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Microbiological profile and antimicrobial sensitivity pattern of culture positive bacterial keratitis: A prospective observational study

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Abstract
Background: Microbial keratitis is suppurative infection of cornea considered as emergency and potential threat to vision. Prompt diagnosis and treatment of microbial keratitis is required to prevent complication and blindness. So present study has been designed to determine bacteriological profile and sensitivity pattern of culture positive bacterial keratitis, risk factor associated it and treatment outcome.
Material and Method: After enrolment of patients a detailed relevant history of patients regarding to mode of injury and predisposing factor were recorded and detailed clinical examination was done. Visual acuity of all patients was tested. Slit lamp biomicroscopy was performed and Corneal ulcer was examined properly. Under local anaesthesia with 4% lignocaine corneal scrap was taken from edge and base of ulcer. Sample was sent for gram stain, 10% KOH wet mount preparation, blood agar and Sabouraud's dextrose agar.
Result: In present study out of 600 specimen 460(76.66%) patients were culture positive out of them 47.33% were fungal and remaining were bacterial(29.33%). Regarding bacteriological profile of culture positive corneal ulcer, we have observed that gram positive bacteria were common than gram negative. Among all staphylococcus aureus was common organism 54(30.68%).
Discussion and Conclusion: From present study we can conclude that bacterial corneal ulcer is less common than funga ulcer. Regarding bacteriological profile of culture positive corneal ulcer, we have observed that gram positive bacteria were common than gram negative. Among all staphylococcus aureus was common organism followed by pseudomonas. Regarding sensitivity of common organism isolated staphylococcus was 100% sensitive to vancomycin and azithromycin, sensitivity to fluoroquinolones were from 60 % to 80 %. More than 80% pseudomonas was sensitive to fluoroquinolones and aminoglycosides. 77.27% patients were recovered at final follow up.

Keywords: Bacterial keratitis, outcome, microbiological profile

Introduction

Microbial keratitis is suppurative infection of cornea considered as emergency and potential threat to vision. Prompt diagnosis and treatment of microbial keratitis is required to prevent complication and blindness [1]. As per world report on vision by world health organisation globally, at least 2.2 billion people have a vision impairment or blindness, of which at least 1 billion have a vision impairment that could have been prevented or has yet to be addressed. Corneal opacity is 5th leading cause of blindness and is estimated to be responsible for 1.5–2.0 million cases of unilateral blindness annually [3, 4, 5].

Most of the time microbial keratitis is associated with predisposing factor and ocular trauma or ocular surface diseases are considered as common predisposing condition but with the use of contact lens the incidence of contact lens related keratitis has increased [6, 7]. The spectrum of bacteria keratitis largely depends upon geographic and climatic factors [8]. After literature search we have found that there is regional variability in sensitivity pattern of microbial agent towards antimicrobial drug and microbiological characteristics of this infection.

Bacterial keratitis is a serious ocular infection so rapid diagnosis and rapid antimicrobial therapy is required with appropriate antimicrobial agent. As there is variability in nature of causative organism and its sensitivity pattern appropriate information about common pathogen and its sensitivity pattern is required.

After going through various literatures we have found that there is variability in organism isolated and its sensitivity pattern. Hernandez-Camarena JC, Graue-Hernandez EO, Ortiz-Casas M, from Mexico City has reported that the most commonly isolated pathogen was

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Staphylococcus epidermidis, and the most common gram-negative isolated species was Pseudomonas aeruginosa. The overall sensitivity for vancomycin of MRSA was 87.5%, whereas 99.6% of the MRCNS were sensitive [9]. Soleimani M, Tabatabaei SA, Masoumi A, Mirshahi R *et al.* has reported that pseudomonas aeruginosa was found to be the most common causative agent in patients with keratitis and Gram negative organisms showed a good sensitivity to levofloxacin, however, 34.1% of S. aureus isolates and 29.7% of coagulase negative staphylococci were resistant to this antibiotic.[10] Acharya, Manisha; Farooqui, Javed Hussain; has reported that the most common gram-positive bacteria isolated were coagulase-negative *Staphylococcus* (56.2%), whereas *Pseudomonas* spp. (64.2%) was the most commonly isolated gram-negative bacteria and maximum sensitivity was seen for moxifloxacin (92.9%). Third generation cephalosporins, vancomycin, and moxifloxacin had good sensitivity for all gram-positive bacteria [11].

