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Etiological Profile and Clinical Outcomes of Bacterial Keratitis from a Tertiary Care Centre in North Kerala

John Britto Augustin[1], Suresh Baboo V. K[2]

[1]Assistant Surgeon, FHC Aralam, Kannur
[2]Additional Professor, Dept. of Microbiology, Government Medical College, Kozhikode

Corresponding Author: Suresh Baboo V. K

ABSTRACT

Corneal ulcer is a leading cause of ocular morbidity and monocular blindness worldwide. To effectively prevent blindness in patients with corneal ulcer, a proper understanding of risk factors predisposing to ulceration, its clinical and microbiological characteristics are essential. Timely identification of aetiological agents causing corneal ulcer and their prompt treatment helps to save the vision.

A cross sectional study was conducted in Government Medical College, Kozhikode, between January 2016 to June 2017 to detect bacterial agents of corneal ulcer and determine the antibiotic susceptibility pattern of those isolates. All patients who were clinically diagnosed as cases of infectious corneal ulcer in the Ophthalmology department, Government Medical College, Kozhikode were included in the study. Corneal scrapings collected from the infected eye were subjected to microbiological examination and culture.

Among the 120 cases, a total of 27 cases were culture positive. 21/120 [17.5%] were bacterial, 6/120 [5.0%] were polymicrobial and 22/120 [18.34%] were fungal. Among bacterial aetiology, *Pseudomonas aeruginosa* was most common [33.34%] followed by Coagulase negative *Staphylococcus*-22.23% and *Streptococcus pneumoniae*-18.51%. Trauma was the major risk factor. Diabetes mellitus, contact lens usage, exposure keratitis were the other comorbidity / risk factors. Out of the total 27 culture positive bacterial corneal ulcer cases none healed completely, 77.78% improved clinically with opacity and 22.22% ended with recurrence/complications.

This study shows majority of infected corneal ulcers are associated with risk factors and the aetiology are mainly due to bacteria. Corneal ulcers responded well to antimicrobials except a few complicated ulcers. Microscopy, culture and clinical correlation helped in adequate management. Thus, prognosis and outcome of corneal ulcers rely on timely identification of their aetiology and prompt treatment.

Key words: Corneal ulcer, bacterial keratitis

INTRODUCTION

Corneal ulcer is a vision threatening disease and a leading cause of ocular morbidity and blindness worldwide [(1), (2)]. It is considered as an ophthalmic emergency which requires prompt treatment. The severity of corneal infection depends on the underlying condition of cornea and pathogenicity of infecting pathogen [(3)]. The incidence and spectrum of corneal ulcer depends on different factors, viz. Geographical and location related factors, and the degree of development of a country. Corneal ulcer is the major cause of monocular blindness in developing countries [(4)]. To effectively prevent blindness in patients with corneal ulcer, a proper understanding of risk factors predisposing to ulceration, and clinical and microbial characteristics of the disease is essential.

Microbial keratitis is an inflammatory condition caused by infection due to bacteria, fungus, virus and protozoa. The role of microbiologist in management

of corneal ulcer is crucial as it has to be differentiated from other non- inflammatory conditions of cornea resulting from immune mediated reactions. It is widely accepted that the bacterial and fungal keratitis have higher incidence in developing countries. The parasite infection is also a cause of ocular morbidity, but with aggressive treatment it can be eradicated.

Diagnosis and treatment of corneal ulcer is challenging as the culture reports are sometimes inconclusive. Microbial keratitis should be detected early and treated timely in order to save the vision. Ocular morbidity such as corneal scarring and subsequent visual loss can be significantly reduced by prompt institution of appropriate therapy guided by the knowledge of the causative agents. [(5)] Severe corneal ulcers might progress to corneal perforation, with poor visual outcome. Therefore, identification of the pathogens and appropriate treatment is critical to a good clinical outcome [(6)]

MATERIALS AND METHODS

A cross sectional study was conducted at Ophthalmology and Microbiology Departments of Government Medical College, Kozhikode between January 2016 to June 2017 after obtaining IRC approval. All patients clinically diagnosed with infectious corneal ulcers presenting to the Ophthalmology Department of Government Medical College, Kozhikode were included in the study. Corneal ulcers of non-infectious aetiology were excluded from the study.

