Original Article

# Pattern of Microbes Associated to Keratitis in Patients Presenting at Liaquat University Hospital

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## ABSTRACT

**OBJECTIVE: To determine & isolate causative organisms in the bacterial keratitis and their sensitivity and resistance to different antibacterial agents.**

**MATERIAL AND METHODS: This was a prospective observational study containing a total of 80 patients (study participants) conducted from March 2011 to October 2012 at Ophthalmology Department of Liaquat University Eye Hospital Hyderabad. After taking informed consent, bacterial isolation procedures were done as: a corneal smear was taken after topical anesthesia, obtained by application of a drop of single dose unit; Proparacaine hydrochloride 0.5% (Alcon, Belgium). A portion of each scrapping was examined microscopically for the presence of bacteria by using Gram staining and the isolated bacteria were tested for their sensitivity and resistance against the different antibiotics.**

**RESULTS: In this study, male were found in the majority and mostly patients were from rural areas. Mostly causative organisms isolated from the bacterial keratitis were Staphylococcus aureus and Staphylococcus epidermidis among the gram positive organisms and Pseudomonas among the gram negative organisms, different types of the antibiotics as Cefazolin, Tobramycin, Ofloxacin, Ciprofloxacin and the Norfloxacin were tried and showed the sensitivity with the percentage of 80%,75.0%,78.75%,73.75% and 72.5%, respectively.**

**CONCLUSION: Mostly causative organisms isolated from the bacterial keratitis were staphylococcus aureus and staphylococcus epidermidis among the gram positive organisms and pseudomonas among the gram negative organisms, different types of the antibiotics as Cefazolin, Tobramycin, Ofloxacin, Ciprofloxacin and the Norfloxacin were tried & showed good response regarding sensitivity.**


**KEY WORDS: Bacterial Corneal ulcer, gram-negative bacteria, Gram-positive bacteria, Therapeutic action.**

## INTRODUCTION

Bacterial keratitis (corneal ulcer) is a sight threatening condition[1,2]. Either untreated or severe bacterial keratitis may result in perforation and endophthalmitis.[3] A corneal ulcer is defined by a corneal infiltrate associated with an overlying epithelial defect.[4] Corneal ulcers generally occur when the normal eye's natural resistance to infection has been compromised from either trauma or contact lens wearing.[5] Bacterial infection accounts for approximately 90% of microbial keratitis.[6] Microbial keratitis increased in prevalence following the introduction of soft lenses in the 1970s.[7] The most common pathogens implicated are staphylococci and pseudomonas.[8,9] While most corneal ulcers in North America are bacterial in origin (accounting for approximately 90% of cases of microbial keratitis) and are most often caused by contact lens wear, trauma (often fungal) is the leading cause of ulcers in developing countries.[10] Many patients have a poor clinical outcome if aggressive and appropriate therapy is not promptly initiated.[11] In the past the mainstay of treatment of microbial keratitis had been combination therapy with two antibiotics (first generation Cephalosporin and an Aminoglycoside), [12] one each with potent gram positive and gram negative coverage. To achieve this broad cover both antibiotics had to be used initially at half hourly intervals. In addition the fortified concentrations caused osmotic damage to the corneal epithelial cells. The pH was always indifferent and the method of preparation meant that the sterility could not be ensured. Fourth generation fluroquinolone (Moxifloxacin and Gatifloxacin) with their wide spectrum covering both gram positive and gram negative bacteria have opened new possibilities in treatment of microbial keratitis.[13] They have broad spectrum, are bactericidal, have a rapid rate of bacterial killing, achieve therapeutic levels in target tissues and have minimal toxicity.[14] The purpose of this study is bacterial identification associated with keratitis and also to assess different antibacterial agent's sensitivity and resistance to the isolated organism.

