

## IDENTIFICATION OF BACTERIA AND THEIR SENSITIVITY PATTERN FROM CORNEAL ULCER PATIENTS

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### Summary

*Corneal ulcer is one of the major causes of blindness in Bangladesh. A total of 280 samples (corneal scraping) of different age groups were examined by microscopy and culture.*

*Microorganism (bacteria&fungus)were recovered form 187 (66.78%) cases.Among these bacteria was recovered in 75 (26.76%) cases. Pseudomonas spp. was the most common 27 (36.00%) bacteria isolated. The next common bacteria was Streptococcus pneumoniae 24 (32.09%) followed by Viridans Streptococcus 8 (10.67%), Staphylococcus aureus 6 (8%) Coagulase negative Staphylococcus 6 (8%), Escherichia coli 2 (2.66%), Corynebacterium spp1 (1.33%) and Klebsiella spp 1 (1.33%). The antibiotic sensitivity pattern of bacterial isolates were found to be variable. Most of the Gram positive bacteria were sensitive to Penicillin, Choloramphenicol, Ciprofloxacin. The Gram negative bacteria were sensitive to Aminoglycosides. Quinolones were found to be most effective drug against majority of the Gram positive and Gram negative bacteria.*

### Key words

Corneal ulcer; microorganism; quinolones

### Introduction

Ocular trauma and corneal ulceration are the significant causes of corneal blindness and may be responsible for 1.5 to 2.0 million new cases of mono-ocular blindness every year<sup>1</sup>. Corneal ulcer causing corneal scarring is a common problem and is one of the major cause of blindness in our country<sup>2</sup>. Suppurative corneal ulcer can be caused by bacteria, fungi & protozoa<sup>3</sup>. The microbial causes of suppurative keratitis vary considerably between continents, countries and also within countries<sup>3</sup>. Bacterial keratitis is a serious ocular infectious disease that can lead to severe visual disability<sup>4</sup>. In a study in south India done by Bharathi *et.al.* from 1999 to 2001 on 1618 eye patients, corneal ulcer was found in 1126 (69.5%) patients. Of 1126 patients 566 (34.98%) had bacterial growth. The predominant bacterial pathogen isolated was *Streptococcus pneumoniae* 41.85% followed by *Pseudomonas aeruginosa* (21.25%)<sup>5</sup>. In Bangladesh bacterial keratitis was found to be varied from 53.5% to 62.4%<sup>6,7</sup>.

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In USA Miami, Florida the predominant bacterial isolates found to be *Pseudomonas aeruginosa* (31%) and *Streptococcus pneumoniae* (29%)<sup>8</sup>. In Bangladesh in the year 1994 Dunlop and co-worker studied 142 cases of suppurative corneal ulcer in Chittagong Eye Infirmary and Training complex, in which 53.5% cases were bacterial of which the most common bacterial isolate was *Pseudomonas species* (24%), followed by *Streptococcus pneumoniae* 17%.

Considering the importance of corneal ulceration as a world wide cause of mono-ocular visual loss, there are surprisingly few studies evaluating the aetiological factors<sup>9</sup>. An understanding of the aetiological agents that occur in specific region are important in rapid recognition, timely institution of therapy, optimal management and prevention of disease entity. So, this study is designed to identify the specific pathogen responsible for the development of infective corneal ulcer and the antibiotic sensitivity pattern of bacteria.

### Materials and methods

#### Study design

It is a cross-sectional study.

#### Study Population

A total of 280 patients who were clinically diagnosed as corneal ulcer of different age and sex groups attended the Out Patient Department of BNSB Eye Infirmary and Training Complex Chittagong and Chittagong Medical College Hospital Indoor and Out door.

#### Place of Study

The study was carried out during the period of July 2005 to 2006. A total of 280 patients of all ages were included in this study. Laboratory works were done in Microbiology Department of Chittagong Medical College and Microbiology Department of BNSB Eye Infirmary and Training Complex.