Each patient was examined with the slit lamp biomicroscope by an ophthalmologist. After staining with fluorescein and with the variable slit on the biomicroscope the details of the corneal ulcer - including the location, size, shape, depth of the ulcer, nature of infiltrate, margins of the ulcer, presence of any satellite lesions, immune ring, corneal vascularization, and hypopyon – were recorded [(8), (7)] A sketch of each ulcer was also drawn on the casebook using standardised frontal and cross sectional diagrams, and the presence or absence of a hypopyon was recorded and the size and height measured in millimetres. A standardized proforma was completed for each patient documenting sociodemographic information as well as clinical findings. Associated ocular conditions such as blepharitis, dacryocystitis, dry eyes, corneal anaesthesia or ocular leprosy were noted. [(8),(9)]

Scrapings from cornea at the site of corneal ulcer were collected by ophthalmologist, after taking detailed clinical history, and examination of the affected eye. Eye was anesthetised with sterile 0.5% tetracaine or 4% lignocaine 2-3 drops. The surrounding skin was wiped with sterile gauze soaked in sterile saline and excess of discharge and debris were removed. A sterile self retaining lid retractor was applied to minimise contamination with conjunctival flora. Using No.15 Bard Parker blade, ulcers were scraped thoroughly and gently from base and edges of ulcer [(10),(11)]

The laboratory procedures used in the diagnosis of infectious keratitis were based on direct visualisation of organisms in the material and inoculation of material on to blood agar under appropriate conditions to allow multiplication of organisms [(12)].

The material scraped first, was directly inoculated onto blood agar, for isolation of bacteria and the last scraping was used to prepare a smear for Gram staining [(13)]. All bacterial cultures were incubated aerobically at 37ºC for 48 hours and discarded if there was no growth. Chocolate agar and Lowenstein Jensen media were used for suspected cases of Pneumococci and Mycobacteria. The corneal scrapings were subjected to Gram stain. Gram stain was examined for pus cells and bacteria.

Samples were streaked on culture plates as ‘C’ or ‘S’ shapes. Bacterial growths in the streaks were identified by Gram stain, and by a battery of biochemical reactions. The criteria adopted for microbial evaluation was that the sample would be

considered positive if any one of the following were met.

a) The growth of the same organism was demonstrated on two or more media on C-streak.
b) The same organism was grown from repeated scrapings.
c) It was consistent with clinical signs.
d) Smear results confirmed the finding from cultures [14]

Antimicrobial susceptibility tests were carried out on isolated bacteria using commercially prepared antibiotic disk (HiMedia) on Mueller Hinton agar plates by the disk diffusion method, as per Central Laboratory Standards Institute (CLSI) guidelines.

RESULTS

A total of 120 cases of infective corneal ulcer have been studied clinically and corneal scrapings of all the 120 cases were processed in Microbiology laboratory, Government Medical College, Kozhikode.

Out of total 120 cases 71[59.17%] were males and 49[40.84%] were females. Majority (29%) of the cases were in the age range of 60-69. (Fig.1)