## MATERIAL AND METHODS

This study was conducted at Liaquat University Eye

Hospital, Ophthalmology Department with the duration of time from March 2011 to October 2012. Data used in this study was based on 80 patients with the 45 male and the 35 female patients while the corresponding percentage was 56.25% and 43.75%, respectively. All the patients having corneal infections with the diagnosis of bacterial keratitis were included in the study, and chronic corneal ulcer patients, patients with fungal, viral and acanthamoeba infection etc were excluded from the study. After taking informed consent, bacterial isolation procedures were done as: a corneal smear was taken after obtaining topical anesthesia by application of a drop of single dose unit Proparacaine hydrochloride 0.5% (Alcon, Belgium). Corneal scrapping was obtained aseptically with a sterile surgical blade No.15 from the base and edges of each ulcer. A portion of each scrapping was examined microscopically for the presence of bacteria by using Gram staining, 100g/L potassium hydroxide (KOH) and Giemsa staining methods. Another portion was inoculated on to blood agar, chocolate agar, Mac Conkey agar, Sarboraud's agar, Brain heart infusion broth respectively, in C shaped streaks and cultured for the potential growth of bacteria, fungi or Acanthamoeba. Most of the time the growth results were obtained within first 24 hours. In case of no response the process was extended to two weeks for slow growing bacteria or fungi. Isolated bacteria were tested by chemical reaction for identification. Isolated bacteria were tested for their sensitivity and resistance against the different following antibiotics as, Cefazolin, Bacitracin, Chloramphenicol, Gentamicin, Neomycin, Polymyxin B, Lomefloxacin, Ciprofloxacin, Norfloxacin, Ofloxacin, Tobramycin, and Fusidic acid. The susceptibility of gram +ve and gram –ve bacteria against the listed antibiotics was noted and classified as 3-plus (most sensitive), 2-plus (moderate), 1-plus (mild sensitive) and negative (resistant). The resistance to antibiotics was evaluated with the standard disc diffusion method according to the modified test recommended by the NCCLS. Antibiotic sensitivity and resistance were noted on attached proforma. Collected information (data) was fed in Statistical Package for Social Sciences (SPSS V .20) for analysis purpose. Simple statistics such as frequency and percentages were computed and reported.

## RESULTS

In all, 80 patients included in the study, male (n=45) were more than the female (n=35), as percentage of male was 56.25% and female was 43.75%, mostly patients (n=41) were found within the age group of 36 to 45 with the percentage of 42.50% and the second most age group (n=16) was above 46 years of the age (20.0%), patients were belonging in the high percentage with rural areas (58.75%) as compared to the urban areas (41.25%) **(TABLE I).**

The gram positive bacteria isolated, Staphylococcus aureus, epidermidis and Staphylococcus pneumonia were found in 18, 14 and 12.50 patients respectively, while gram negative organism isolated were Pseudomonas (n=15), Escherichia coli (n=6) and Proteus mirabilis (n=5), the percentage values were computed 18.75%, 7.50% and 6..25% respectively **(TABLE II).**

Different types of antibiotics were tried to assess the sensitivity and resistance. The sensitivity and resistance were assessed and reported in percentage **(TABLE III).**

**TABLE I: BASELINE CHARACTERISTICS OF PATIENTS (n=80)**

| Age Group | Frequency | % |
|---|---|---|
| 16-25 | 08 | 10% |
| 26-35 | 15 | 18.75% |
| 36-45 | 41 | 51.25% |
| >45 | 16 | 20.0% |
| Total | 80 | 100% |
| **Male/Female** | | |
| Male | 45 | 56.25% |
| Female | 35 | 43.75% |
| Total | 80 | 100% |
| **Residential status** | | |
| Rural | 47 | 58.75% |
| Urban | 33 | 41.25% |
| Total | 80 | 100% |

**TABLE II: ISOLATED BACTERIAL STATUS FROM PATIENTS (n=80)**

| Organism | Frequency | % |
|---|---|---|
| **Gram – positive** | | |
| Staphylococcus aureus | 18 | 22.50% |
| Enterococcus faecalis | 04 | 5.0% |
| Streptococcus pneumoniae | 10 | 12.50% |
| Staphylococcus epidermidis | 14 | 17.50% |
| Mixed | 06 | 7.50% |
| **Gram – negative** | | |
| Pseudomonas | 15 | 18.75% |
| Escherichia coli | 06 | 7.50% |
| Proteus mirabilis | 05 | 6.25% |
| Klebsiella spp | 02 | 2.50% |
| **Total** | **80** | **100%** |