#### Criteria for selection of patients

The patients were selected by an ophthalmologist according to the clinical features—pain, watering, photophobia, redness of the eye, foreign body sensation, dimness of vision.

#### Inclusion criteria

Bacterial and fungal corneal ulcer cases clinically diagnosed by an ophthalmologist were included in this study.

### Exclusion criteria

Herpetic ulcer, neuroparalytic keratitis, interstitial keratitis, ulcer associated with autoimmune conditions (e.g; mooren ulcer) and of course patient refusal<sup>6</sup> as well as other viral corneal ulcer cases clinically diagnosed by an ophthalmologist were excluded in this study

### Clinical Examination of the patients

All the patients had undergone thorough slit-lamp biomicroscopic examination by an ophthalmologist. The epithelial defects were stained with fluorescent dye and size of the ulcer were measured in the slit on the bio-microscope and recorded in millimeters and a drawing was made for patient record by the ophthalmologist.

### Collection of samples

Samples were collected by an ophthalmologist after a detailed ocular examination, corneal scrapings were collected under aseptic conditions from each ulcer. The procedure was performed under the magnification of a slit lamp 5 minutes after instillation of local anaesthetic (0.4%) Oxybuprocaine without preservative using a sterile Bard parker blade no 15<sup>5,10,11,12</sup>. Great cares were taken not to touch the lashes or the lids. Several scrapings were made. The material scraped from the leading edge and the base of each ulcer and was directly inoculated on the surface of solid media Blood agar media, Chocolate agar media, MacConkey's agar media in a row of 'C' shaped streaks at the side of the patient. Subsequent scrapings were spread on a precleaned labeled slide in a thin even manner to prepare smear for Gram staining .

### Laboratory Procedure

The samples for bacteriological study were subjected to direct smear examination by microscopy, culture and sensitivity testing. Growth on the 'C'-streaks is considered significant, while growth off the "C"-streaks is considered contamination<sup>13</sup>.

The media were incubated immediately. MacConkey's agar media were incubated for 24 to 48 hours at 37<sup>0</sup>C in aerobic conditions. The Blood agar (5% to 10% sheep blood) media and Chocolate agar media were incubated in candle jar at 37<sup>0</sup>C for 24 to 72 hours. After 24 hours of incubation, any growth of bacteria was observed and colonies from the primary culture media were sub-cultured in suitable media for isolation and identification. Identification of the organisms done as per standard method. All the isolates obtained by culture were tested for antimicrobial susceptibility by disk diffusion method against different antimicrobial agents<sup>14</sup>.

### Method of antibiotic sensitivity testing<sup>14,15</sup>.

The inoculum used for disc sensitivity adjusted 0.5 Mc Farland standard . For *S.pneumoniae* blood agar media was used. Mueller Hinton agar plates were used for other bacteria The plates were incubated in candle extinction jar at 37<sup>0</sup>C for *S. pneumoniae* . Mueller Hinton agar plates were incubated aerobically at 37<sup>0</sup>C. Antibiotics used were Penicillin(P), Cholaramphenicol (C), Gentamicin (CN), Tobramycin (Tob), Neomycin (N), Ciprofloxacin (CN), Lomefloxacin (LO), Ceftriaxone (CRO), Tetracycline (T).

### Interpretation of zone size

Inhibition zone produced by each drug was considered in three susceptibility categories namely sensitive(S), Intermediate sensitive(IS) and resistant (R) according to NCCLS, guideline(1994). Zone of inhibition was compared with standard values(NCCLS,1994) Against the reference strains of *E.coli* ATCC No.25922 & *S.aureus* ATCC No.25923. Diameter of complete zone of inhibition was measured by using a ruler on the underside of the plate in mm.