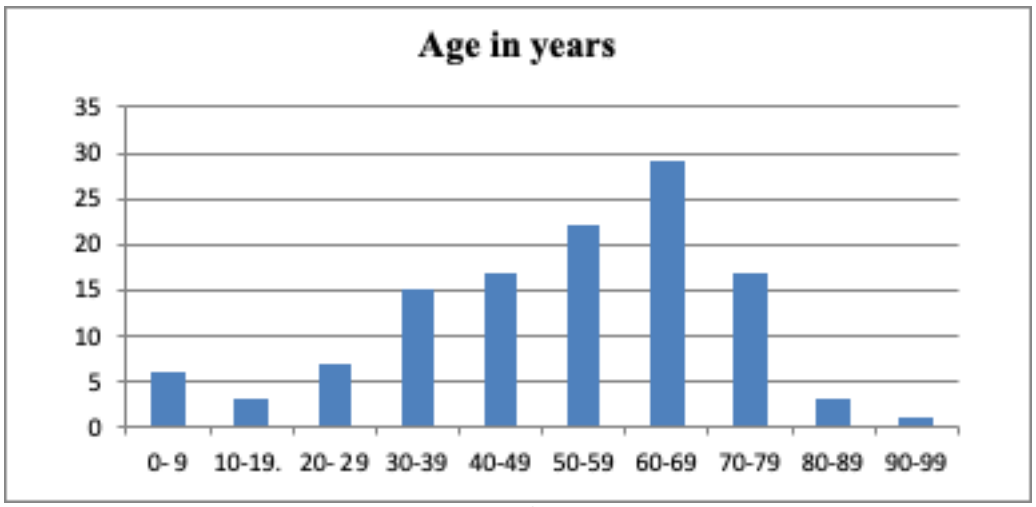

Fig: 1 Age distribution of the study population.

Manual labourers (38.3%) accounted for majority of cases.14.2% of cases were agricultural workers. Majority [62.96%] had history of trauma of some kind. (Table:1)

Table: 1 Objects of trauma

Objects of trauma	No. of positive bacterial cases	Percentage
Unknown foreign body exposure	7	25.92
Wooden twigs, sticks and concrete particles and cement	6	22.22
Previous surgeries and contact lens usage	3	11.11
Metal pieces	1	3.70
No trauma	10	37.04
Total	27	100

Diabetes mellitus and hypertension were the most common comorbidities associated with those cases where there was no history of trauma. No history of trauma in 10/27 [37.04%] cases. The most common clinical features were pain [86.7%] and redness [75%] followed by watering [50%] and hypopyon [25.93%].

Left eye involvement was slightly more than right eye. Ulcers are central than peripheral. 24/27 ulcers had ulcer size <6mm.21/27 ulcers involved stroma. More than half of ulcers had regular margins.

Among a total of 36 suspected bacterial keratitis only 27 were positive by culture, rest were positive only by microscopy. Out of 27 cases positive by culture, pure bacterial isolates were obtained in 21 cases. In 6 cases, bacteria were isolated along with fungi. (Fig: 2)

Out of 21 cases were pure growth was obtained; presence of bacteria could be established both by microscopy and culture in seven cases, whereas culture alone was positive in 14 cases.

In six cases, where bacterial growth was obtained along with fungi, presence of bacteria could be established both by microscopy & culture in four cases whereas in two of these cases bacteria were obtained only in culture.

Out of 36 suspected cases, nine were microscopy positive and culture negative.