**TABLE III: ANTIBIOTIC SENSITIVITY AND RESISTANCE (n=80)**

| Antibiotics | Sensitivity | | Resistance | | Total | |
|---|---|---|---|---|---|---|
| | Frequency | % | Frequency | % | Frequency | % |
| Tobramycin | 60 | 75.0% | 20 | 25% | 80 | 100% |
| Ofloxacin | 63 | 78.75% | 17 | 21.25% | 80 | 100% |
| Ciprofloxacin | 59 | 73.75% | 21 | 26.25% | 80 | 100% |
| Fucidic acid | 43 | 53.75% | 37 | 46.25% | 80 | 100% |
| Gentamycin | 54 | 67.50% | 26 | 32.50% | 80 | 100% |
| Cefazolin | 64 | 80% | 16 | 20% | 80 | 100% |
| Chloramphenicol | 54 | 67.50% | 26 | 32.50% | 80 | 100% |
| Neomycin | 34 | 42.50% | 46 | 57.50% | 80 | 100% |
| Norfloxacin | 58 | 72.5% | 22 | 27.50% | 80 | 100% |

**FIGURE I: STAPHYLOCOCUS AUREUS INFECTION**

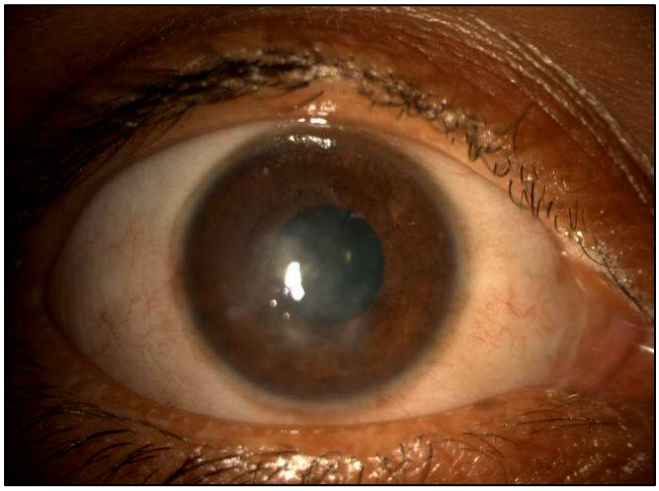


**FIGURE II:**
**E.COLI WITH FUNGAL (MIXED) INFECTION**

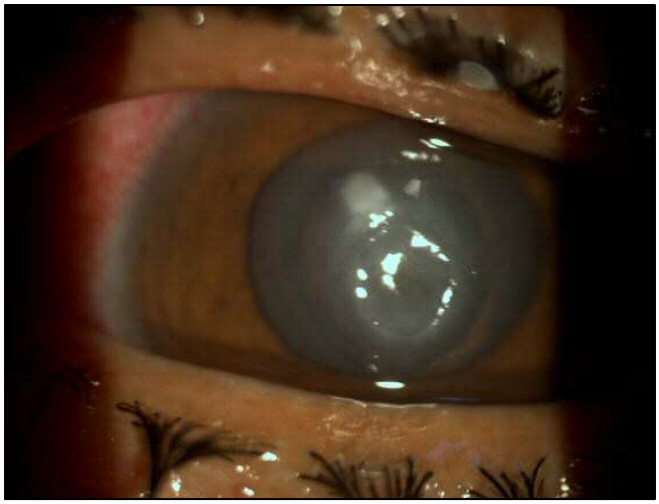


**FIGURE III: PSEUDOMONAL INFECTION**

**FIGURE IV: STAPHYLOCOCUS INFECTION**

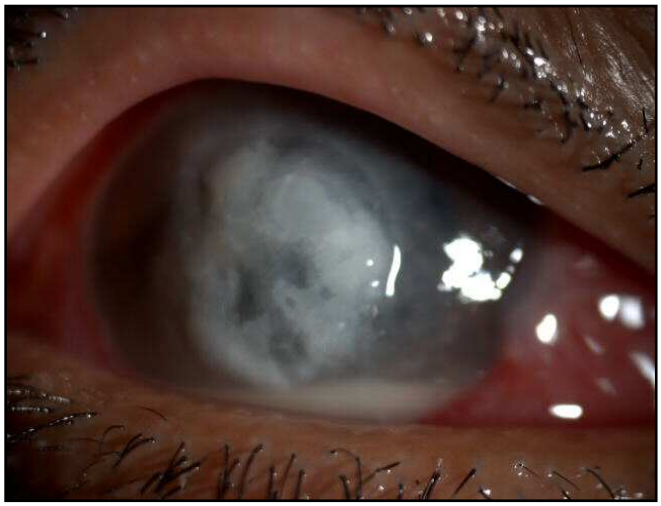


**FIGURE V: STAPHYLOCOCUS WITH FUNGAL (MIXED) INFECTION**

**FIGURE VI: PROTEUS MIRABILIS INFECTION**

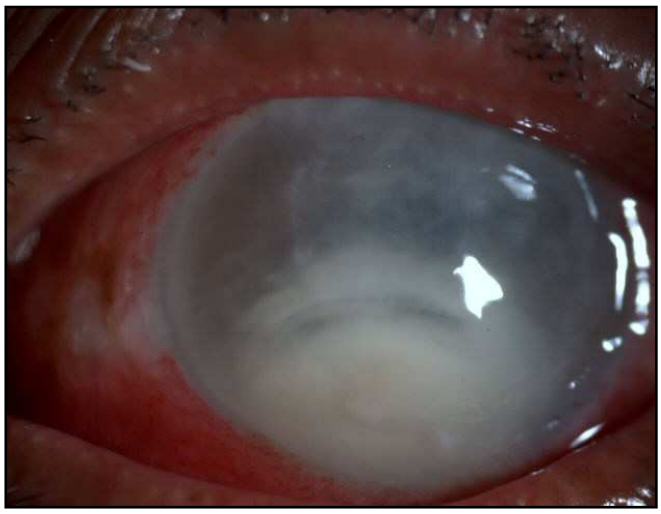


**FIGURE VII: HYPOPYON ULCER**

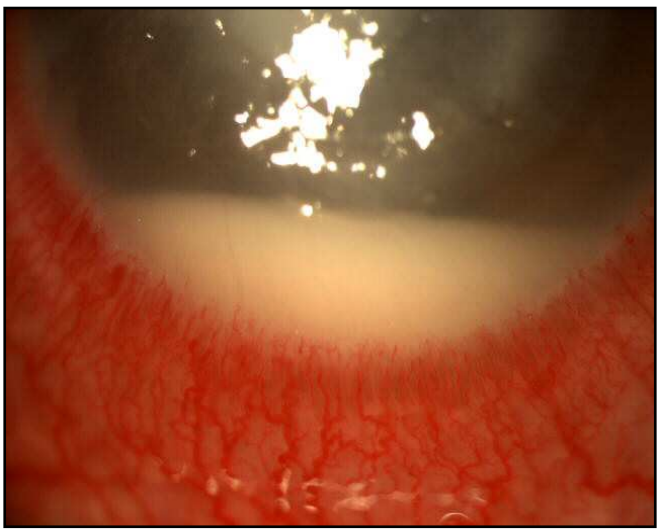
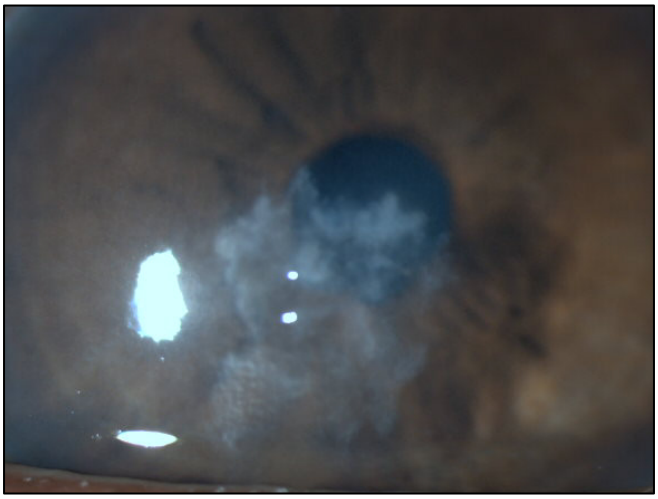