### Results

Specimens were examined by microscopy and culture. Of 280 samples studied 187 (66.78%) cases were found positive for either fungus or bacteria. Bacteria were isolated in 75 (26.78%) cases. (Table I) Among 75 bacterial isolates, *Pseudomonas spp.* was most common 27(36%) followed by *Streptococcus pneumoniae* 24 (32%). Other isolates were *Viridans streptococcus* 8 (10.67%), *Staphylococcus aureus* 6 (8%), coagulase negative *Staphylococcus* 6 (8%), *E.coli* 2 (2.67%), *Corynebacterium spp.* (1.33%) and *Klebsiella spp.* (1.33%). (Table II)

**Table I :** Distribution of positive cases (Fungus and bacteria) among study population (n=280)

Name of Isolates	No of Positive Cases	Percentage (%)
Fungus	112	40
Bacteria	75	26.78
Total	187	66.78

**Table-II:** Distribution of Bacterial isolates among corneal ulcer patients (n =75)

Name of Bacteria	Total	Percentage
<i>Pseudomonas spp.</i>	27	36.00
<i>Streptococcus pneumoniae</i>	24	32.00
<i>Viridans streptococcus</i>	08	10.67
<i>Staphylococcus aureus</i>	06	8.00
Coagulase negative		
<i>Staphylococcus</i>	06	8.00
<i>Escherichia Coli</i>	02	2.67
<i>Corynebacterium spp</i>	01	1.33
<i>Klebsiella spp.</i>	01	1.33
<b>Total</b>	<b>75</b>	<b>100</b>

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Table III shows the antibiotic sensitivity pattern of Gram positive bacteria. Here among 24 isolated *S.pneumoniae* almost all of them were highly sensitive to Ciprofloxacin (95.83%) and Chloramphenicol (83.33%). Most of them were also sensitive to Penicillin (66.67%), Lomefloxacin (62.50%). Where as most of the *S.pneumoniae* were seen resistant to Neomycin (83.33%), and Tobramycin (83.34%). Few of the *S.pneumoniae* were also resistant to Tetracycline (37.5%). All of the isolated *Viridans Streptococcus* were found sensitive to Ciprofloxacin (100%) and Chloramphenicol (100%). Most of them were sensitive to Penicillin (62.5%) and Tetracycline (50%). They were however mostly resistant to Tobramycin (75%), Neomycin (75%) and Gentamicin (87.5%).

Among the 6 isolated *S. aureus* all of them were 100% sensitive to Ciprofloxacin. Most of them were moderately sensitive to Lomefloxacin (66.66%), Tobramycin (66.66%), Chloramphenicol (50%) Neomycin (66.66%), and Gentamicin (50%). Almost all *S. aureus* were resistant to Penicillin (83.33%) and Tetracycline (66.66%).

Among the 6 isolates of Coagulase negative *Staphylococcus (S.epidermidis)* most of them were highly sensitive to Ciprofloxacin (83.33%) as well as Tobramycin (83.33%) and Gentamicin (66.67%). *S. epidermidis* were moderately sensitive to Chloramphenicol (50%), Tetracycline (50%), Neomycin (66.66%), and Lomefloxacin (66.67%). Penicillin was found to be non-effective in case of *S. epidermidis*.

