) and Coagulase negative Staphylococcus (CONS)(I. 14.3%) and Citrobacter koserii (1, 4.3%).

In Group 8, Methicillin sensitive staph aureus (2), Streptococcus pneumoniae (2). Streptococcus vlridans (2) were equally isolated, accounting for 28.6% each followed by Coagulase negative Staphylococcus (CONS) (I, 14.3%)

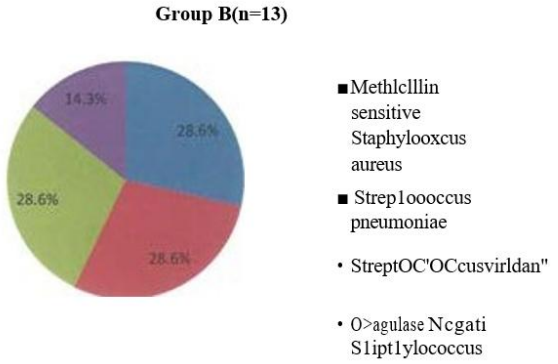

Figure 2: Pie chart showing bacterial isolates on BHI in Group B

Table 4: Gram stain and Culture association

	Culture positive	Culture negative	p value
Gram stain positive	6(26.1%)	0(0%)	0.07
Gram stain negative	8(34.8%)	9(39.1%)	
Total	14(60.9%)	9(39.lo/,)	

A total of 6 (26.1%) cases were both gram stain posi1ivc and culture positive. None of the cases were gram stain positive and culture negative. 34.8% cases were gram stain negative and culture positive. 39.1% cases were both gram stain negative and culture negative. The p-value
Statistically insignificant.

Table 5· Direct culture and Enrichment culture on BHI association

	Culture positive on direct culture	Culture negative on direct culture	p-value
Culture positive by BHI	I I	3	0.001
Culture negative by BHI	0	9	

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Total	20	3	

11/11•100= 100%
Specificity of BHI
9/12•I00= 75%
Positive predictive value 11/14•100= 78.57%
Negative predictive value 9/<1•JOO-, I 00%
Sensitivity of BHI (Enrichment culture) was I 00% as compared 10 direct culture wi1h specificity of 75%. The difference was statistically significant (0.001) with 8HI being better culture media [Figure].

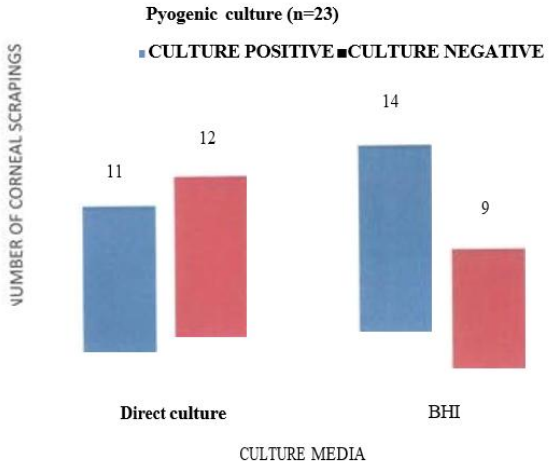

Figure 3: Bar diagram showing comparison of culture results by Direct culture method and Enrichment culture (BHI) method

Culture of conjunctival sac uninvolved eyc:-
Out of 23 subjects, 3(13%) subjects had yielded growth of Methicillin sensitive staph aureus (M.SSA) from their conjunctival flora and rest 20(87%) subjects showed no aerobicbac1erial growth.

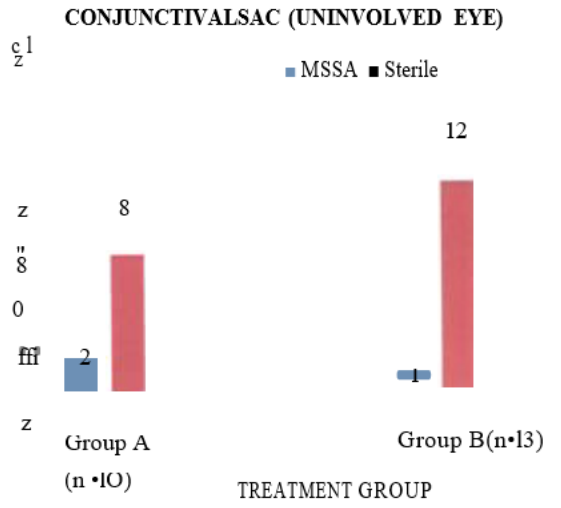

Figure 4: Bar diagram showing bacterial culture or Conjunctival sac (Uninvolved eye)