**FIGURE VIII: STREPTOCOCAL INFECTION**



## DISCUSSION

Bacterial keratitis is the most common vision threatening ocular infection in the all age groups that ophthalmologists encounter worldwide.[15] Microbial keratitis was noted relatively more in males than the females in this study. Similar percentage was found in an Indian study, the male/ female ratio with the percentage of 65.9% and 34.1% respectively.[16]Another study from Pakistan also reported similar results as percentage of male patients was 64.9% against females (35.1%).[17] In this study, patients were found with the age group of 36 to 45 with the percentage of 51.25% and the second most common age group was above 46 years of the age with percentage of 20.0%. Above referred study from Pakistan reported the mean age of the patients was as 42.8 ± 21.9 years.[17] This study showed that the patients belonged to rural areas with majority (58.75%) as compared to the urban areas (41.25%). On the other hand, another study reported that majority of patients was from rural areas (69%), and only 31% from urban areas.[18] According to the results of this study, isolated bacteria among the gram positive, Staphylococcus aureus , Staphylococcus epidermidis and Streptococcus pneumonia were commonly found with the percentages of 22.50%, 17.50% and 12.50% respectively, and among the gram negative the most important organisms were Pseudomonas, Escherichia coli and Proteus mirabilis while their percentage values were computed as 18.75%, 7.50% and 6.25% respectively.

These results are comparable with the results of some other studies as Ibrar Hussain et al reported that among the isolates bacteria Gram positive cocci were the most common bacteria (17/35 - 48.5%) with Staphylococcus aureus in 11 (31.4%), Staphylococcus epidermidis in 5 (14.3%) and Streptococcus pneumoniae in 1 case (2.8%). Gram negative bacilli were

isolated in 6 positive cultures (17.1%) with Pseudomonas aeruginosa in 5 cases (14.3%).[17] A study from our country also showed that the Staphylococcus aureus and Pseudomonas aeruginosa were the main organisms isolated.[19] In a study of India, gram positive cocci were isolated in 46.8% positive cultures with preponderance of Streptococcus pneumoniae (26.4%).[20] In this study, Pseudomonas aeruginosa was isolated in 14% cultures. Another study from South India has mentioned Staphylococcus epidermidis as the most common type of isolated bacteria (42.3%).[21] Some other studies reported that Pseudomonas aeruginosa was the most common isolated Gram negative bacillus.[22,23] According to Wong et al polymicrobial infection was identified in 33% of cases.[23]

When different antibiotics sensitivity and resistance were assessed mostly antibiotics were sensitive in the high percentage and resistance were found in low percentage of antibiotics. Similar results were noted in a study Chloramphenicol, Cefazolin, and new Fluoroquinolones (particularly Ofloxacin) were very effective against Gram positive bacteria. In contrast, many of bacteria are resistant to Polymyxin, similar to the effectiveness against Gram positive bacteria, as the new fluoroquinolones were also effective against Gram negative bacteria. Aminoglycosides (tobramycin, Neomycin, and Gentamicin) also provided a broad spectrum of activity against Gram negative pathogens. Bacitracin, Cefazolin, Chloramphenicol, and Fusidic acid, on the contrary, had little effect against these bacteria.[24]A recent report has shown a rapid increase in Staphylococcus aureus resistance to ciprofloxacin, with a 5.8% resistance in 1993, 9.4% in 1994, 11.4% in 1995, 26.5% in 1996, and 35% resistance in 1997; a similar rate of resistance progression was observed with ofloxacin.[25]

## CONCLUSION

It was inferred from the study that majority of the patients with bacterial keratitis belonged to rural areas and mostly causative organisms isolated were Staphylococcus aureus and Staphylococcus epidermidis among the gram positive organisms and Pseudomonas among the gram negative organisms, different types of the antibiotics as Tobramycin, Ofloxacin, Ciprofloxacin and the Norfloxacin had shown the sensitivity as high as 70%.

## ACKNOWLEDGEMENTS

Authors extend thanks to Dr. Aijaz Ali Khooharo, Associate Professor and Incharge Director, Advanced Studies, Sindh Agriculture University Tandojam for his input in interpretation of results.

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