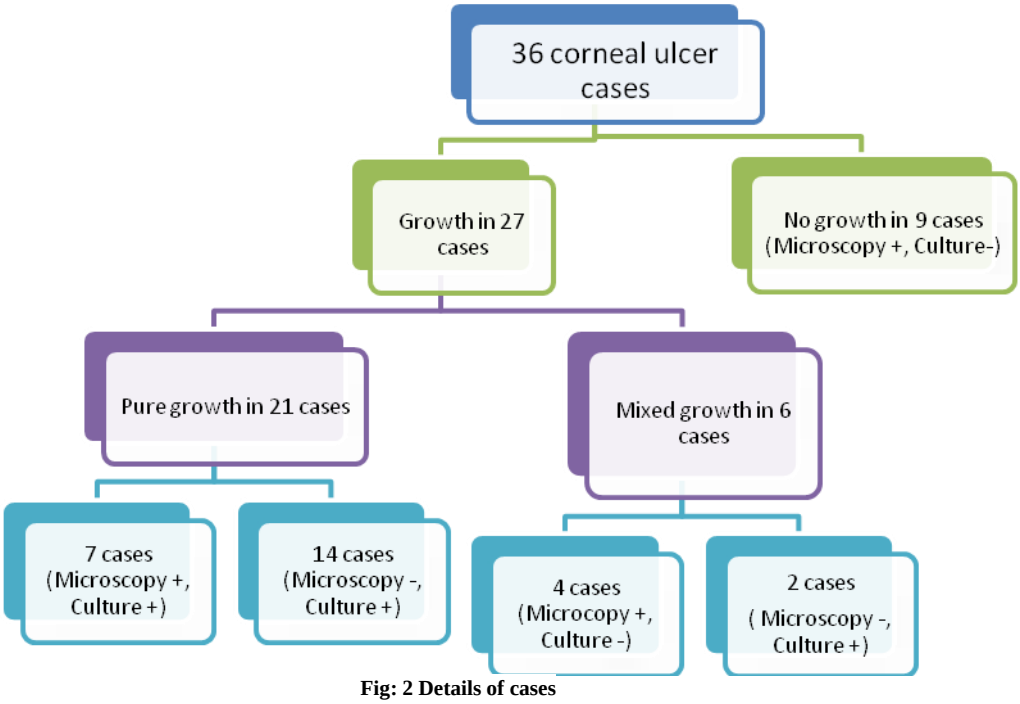

Fig: 2 Details of cases

Microbiological profile of Corneal ulcer

Table 2: Microbiological profile of corneal ulcer

Isolates	No. of cases	Percentage
Bacteria	21	17.5%
Fungus	22	18.34%
Parasite	0	0%
Mixed isolates[bacterial+fungal]	6	5%
Organism not isolated	71	59.17%
Over all total	120	100.0%

Total Bacterial profile

Table 3: Total bacterial profile

Bacteria	No. of cases	Pure isolates	%	Mixed isolates	%	Total Percentage	Valid percentage
P.aeruginosa	9	8	6.6	1	0.83	7.5	33.34
CoNS	6	2	1.66	4	3.33	5	22.23
S.pneumoniae	5	4	3.33	1	0.83	4.2	18.51
MRSA	2	2	1.7	0	0	1.7	7.41
Diphtheroids	2	2	1.7	0	0	1.7	7.41
Acinetobacter spp	1	1	0.8	0	0	0.8	3.7
E .coli	1	1	0.8	0	0	0.8	3.7
Micrococci	1	1	0.8	0	0	0.8	3.7
Total	27	21	17.45	6	4.99	22.5	100

Pseudomonas aeruginosa was the most common bacterial isolate [33.34%] followed by CoNS (22.23%).

Three contact lens had been cultured from suspected cases of corneal ulcer and one yielded *Pseudomonas aeruginosa* both from scrapings and contact lens culture, other two were sterile.

Mixed isolates

Table 4: Mixed isolates [bacterial + fungal]

Mixed isolates	No. of Cases	Percentage	Valid percentage
CoNS+Fusarium solani	2	1.7%	33.34
Pneumococci+Aspergillus fumigatus	1	0.8%	16.67
Pseudomonas aeruginosa+Acremonium	1	0.8%	16.67
CoNS+Scedosporium apiospermum	1	0.8%	16.67
CoNS+Cylindrocarpon	1	0.8%	16.67
Total	6	4.9%	100

Antibiotic sensitivity pattern [%] of Gram Negative Bacterial isolates

Table 5: Antibiotic sensitivity pattern [%] of Gram negative bacterial isolates

	G	A	M	Cf	Cfs	Cip	Tax	Pip	Ptz	Cefta	Net	Mr
Acinetobacter baumanii[1]	100 [1]	0 [0]	100 [1]	0 [0]	100 [1]	0 [0]	0 [0]					
Pseudomonas aeruginosa[9]	22.23[2]		55.56[5]		50[1]	22.23[2]		55.56[5]	66.67[6]	55.56[5]	66.67[6]	100[3]
E .coli[1]	100 [1]	0 [0]	100 [1]	100 [1]	100 [1]	100 [1]	100[1]					

G-gentamicin, A-ampicillin, M-amikacin, Cf-cefazolin, CFS-cefoperazone sulbactam, Cip-ciprofloxacin, Tax-cefotaxime, Pip-piperacillin, Ptz-piperacillin tazobactam, Cefta-ceftazidime, Net-netilmycin, Mr-meropenem

E.coli and *Acinetobacter* sp were found to be susceptible to all antibiotics tested. Approximately 50% of *Pseudomonas aeruginosa* isolated were resistant to the routine antibiotics tested.