**Table III :** Antimicrobial Sensitivity pattern of Gram positive bacteria isolated from patients of corneal ulcer

Name of the Anti microbial agents n=6 (8%)	Sensitivity pattern*	Pneumo-coccus n=24 (32%)	Viridans Streptococci n-8 (10%)	Staphylo coccus aureus n=6(8%)	Coagulase negative staphylococcus	Corynebacterium (diphtheroid) n=1(1.33%)
P	S	16 (66.67)	5 (62.5)	0	2 (33.33)	1(100)
	IS	5 (20.83)	3 (37.5)	1(16.67)	0	0
	R	3 (12.50)	0	5(83.33)	4(66.67)	0
CL	S	20 (83.33)	8 (100)	3(50.00)	3 (50.00)	1 (100)
	IS	1 (4.17)	0	1 (16.67)	0	0
	R	3 (12.5)	0	2 (33.33)	3 (50.00)	0
CIP	S	23 (95.83)	8 (100)	5 (83.33)	5 (83.33)	1 (100)
	IS	0	0	1 (16.67)	0	0
	R	1 (4.17)	0	0	1 (16.67)	0
Te	S	13 (54.17)	3 (37.50)	1(16.67)	3 (50)	1 (100)
	IS	2 (8.33)	1 (12.50)	1 (16.67)	0	0
	R	9 (37.5)	4 (50.00)	4 (66.66)	3 (50)	0
CN	S	1 (4.17)	0	3 (50.00)	4 (66.67)	1 (100)
	IS	3 (12.50)	1 (12.5)	1 (16.67)	0	0
	R	20 (83.33)	7 (87.5)	2 (33.33)	2 (33.33)	0
Lo	S	15 (62.50)	3 (37.50)	4 (66.66)	4 (66.67)	1 (100)
	IS	0	0	0	0	0
	R	9 (37.50)	5 (62.50)	2 (33.34)	2 (33.33)	0
Tob	S	4 (16.66)	1 (12.50)	4 (66.66)	5 (83.33)	
	IS	0	1 (12.50)	0	0	
	R	20 (83.34)	6 (75.00)	2 (33.34)	1 (16.67)	
N	S	1 (4.17)	0	4 (66.66)	4 (66.66)	
	IS	3 (12.5)	2 (25.00)	1 (16.67)	1 (16.67)	
	R	20 (83.33)	6 (75.00)	1 (16.67)	1 (16.67)	

\*S = Sensitive  
R = Resistant  
IS = Intermediate Sensitive  
Te = Tetracyclin

P = Penicillin  
CL = Cholramphenicol  
CIP = Ciprofloxacin  
N = Neomycin

CN = Gentamicin  
LO = Lomefloxacin  
Tob = Tobramycin

Figures within parenthesis indicate percentage.

The Diptheroid isolated was mostly sensitive to all of the antibiotic panels used for Gram-positive bacteria except Lomefloxacin to which it was found to be resistant (100%).

The antibiotic sensitivity pattern of the Gram-negative bacteria are shown in Table IV. In this study all the 27 *Pseudomonas spp.* were 100% resistant to Penicillin. Most of the *Pseudomonas spp.* were highly susceptible to Gentamicin (81.48%), Lomefloxacin (81.48%) and Tobramycin (74.06%). It is also seen that *Pseudomonas spp.* were moderately sensitive to Ciprofloxacin (55.55%), Ceftriaxone (44.44%). Most of the *Pseudomonas* were found resistant to Tetracycline (85.18%), Chloramphenicol (77.78%) and Neomycin (74.06%). The two isolates of *E.coli* were 100% sensitive to Chloramphenicol, Ciprofloxacin, Gentamicin, Lomefloxacin, Ceftriaxone and 50% sensitivity were seen in Tobramycin and Neomycin. *Klebsiella* was 100% sensitive to most of the antibiotics except Neomycin, to which it was found to be resistant (100%). It is also seen that *E.coli* (2) and *klebsiella* (1) were 100% resistant to Penicillin.

**Table IV :** Antimicrobial sensitivity pattern of Gram negative bacteria isolated

Name of antimicrobial Agents	Sensitivity Pattern	Pseudomonas n=27 (36.00%)	E.Coli N=02 (2.66%)	Klebsiella N=1 (1.33%)
P	S	0	0	0
	IS	0	0	0
	R	27 (100)	2 (100)	1 (100)
CL	S	2 (7.40)	2 (100)	1 (100)
	IS	4 (14.82)	0	0
	R	21 (77.78)	0	0
Cip	S	15 (55.55)	2 (100)	1 (100)
	IS	5 (18.52)	0	0
	R	7 (25.93)	0	0
Te	S	1 (3.70)	0	1 (100)
	IS	3 (11.12)	0	0
	R	23 (85.18)	2 (100)	0
CN	S	22 (81.48)	2 (100)	1 (100)
	IS	1 (3.70)	0	0
	R	4 (14.82)	0	0
Lo	S	22 (81.48)	2 (100)	1 (100)
	IS	0	0	0
	R	5 (18.52)	0	0
Tob	S	20 (74.06)	1 (50)	1 (100)
	IS	3 (11.12)	0	0
	R	4 (14.82)	1 (50)	0
CRO	S	8 (29.63)	2 (100)	1 (100)
	IS	12 (44.44)	0	0
	R	7 (25.93)	0	0
N	S	3 (11.12)	1 (50)	0
	IS	4 (14.82)	0	0
	R	20 (74.06)	1 (50)	1 (100)

