*IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*
*e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 14, Issue 6 Ver. II (Jun. 2015), PP 70-76*
*www.iosrjournals.org*

# Epidemiological and Microbiological Profile of Infective Keratitis in a Referrel Centre, Bhubaneshwar, Odisha

Dr. Soumyadeep Seal, Dr. Prasenjit Bhawmik, Dr. Bianca Sau,
Dr. Priyadarshini Bhoi, Dr. J.P Mittra.
*Dept Of Microbiology, Hi Tech Medical College and Hospital, Odisha.*

***Abstract:*** *Infective keratitis is one of the leading causes of avoidable blindness in developed nations. Definitive diagnosis is by microbiological culture. So, knowledge of local etiological agents and their susceptibility helps to initiate prompt treatment and control the disease.*

***Aim:*** *To determine frequency of infective keratitis (bacterial and fungal) in patients of Hi Tech Medical College and Hospital, Bhubaneshwar and analyze its characteristics, aetiology, sensitivity patterns, risk factors.*

***Materials and Methods:*** *A prospective analysis for six months of clinical samples of 50 patients with keratitis was conducted at HI Tech Medical College and Hospital , Bhubaneshwar. These were subjected to standard microbiologic processing .Relevant information was recorded using standard proforma.*

***Results:*** *The growth analysis deduced 48%, 28%, 14 % and 10% had bacterial , fungal, viral and mixed flora respectively. Majority of patients were urban and elderly. Pre-existing ocular disorders and topical steroid usage were the predominant risk factors. Coagulase-negative Staphylococcus (41%) was the common bacterial isolate while Candida species (44.5%) the most common fungal isolate. Amikacin and gatifloxacin were the most effective antibiotics against bacterial isolates. There was no significant difference in susceptibility patterns of 8-methoxyfluoroquinolones among gram-negative pathogens. Resistant isolates of Coagulase-negative staphylococci to 8-methoxyfluoroquinolones were reported. Clinical outcome was better in patients with bacterial keratitis though they required more surgical interventions.*

***Conclusion:*** *Our study shows bacterial keratitis is more common than viral or fungal**.** Our study also found that pre-existing ocular diseases and topical steroid usage were the common risk factors for infective keratitis.*

## I. Introduction

Infective keratitis (microbial keratitis) is infection of the cornea caused by a wide spectrum of microbial agents and should be considered a medical emergency.1 A rapid aetiological diagnosis helps in initiating an aggressive specific treatment which could prevent untoward sequels.2

Microbial keratitis is one of the leading cause of avoidable blindness in developing nations.surveys in many parts of worlds have revealed that corneal scarring is an important cause of blindness and visual impairment and blindness survey in Nepal (3,4) too showed that corneal trauma and ulceration are second leading cause of unilateral blindness after cataract and are responsible for 7.9% of all blindness.

With the overall decline in causes of blindness like trachoma, onchocerciasis and leprosy, the World Health Organization (WHO) has perceived that corneal blindness due to microbial keratitis is emerging as a principal reason for visual inability**5**and that it is a "silent epidemic" happening unnoticed around the world.**6**The etiological and epidemiological features of infective keratitis [IK] depend on host factors, geographical location and the climate. Several risk factors like age, sex, immune status and socio-economic background determine the pathogenesis of IK.5 Therefore, knowledge of above features and local organisms and resistance patterns help in rapid identification and appropriate selection of antimicrobial therapy.

Most of the studies about microbial keratitis have primarily evaluated epidemiological features, predisposing Factors and clinical features of corneal ulceration 7 (Whitcher et al, 1997; Srinivasan et al, 1997; Norina et al, 2008; Upadhyaya et al, 1991; Williams et al, 1987), but this study, along with the epidemiological pattern and identification of causative microorganisms, tried to include the sensitivity pattern of bacterial isolates, treatment modalities and their outcome. 8 (Dhakhwaet al Nepal 2012 ;).

## II. Materials And Methods

**Study Design:** Prospective study.

**Settings:**Tertiary eye care center at Hi tech medical college, in Bhubaneshwar, Odisha. Study population: A total of 50 patients with infective keratitis were enrolled during the study period from October 2014 to March 2015.

**Inclusion criteria:**All patients with clinical findings of infective keratitis, presenting at HMCH during the study period, were included. Corneal ulceration was defined as a disruption of the epithelium with involvement of corneal stroma.6

**Exclusion criteria:**Viral ulcers, neurotrophic ulcers, healing ulcers and ulcers resulting from autoimmune disorders were excluded.

**Study tools:**Relevant information about demographics, clinical features, treatment, risk factors etc was recorded using standard proforma.