So present study has been designed to determine bacteriological profile and sensitivity pattern of culture positive bacterial keratitis, risk factor associated it and treatment outcome.

Material and Method
This is a prospective observational study conducted in the department of ophthalmology Konaseema institute medical science Amalapuram Andhra Pradesh India from October 2017 to June 2021.

Selection of patients
During our study period we have enrolled 600 patients with corneal ulcer attending outpatient department of ophthalmology were enrolled for this study as per exclusion and inclusion criteria.

Selection criteria
Patients were selected based on following inclusion and exclusion criteria,

Inclusion criteria
All age
Both sex
Culture positive cases of corneal ulcer

Exclusion criteria
Culture negative
Perforated or bacterial keratitis with impending perforation
Patients not given consent

Sample size: Based on incidence of treatment outcome as reported by Gopinathan, Usha & Sharma, Savitri & Garg, Prashant & Rao, Gullapalli *et al.* to be 76 %, alpha error to be 0.05, beta error to be 0.02, power of study 0.8 and expected incidence in the study to be 68.4 % sample size was calculated to be 261.

Ethics: Present study is approved by the institutional ethics committee. Written informed consent was obtained from all patients or relatives of patients before enrolling them for study.

Method
After enrolment of patients a detailed relevant history of patients regarding to mode of injury and predisposing factor were recorded and detailed clinical examination was done.

Visual acuity of all patients was tested. Slit lamp biomicroscopy was performed and Corneal ulcer was examined properly. Under local anaesthesia with 4% lignocaine corneal scrap was taken from edge and base of ulcer. Sample was sent for gram stain, 10% KOH wet mount preparation, blood agar and Sabouraud's dextrose agar. Positive bacterial culture was subcultured on Mullar Hilton agar from sensitivity and Clinical and laboratory standards institute guidelines was used for interpretation of sensitivity. Empirical antibiotic therapy was started was started based on gram stain report and other supportive treatment like cycloplegic and analgesics were given to each patients. Each patient enrolled for this study was followed on regular interval. In each visit uncorrected visual acuity (UCVA) and complete ocular examination was done. Ulcer was examined for feature of healing and antibiotic was changed if required based on sensitivity report. Patients were followed every 15 days till ulcer healed.

Statistical analysis: Data were recorded in excel sheet and statistical Analysis was done with software SPSS-14 version. Qualitative data were calculated as percentage and proportions. Quantitative data were expressed as mean ±SD.

Result
During our study period of three years and nine months we have enrolled 600 patients with corneal ulcer as per selection criteria.

Table 1: Clinicodemographic profile of patients with corneal ulcer

Variables		Number	Percentage
Age(years)	Less than 20	140	23.3
	21 to 50	224	37.3
	More than 50	236	39.3
Sex	Male	360	60
	Female	240	40
Causative factor	Trauma	486	81
	Contact lens	60	10
	Iatrogenic	40	6.67
	Immunocompromised	14	2.3
Size of ulcer	Less than 3mm	28	15.90
	3mm to 6mm	132	75
	More than 6 mm	16	9.09
Location of ulcer	Central	30	17.04
	Paracentral	112	63.63
	peripheral	34	19.31
Depth of ulcer	0 to 33%	68	38.63
	33% to 66%	74	42.04
	More than 67%	34	19.31
Association with hypopyon	Present	22	12.5
	Absent	152	87.50

As per table 1, regarding Clinicodemographic profile of the patients, mean age of the patients with corneal ulcer was 48.64±24.22 years. The numbers of patients having age group less than 20 years was 140 (22.3%), between 21 to 50% were 224(37.3%) and rest were above 51 years. There was male predominance. Trauma was most common causative factor 486(81%). Contact lens was etiology of ulcer in 60(10%) patients. In 40 (6.67%) patients corneal ulcer was iatrogenic and remaining patients with corneal ulcer decreased immunity was the etiology. Regarding size of ulcer, it was less than 3 mm in 28(15.9%) patients, between 3 mm to 6 mm in 132(75%) patient and remaining were more than 6 mm in size. Most common location of ulcer was Paracentral 112(63.63%), central location was in