Antibiotic sensitivity pattern [%] of Gram Positive Bacterial isolates

Table 6: Antibiotic sensitivity pattern [%] of Gram positive bacterial isolates

	P	A	G	E	Ceftri	Va	CO	Cf	Cn	M	Lz	Rif	Tetra
Streptococcus pneumoniae[5]	100[5]	100[5]	80 [4]	60[3]	100 [5]	100 [5]							
CoNS[6]	0 [0]		50 [3]	0 [0]		100 [6]	50 [3]	33.34 [2]	33.34[2]	83.34 [5]	100 [6]		
MRSA[2]	0 [0]		100 [2]	0 [0]		100[2]	50 [1]	0	0	100 [2]	100[2]		
Diphtheroids[2]	50 [1]	50 [1]	50 [1]	0 [0]		100 [2]						100 [2]	50 [1]

P-penicillin, A-ampicillin, G-gentamicin, E-erythromycin, Ceftri-ceftriaxone, Va-vancomycin, CO-cotrimoxazole, Cf-cefazolin, M-amikacin, Lz-linezolid, Rif-rifampicin, Tetra-tetracycline

Table 7: Aetiology Vs Clinical outcome

Aetiology	Ulcer healed without opacity	Improved clinically	Recurrence/complication	Total
Bacterial	0	16	5	21
Mixed [Bacterial +fungal]	0	5	1	6
Not isolated/sterile	0	0	0	93
Total	0	21	6	120

Of the 120 cases, none healed completely, 21 improved clinically with opacity and 6 had recurrence/complication. Nine cases where microscopy alone was positive healed completely.

DISCUSSION

A total of 120 clinically diagnosed cases of corneal ulcer were studied in Ophthalmology department, during January 2016- June 2017 and their corneal scrapings were sent to Microbiology department of Government Medical College, Kozhikode.

In this study, cases belonged to different age groups from 0-99 years. Median age was 55 and higher incidence noted in 60-69 years, which was closely followed by 50-59 years. Males were affected more than females in our study. The cases were most common among manual labourers accounting to increased ocular trauma in such cases. Manual labor is a common occupation in this part of Kerala. There is association between non agricultural workers or manual labourers with corneal ulcers, especially bacterial ulcers in this study. MJ Bharathi et al in their study observed that there was significantly higher incidence of bacterial keratitis among patients other than agricultural workers [(15)] In the present study, Pseudomonas ulcers were seen during summer and autumn. Trauma is the leading risk factor in this study. Unknown foreign body exposure [25.92%], trauma due to wooden twigs and sticks, concrete particles and cement [22.22%], previous surgeries and contact lens usage [11.11%] and finally metal pieces [3.70%] were the common objects of trauma. Diabetes mellitus was an important risk factor in injured and non injured cornea. Ranjini and Waddepally found that the most common associated risk factors in their study were trauma followed by diabetes mellitus and contact lens usage which is comparable with other studies [(5)]. Stapleton et al observed, Contact lens wear was a common predisposing factor in microbial keratitis and was one of the preventable risk factors for corneal infection in a working age population [(16)] Cumurcu et al noticed, the patient in their study used multipurpose solution for cleaning, disinfecting, and storing daily-wear soft contact lenses. The extended wear may have been a cause for microbial [*Klebsiella pneumoniae*] keratitis [(17)]. In this study *Pseudomonas aeruginosa* was isolated from 1 out of 3 contact lens culture. Stapleton et al observed *Pseudomonas aeruginosa* remains the commonest cause of contact lens-related corneal infection probably because of its unique virulence characteristics and ability to survive in the contact lens/storage case/ocular environment. [(16)] We observed that, left eye was slightly more involved than right eye. Pain and redness were the common symptoms in this study followed by watering, photophobia, foreign body sensation, defective vision, and hypopyon. Central corneal ulcers were more common, followed by inferotemporal. In this study, out of 21 pure bacterial cases, seven were microscopy [Gram stain] and culture positive, 14 were microscopy negative, but culture positive. Interestingly, nine were microscopy positive and culture negative. These might be due to prior treatment with antibiotics in this study, Out of 120 cases, there were 27 isolates [22.50%] bacterial. The bacterial isolates were *Pseudomonas aeruginosa* [33.34%], CoNS [22.23%], followed by *Streptococcus pneumoniae* [18.51%]. The six (6) mixed isolates were *Streptococcus pneumoniae+Aspergillus fumigatus*-1 out of 6[16.67%], *Pseudomonas aeruginosa+Acremonium*-1[16.67%], CoNS+*Fusarium solani*-2 [33.34%], CoNS+*Scedosporium*

apiospermum-1 [16.67%], CoNS+ *Cylindrocarpon* -1 [16.67%]. The most common bacterial isolate in this study was *Pseudomonas aeruginosa* [33.34%]. Kampitak et al observed in their study that the most common bacteria was *Pseudomonas aeruginosa* (55%) and most common fungus was Fusarium species (33%).[6]