S = Sensitive  
R = Resistant  
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P = Penicillin  
CL = Chloramphenicol  
CIP = Ciprofloxacin  
N = Neomycin  
CRO = Ceftriaxone  
CN = Gentamicin  
LO = Lomefloxacin  
Tob = Tobramycin

Figures within parenthesis indicate percentage.

## Discussion

Corneal ulcer causing corneal scarring is a common problem and is one of the major cause of blindness in our country<sup>2</sup>. In the present study we have studied 280 samples of corneal ulcer patients, of which bacterial isolates were 75 (26.78%). Among the bacterial isolates *Pseudomonas* was the major pathogen isolated 27 (36%) which was nearer to the study<sup>16</sup>. The next common isolate was *S.pneumoniae* 24 (32%). The result was closely related in Nepal, but higher than Bangladesh<sup>6</sup>.

Among the Gram positive isolates the other bacterial isolates were *Viridans streptococci* 8 (10%) *Staphylococcus aureus* 6 (8%) and coagulase negative *Staphylococcus* or *Staphylococcus epidermidis* 6 (8%). Many investigators found these organisms causing corneal ulcer. Gomes et al., (1989)<sup>7</sup> isolated *Viridans streptococcus* 10 (8%), *S aureus* 11 (8.8%), *S epidermidis* 24 (19.2%) which is almost similar to this study. Isolated *V. streptococci* in 21 (5.2%), *S. aureus* 43 (10.8%) and *S. epidermidis* 48 (11.5%) cases respectively<sup>1</sup>. Our study is in conformity with these reports.

The other less common organisms isolated from the keratitis patients in this study were *E. coli* 2 (2.66%), *Klebsiella* 01 (1.33%), *Corynebacterium spp* 01 (1.33%). Many investigators working in this field also found such organisms in varying percentages and proved their role in producing bacterial keratitis<sup>5,7,8,10</sup>.

The antibiotic sensitivity pattern of the bacterial isolates from corneal ulcer were found to be variable<sup>7</sup>. Most of the Gram-positive organisms were sensitive to Penicillin, Chloramphenicol, Ciprofloxacin in our study which is similar to the studies<sup>7,17</sup>. In this study most of the *Pneumococci* 20 (83.33%) were resistant to Neomycin which conform with the studies done where they found most of the *Pneumococci* were resistant to Neomycin<sup>18</sup>.

In this study it is seen that most of the Gram-negative organisms were sensitive to Aminoglycosides (Gentamicin and Tobramycin). Similarly described Gentamicin as highly effective against Gram-negative bacilli<sup>19</sup>. *Pseudomonas* had remained highly sensitive (81.48%) to Gentamicin. Same picture was seen in Hong Kong & South Florida<sup>8,20</sup>.

Quinolones (Ciprofloxacin & Lomefloxacin) were found to be most effective drug against most of the Gram-positive and Gram-negative bacteria in this study. The same picture was depicted<sup>17</sup>.

#### Conclusion

Corneal ulcer is one of the major causes of preventable blindness in Bangladesh. Both of *Pseudomonas* and *Pneumococcus* were the most commonly isolated bacterial pathogen. *Pseudomonas* usually produce severe hypopyon keratitis and all of them were resistant to most of the commonly used antibiotics. Aminoglycosides & Ciprofloxacin were the only antimicrobial agents effective against this pathogen. Therefore Gentamicin or Ciprofloxacin singly or in combination may be prescribed in the emergency management of severe bacterial keratitis before obtaining sensitivity reports.

#### Disclosure

All the authors declared no competing interests.

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