30(17.04%) patients, in remaining patients it was peripheral. Site and depth classification was based on previous literature [13]. Depth of ulcer was less than 33% in 68(38.63%) patients, between 33 to 66 % in 74(42.04%)

patients and remaining patients have depth of corneal ulcer more than 67%. Corneal ulcer associated with hypopyon in 22(12.5%) patients.
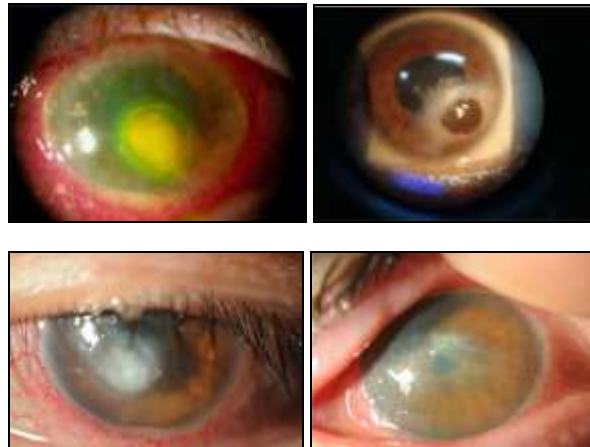

Fig 1: bacterial corneal ulcer

Table 2: Profile of corneal ulcer under study

Variable	Number(percentage)
Total number of specimen	600
Culture positive	460(76.66)
Fungal	284(47.33)
Bacterial	176(29.33)

In present study out of 600 specimen 460(76.66%) patients were culture positive out of them 47.33% were fungal and remaining were bacterial (29.33%).

Table 3: Microbiological profile of culture positive corneal ulcer

Spectrum of bacteriological isolates		Number (n=176)	Percentage
Gram positive (102)	Staphylococcus aureus	54	30.68
	Streptococcus	36	20.45
	Cons	6	3.4
	Corynebacterium	2	1.12
	Others	4	2.27
Gram negative (74)	Pseudomonas	52	29.54
	Klebsiella	6	3.4
	E.Coli	4	2.27
	Moraxella	6	3.4
	Acinatobactor	6	3.4

Regarding bacteriological profile of culture positive corneal ulcer, we have observed that gram positive bacteria were common than gram negative. Among all staphylococcus aureus was common organism 54(30.68%), followed by pseudomonas 52(29.54%), CONS was isolated from 6(3.4%) sample, Corynebacterium was present in 2(1.12%), Klebsiella was present in 6(3.4%), E. Coli was isolated from 4(2.27%) patients Acinetobacter and Moraxella was isolated from 6(3.4%) specimen each.

Fig 2: Culture of CONS, Pseudomonas and Streptococcus

Table 4: Antimicrobial Sensitivity of common organism isolated

Drugs	Sensitivity of Common organism isolated(number and percentage)		
	Staphylococcus (n=54)	Streptococcus (n=36)	Pseudomonas (n=52)
Cefazolin	37(68.51)	27(75)	
Amikacin			42(80.76)
Gentamycin	38(70.37)		41(78.84)
Chloramphenicol	44(81.48)	25(69.44)	
Tetracycline		21(58.33)	
ciprofloxacin	32(59.25)	25(69.44)	43(82.69)
Ofloxacin	37(68.52)	25(69.44)	47(90.38)
Gatifloxacin	44(81.48	28(77.77)	48(92.70)
Moxifloxacin	34(62.96)	30(83.33)	46(88.46)
Vancomycin	54(100)	34(94.44)	
Azithromycin	54(100)	32(88.89)	

Regarding sensitivity of common organism isolated staphylococcus was 100% sensitive to vancomycin and azithromycin, sensitivity to fluoroquinolones were from 60 % to 80 %.

Streptococcus was sensitive to vancomycin (94, 4%), azithromycin (88.89%) and Moxifloxacin it was 83.33% sensitive. More than 80% pseudomonas was sensitive to fluoroquinolones and aminoglycosides.

Table 5: Outcome of treatment

Ulcer healing		Number (%)
After 20 days	Improved	112(63.63)
	No response	34(19.31)
	Deteriorated	30(17.04)
At final follow up	Improved	136(77.27)
	Not improved	40(17.04)

There was improvement in corneal ulcer after 20 days of treatment and 77.27% patients were recovered at final follow up.