In the current study, 7 out 9 *Pseudomonas aeruginosa*, had h/o trauma [surgical and non surgical], 1 had radiation history and one without trauma. Three had diabetes mellitus as additional risk factor. In one case, Pseudomonas was isolated along with fungal growth. Ulcers were <6mm [except one>6mm], stroma involved, 2 with stromal abscess, 1 was perforated, 3 with hypopyon, central or peripheral ulcers with most of them having regular margins. Gram stain was positive in one case with only presence of pus cells in all others. *Pseudomonas aeruginosa* was isolated from contact lens culture and scrapings in one case. All were started empirically on fortified gentamicin or amikacin along with fortified cefazolin or moxifloxacin topicals. According to the antimicrobial sensitivity, oral ciprofloxacin, injectables ciprofloxacin, ceftazidime or piperacillin-tazobactam were added. In case of mixed infection, antifungals were added. Most of the ulcers [66.67%] responded well and 44.44% were multidrug resistant, one of which later required scleral exploration. Outcome was poor for 3 ulcers, others improved. Leck et al observed, *Pseudomonas species* were the commonest bacterial isolates from corneal ulcers in Ghana (52.5%); *P aeruginosa* being the commonest reported species.[18] Kampitak et al observed in their study that, in the total 35 cases in contact lens wearers, 29% (10/35) of cultures from corneal scraping were positive. Nine out of 10 cases were *Pseudomonas aeruginosa* and only one case was *Acinetobacter baumannii* [6] Al-Mujaini et al noticed that within 2-5 days of Pseudomonas infection, an untreated corneal ulcer may lead to descemetocele formation and perforation of the cornea[19]

Streptococcus pneumoniae, was isolated in 5 cases of which four had h/o trauma- surgical and non surgical. One was isolated along with fungal growth. Ulcers were <6mm, with regular margins, stroma involved, peripheral and paracentral ulcers. 1 had diabetes mellitus as additional risk factor. Microscopy was positive for 2 ulcers and showed Gram positive, lanceolate diplococci. All were started on fortified gentamicin and fortified cefazolin or moxifloxacin topicals on Gram smear results or empirically. In one of the Pneumococcal ulcer, prognosis was worse because of panophthalmitis. Others improved. Natamycin was added for the case with fungal growth and improved. Bharathi et al in their study, observed, the most commonly isolated organism was *Streptococcus pneumoniae*, (41.85%) followed by *Pseudomonas aeruginosa* (21.24%) [4] Suwal et al says that, *S. pneumoniae* is the major biological agent causing corneal ulcer in developing as well as industrial nations. The production of virulence factor pneumolysin favors *S. pneumoniae* to establish infection in corneal epithelium [20]

In this study, 2 out of 6 CoNS were isolated alone, rest of the 4 Corneal ulcer caused by CoNS were isolated along with fungal growth. The first two cases had no h/o trauma and latter 4 had. There were no other risk factors. All cases of isolated CoNS [2 cases], ulcers were <6mm, with regular margins, not involving stroma and central ulcers. Gram stain showed Gram positive cocci in both. In corneal ulcers, with bacterial and fungal isolation, they were <6mm except one, with irregular margins and mostly central ulcers involving stroma. Hypopyon was seen in 2 of the six cases. Gram positive cocci were visualised in all except one. 10% KOH was positive for 2 cases. Out of all CoNS isolated, 4 were cefoxitin resistant. All were started on fortified gentamicin or amikacin and fortified cefazolin or moxifloxacin topicals. All ulcers improved with the mixed isolates requiring addition of Natamycin. One of

these cases required addition of intralesional, intracameral and topical voriconazole. CoNS is a potential organism which can cause keratitis. Bourcier et al observed, Gram positive bacteria were predominant (83% of all positive cultures), mainly coagulase negative *Staphylococcus* species [(3)]. Kaliamurthy et al observed that, *S. epidermidis* continues to be a very important bacterial cause of keratitis [(21)]