**Clinical procedure:**Every patient was examined on the slit-lamp biomicroscope. The size of the epithelial defect after staining with fluorescein was measured with the variable slit on the slit-lamp and recorded in millimeters on a standardized form. After a detailed ocular examination, corneal scrapings were performed under aseptic conditions on each ulcer by ophthalmologist using a flame sterilized Kimura spatula. Scrapings were performed in the slit lamp after instillation of 4 % lignocaine (lidocaine). Material was obtained from scraping, the leading edge and the base of each ulcer, was inoculated directly onto Blood agar, and Sabaraud-Dextrose agar (SDA). Material from the corneal scraping was also taken on two separate glass slides for smear: one for Gram stain and the other for microscopic examination in the clinic as a KOH wet mount. All KOH smears were then sent to the Central laboratory for confirmation.

**Laboratory procedure:**All bacterial cultures were incubated aerobically at 37 °C. Cultures on blood agar were evaluated at 24 hours and then discarded if there was no growth. Fungal cultures inoculated onto SDA were incubated at 27°C, examined daily, and discarded after 2 weeks if no growth was present in culture. Microbial cultures were considered positive only if growth of the same organism was demonstrated on two or more solid media; or there was semi confluent growth at the site of inoculation on one solid medium associated with the identification of the organism of appropriate morphology and staining characteristics on Gram stain or KOH mounted corneal smears.

The specific identification of bacterial pathogens was based on microscopic morphology, staining characteristics, and biochemical properties using standard laboratory criteria. Fungi were identified by their colony characteristics on SDA. All culture positive samples were tested for their sensitivity pattern with commonly available/ used antimicrobials.

Treatment decision was based on clinical judgment and response to empirical treatment. Culture & sensitivity pattern was taken into account after 48 hours when culture sensitivity report was available of bacterial growth.

The susceptibility testing was done by both Kirby Bauer's disc diffusion and broth dilution methods as per Clinical and Laboratory Standards Institute guidelines.8The isolates were considered significant according to the criteria described by Bharathi et al.9-11

## III. Results

The total number of samples processed during the study period was 50 and the number of positive samples (bacterial and fungal) was 24 and 14 (48% and 28%). Only the culture confirmed cases were selected for analysis.

**Epidemiological findings:**

The mean age of the study population was 49.46 years (range 1-60 years). The study showed slightly more preponderance for males (n=17, 57%). 16 patients hailed from urban and 14 from rural area. The occupational group analysis revealed significantly high incidence among professionals followed by labourers.

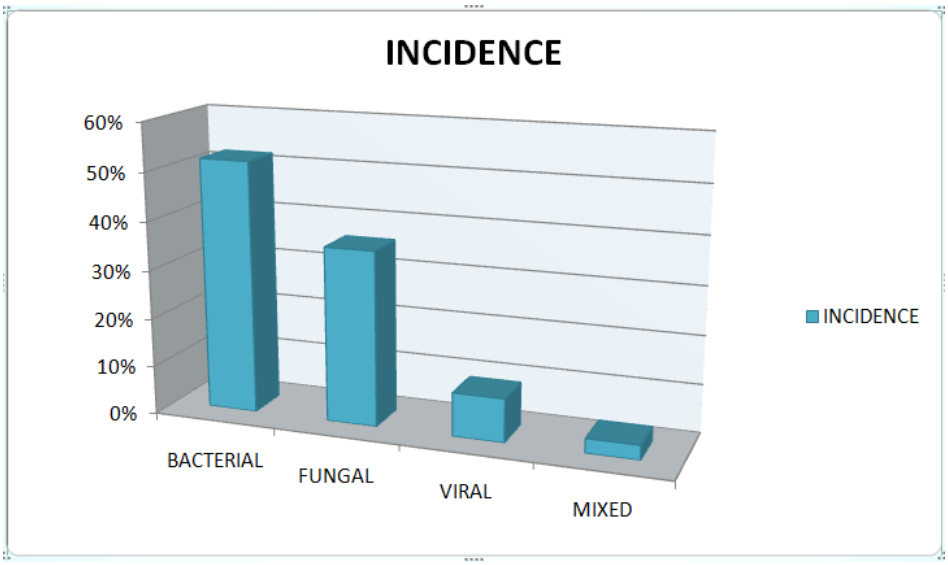



**Fig 1: Incidence various keratitis in 50 cases .**

**Epidemiological characteristics**

50 cases with the clinical diagnosis of corneal ulcers were enrolled in this study 32 cases were males and 18 were females. Ulceration occurred most frequently in the age group of 30-39 years in 29 cases, followed by 21 cases in the age group of less than 30 years. (Fig. 2).