Discussion

In present prospective observational study to determine bacteriological profile and sensitivity pattern of culture positive bacterial keratitis, risk factor associated it and treatment outcome 600 patients were enrolled as per

selection criteria, mean age of the patients with corneal ulcer was 48.64±24.22 years and most of the patients were above 20 years of age with male predominance. This finding corroborates with the study of Srinivasan, Muthukumarasamy & Gonzales, CA *et al.* and Keshav BR; Zacheria G; Ideculla T; Bhat V; Joseph M. *et al.* [14, 15]. Trauma was most common causative factor 486(81%) this finding is supported by the work of Musch DC, Sugar A, Meyer RF *et al.* and Titiyal JS, Negi S, Anand A, Tandon R, Sharma N, Vajpayee RB *et al.* [16, 17]. Most of the ulcer were 3 mm to 6 mm in size and Paracentral in location, this finding is similsr to the study of Mascarenhas J, Srinivasan M, Chen M, Rajaraman R, Ravindran M *et al.*, Khare P, Shrivastava M, Kumar K *et al.* and Brzheskaya I.V., Somov E.E [13, 18, 19].

Corneal ulcer associated with hypopyon in 22(12.5%) patients. Bourcier T, Thomas F, Borderie V, Chaumeil C, Laroche L *et al.* has reported that hypopyon was found in 1.6 to 7% patients. Tarekegn Wuletaw, Mekuanint Geta, Adane Bitew, Wossen *et al.* has reported that the majority of participants (80%) had ulcers larger than 3 mm, and 46% of cases presented with hypopyon which doesn't support our study.) Alex Lap-Ki Ng, Kelvin Kai-Wang To, Chile Chi-Lai Choi *et al.* has reported that hypopyon was found in 30 cases (13%) which support our study.

Out of all cases of corneal ulcer fungal etiology was common than bacterial (49.33% vs 27.33%) and 76.66% patients were culture positive. Ranjini CY, Waddepally VV *et al.* has reported that out Of 117 positive cases, 52 (44.5%) were bacterial, 58 (49.5%) were fungal and 7 (6%) patients showed mixed bacterial and fungal infection this finding support our study.

Regarding bacteriological profile of culture positive corneal ulcer, we have observed that gram positive bacteria were common than gram negative. Among all staphylococcus aureus was common organism 54(30.68%), followed by pseudomonas 52(29.54%). Ranjini CY, Waddepally VV has reported that *Staphylococcus aureus* was the most common isolated bacteria followed by pseudomonas, this finding is similar to our study [22].

Regarding sensitivity of common organism isolated staphylococcus was 100% sensitive to vancomycin and azithromycin, sensitivity to fluoroquinolones were from 60 % to 80 %.

Streptococcus was sensitive to vancomycin (94, 4%), azithromycin (88.89%) and Moxifloxacin it was 83.33% sensitive. More than 80% pseudomonas was sensitive to fluoroquinolones and aminoglycosides. This finding is supported by the study of Ranjini CY, Waddepally VV *et al.* and Das S, Samantaray R, Mallick A, Sahu SK, Sharma S *et al.* [22, 23].

There was improvement in corneal ulcer after 20 days of treatment and 77.27% patients were recovered at final follow up. Gopinathan, Usha & Sharma, Savitri & Garg, Prashant & Rao, Gullapalli *et al.* has reported that Corneal healed scar was achieved in 75.5%, 64.8%, and 90.0% of patients with bacterial, fungal, and *Acanthamoeba* keratitis respectively this finding support our study [12].

Conclusion
From present study we can conclude that bacterial corneal ulcer is less common than funga ulcer. Regarding bacteriological profile of culture positive corneal ulcer, we have observed that gram positive bacteria were common than gram negative. Among all staphylococcus aureus was

common organism followed by pseudomonas. Regarding sensitivity of common organism isolated staphylococcus was 100% sensitive to vancomycin and azithromycin, sensitivity to fluoroquinolones were from 60 % to 80 %. More than 80% pseudomonas was sensitive to fluoroquinolones and aminoglycosides. 77.27% patients were recovered at final follow up.

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