Antibiotic sensitivity pattern of pathogenic bacterial isolates:

Table 6: Antibiotic sensitivity of gram positive isolates

S No.	Organisms	PEN (lµg)	VAN (30s•sl	GEN (IOµg)	err (5µg)	eEr (30µg)	AZM (15µg)	ex (30µg)
I.	Staphylococcus aereus	4	4	4	4	3	0	4
		(IOO%)	(100%)	(100%)	(100%)	(75%)	(0%)	(I00'/4)
2.	Streptococcus pneumonia(n=3)	0 (0%)	3 (100%)	3 (100'/o)	3 (I00'/4)	3 (100%)	0 (0%)	2 (66.7%)
3.	Streptococcus viridian(n=2)	2 (100%)	2 (100%)	I (50%)	2 (100%)	I (50%)	0 (0%)	2 (100%)
4.	CONS(n=Z)	I (50%)	2 (100'/o)	I (SO%)	2 (100%)	I (SO%)	I (50%)	I (SO%)
	Total	7	11	9	11	8	I	9
	isolates(n II)	(63.7%)	(I00%)	(81.8%)	(100%)	(72.7%)	(50%)	(81.8%)

PEN (Penicillin). VAN(Vancomycin), GEN(Gentamicin),CIP(Ciprofloxacin), CEP(Cephalothin),AZM(Azithromycin).
CX(Ccfoxitin) Gram positive bacterial isolates are 100%sensitive to Vancomycin and Ciprofloxacin followed by,82% sensitive to (gentamicin and Ccfoxitin, 72.7% sensitivity to Cephalothin and 64% sensitivity to Penicillin. These isolates are resistant to zithromycin cxcept for CONS which had shown 50% sensitivity to it.

Table 7: Antibiotic sensitivity pattern of gram negative isolates

S No.	Organisms	PIP (IOOµg)	CEP (3011S)	CAZ (30µg)	GEN (3011g)	CIP (S11g)	AMC (30µg)	JPM (IOµg)
I.	Pseudomonas aeruginosa (n=2)	2 (100%)	0 (0%)	2 (100%)	2 (100%)	2 (100%)	0 (0%)	2 (100%)
2.	Citrobactor koserii(n= I)	0 (0'/4)	0 (0%)	I (100'/4)	I (100%)	1 (100%)	I (100%)	I (100'/4)
	Total (n=3)	2 (66.7%)	0 (0%)	3 (100%)	3 (100'/4)	3 (100%)	I (33.3%)	3 (100%)

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lPIP{Pipcrncillin),CEP(Ccphalothin), CAZ (Ccfin, idime). GEN (Gentamicin),CIP(Ciprofloxacin), AMC(Amoxyclav), IPM(lmipencm)

Gram ncgative isolates are 100% sensitive to Ccftazidime, Gentanticin. Ciprofloxacin and Imipenem followed by 66.7% sensitivity to Piperncillin sad 33.3% sensitivity to Amoxyclav, These isolates had shown resistance to Cephalothin.

Image 1: Blood agar showing growth of Methicilin sensitive Staphylococcus aureus.

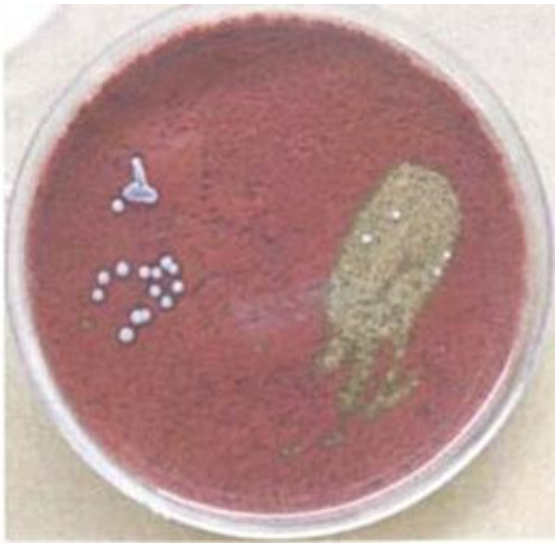

Image 2: Chocolate agar showing growth of Methicillin sensitive Staphylococcus aureus.