MRSA had been isolated from two cases, both had no h/o trauma, one had both diabetes mellitus and contact lens usage as risk factors. Ulcers were one < 6mm, other >6mm, with regular margins, and central or paracentral ulcers, with and without involvement of stroma respectively, both with hypopyon. Gram stain showed Gram positive cocci in clusters in one case. Coagulase test was positive and cefoxitin was resistant for both. One was started empirically on moxifloxacin and latter one on fortified gentamicin and fortified cefazolin. Later both were changed to fortified vancomycin on culture reports and ulcer improved. Hsiao et al observed in their study that the most common predisposing factor for both CA-MRSA and HA-MRSA keratitis was ocular surface disease, which accounted for 80.7% of the MRSA keratitis cases. No significant difference in clinical manifestations was observed between the CA-MRSA and HA-MRSA keratitis cases in their study. [(22)]

Diphtheroids were isolated from 2 cases. One had history of exposure keratitis and the other glaucoma. Ulcers were <6mm diameter, irregular or regular, with one involving stroma. Gramstain showed Gram positive bacilli in both cases. They were isolated from blood agar on C-streaks and showed enhanced growth on Blood agar with Tween 80. Both were started on moxifloxacin or fortified amikacin and oral ciprofloxacin according to the clinical presentation. Earlier one was changed to fortified vancomycin on antibiotic susceptibility testing and both ulcers improved. Rubinfeld et al studied a series of eight cases of apparently infectious keratitis associated with heavy growth of diphtheroids on cultures of ulcer scrapings. All of these cases included indolent ulcers that occurred almost exclusively in elderly patients. All patients had preexisting ocular conditions that compromised the corneal surface. [(23)]

Acinetobacter baumannii was isolated from a 5 day old newborn who had congenital buphthalmos without a h/o trauma. The ulcer was irregular, peripheral and perforated penetrating beyond stroma. Gram stain was negative. Cefotaxime and amikacin was started empirically but cefotaxime was found to be resistant and was changed but prognosis was poor. Broniek et al noticed that, keratitis caused by *Acinetobacter* spp. is rare and may result from trauma, contact lens wear or ocular surgery, like penetrating keratoplasty- PKP or cataract surgery.[(24)]

E.coli was isolated from one case, which had h/o trauma and had diabetes mellitus as additional risk factor. Ulcer was <6mm with regular margins with stromal involvement and central ulcer. Microscopy was negative and started on moxifloxacin topical and ciprofloxacin oral and it was found to sensitive on antimicrobial susceptibility testing. Ulcer improved on antibiotic treatment. Bharathi et al in their study noticed, *Enterobacter* species was found in (1.92%) whereas other Gram negative bacilli like *Proteus* species, *Klebsiella* species, *Haemophilus* species and *Escherichia coli* were isolated only rarely[(4)] Al-Mujaini et al observed that, *Klebsiella, Escherichia coli* and *Proteus* keratitis are common in compromised corneas with chronic epithelial disease, often without any history of trauma. A typical lesion has an indolent course with mild anterior chamber reaction [(19)]

Micrococci were isolated from one case, in which there was h/o trauma. Ulcer was <6mm, with irregular margins, stroma involved, peripheral ulcer. Microscopy was negative but correlated with clinical presentation. Isolates were furazolidone resistant and bacitracin sensitive.

Moxifloxacin topical and oral ciprofloxacin was started and the ulcer improved. Mallareddy observed in their study, that among 40 bacterial isolates *Pseudomonas aeruginosa* was the predominant bacterial isolate (22.5%) followed by *Staphylococcus aureus* (20%), *Streptococcus pneumoniae* (20%) and *Micrococcus species* (20%) [25]

CONCLUSION

To conclude with, bacterial keratitis has an acute presentation, can be aggressive with delay in prompt management. Therefore, early identification and prompt timely treatment of corneal ulcer gives good clinical outcome, thereby saves the vision.

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