Image 3: MacConkey agar showing growth of Pseudomonas aeruginosa.

DISCUSSION

Direct microscopy (gram staining) was positive in 26.1% cases which was higher than study done by Verma et al (12.9%) and lower than Amirutha et al (48%). Culture was positive in 60.9% of the cases. Similar results were observed by Chhangte et al (68.4%) and Amrutha et al" (67%).

Higher incidence was seen in study done by Change et al (42.8%) and Tewari et a1 7(54%). Lower incidence was seen in study done by Patel at al,[7] (16%) However. Streptococcus pneumonioe was the predominant species in study done by Bamthi at al. ln this study Streptococous pneumonia (27.3% or total Gram-positivc cocci isolates) was the second most common bacterial isolate found. followed by Streptococcus viridans (18.2% of total Gram-positive cocci isolates) and Coagulase negative Staphylococcus (18.2% of total Gram-positive cocci isolates).

Similar results were seen in study done by Tewari et al,[7] This is in contrast to studies done by Bosak at al,[2] Barathi et al and Srinivasan et al, which have demonstrated a 4.0-12.5% incidence of gram positive bacilli.

In current study. most common bacterial commensal isolated from conjunctival sac of uninvolved eye in both the groups was Methicillin sensitive staph aureus (MSSA) (13.1% of total conjunctival swabs taken) however., in study done by Shanna et al in lower to mid Himalayan region of Shimla hills, found that Staphylococcus spccies (60%) was the predominan1 bacterial isolates with Staphylococcus epidermidis (29%) being the most common commensal organism in conjunctival flora. Similar results were obtained by Kanhika et al (32%,) and Sthapit et al" (5I%),

Hospilal based antibiotic sensitivity pattern for bacterial keratitis should be establishcd and followed for every case. As evident from sensitivity results, gram positive isolates were 100% sensitive to fluoroquinolones (Ciprofloxaein) and Vancomycin, It was followed by aminoglycoside

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(Gemamicin) and cephalosporins (Ccphalothin). Most of tbc isolates were rosistant to macrolides (Azithromycin). So, first line of drugs for gram positive isolates remains ciprofloxacin. Vancomycin should be kept as a reserve drug in drug resistant cases.

Gram nega1ive isolates were I00% sensitive to fluoroquinolones (Cipronoxacin), aminoglycosides (Gentamicin), third generation cephalosporins (Ccftazidimc). and lmipencm. On the other hand, gram negative isolates did not yield good sensitivity Pattern of Amoxyclav combination and were resistant to first generation cephalosporin (Cephalothin).

Thus, it can be established from sensitivity pattern that fluoroquinolones are a better alternative over cephalosporins and aminoglycosides in treatment or bacterial kcratitis. Vancomycin. Cefiazidime and lmipenem can be used as higher drugs. reserved for resistant cases. In s1udics done by Tewari et al and Amrutha et al, 1he gram positive isolates were sensi1ive to ciprolloxacin.

Culture and antibiotic sensitivi1t is necessary for microbiological confim1ation of pathogenic organisms and initiation of appropriate drug regimen based on sensitivity reports is an ideal approach. In rural settings, where microbiological facilities are unavailable or till the culture reports are awaited, intervention must be initiated as corneal ulceration is an urgency and can lead 10 blinding complica1io11s if tl1erapy is delayed.

CONCLUSION

Corneal ulcer is more common in middle aged agriculturist men. Staphylococcus aurous is most common isolates. Routine Microbiological examination of the patients with corneal ulcer is necessary so as to analyse and compare the changing trends in the microbial etiology and their susceptibility pattern. All the aerobic gram positive cocci were sensitive to vancomycin, tobramycin 100% each, to be followed by amikacin and Gentamicin.

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