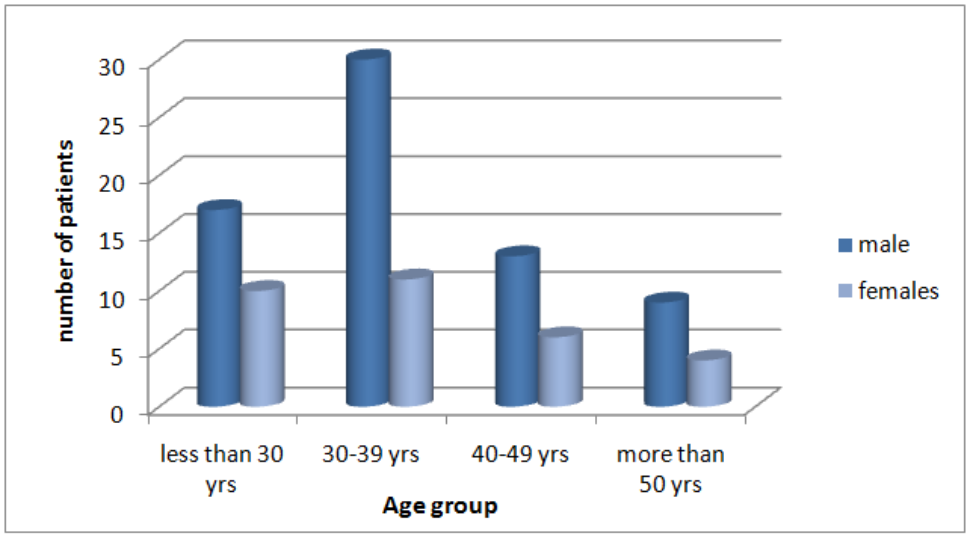



**Fig 2: Age and sex distribution of 50 patients with corneal ulceration**

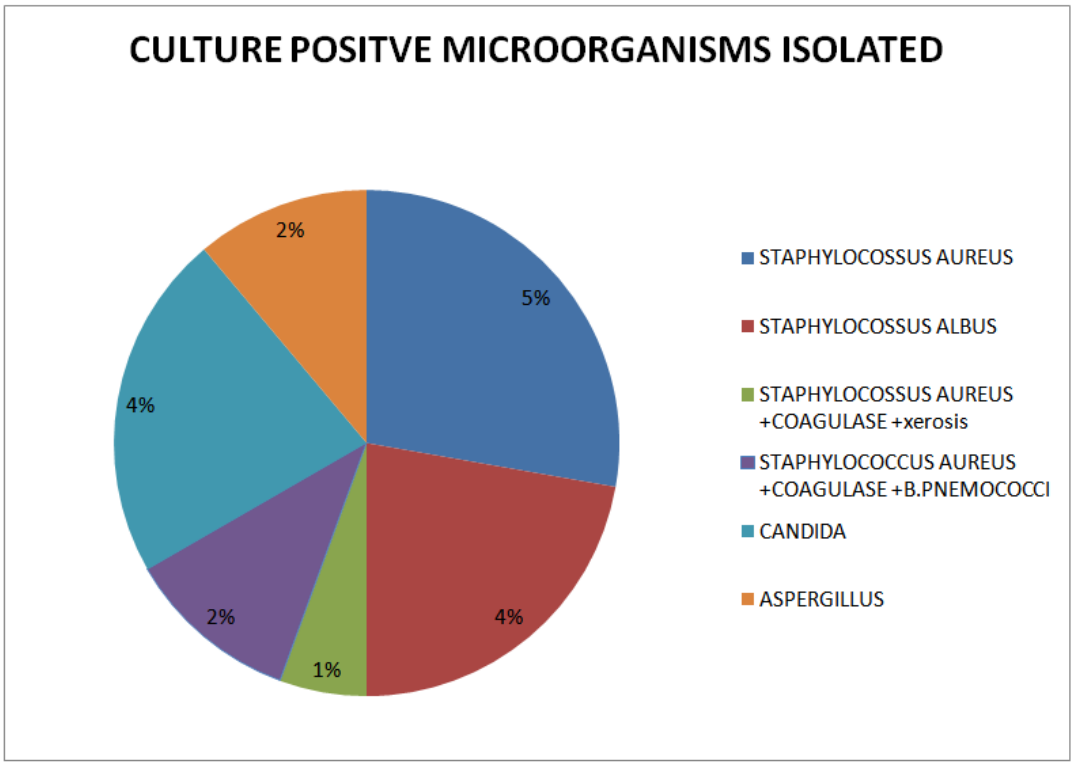
**Predisposing factors/ risk factors:** Patients with history of trauma or injury by vegetative material are at more risk for development of corneal ulcer than other risk factors. (table.1).

| S. NO | RISK FACTOR | % AT RISK |
|---|---|---|
| 1 | ACUTE CONJUNCIVITIS | 26 |
| 2 | TRAUMA | 44 |
| 3 | DACROCYSTITIS | 16 |
| 4 | SYSTEMIC DISORDERS | 9 |
| 5 | UNDETERMINED | 5 |
| TOTAL | | 100 |

**Microbiological findings:**

| GROWTH PATTERN | No. Of cases | % of cases |
|---|---|---|
| Pure Bacterial Growth | 24 | 48 |
| Pure Fungal Growth | 14 | 28 |
| Viral Growth | 7 | 14 |
| Mixed growth | 5 | 10 |

**Microbial diagnosis** Cultures were positive and fulfilled the criteria established for the presence of infection. shows 12% cultures, exhibited pure bacterial growth, although 3% cultures were mixed type. Pure fungal growth was present in 6% all cases of corneal ulcers .

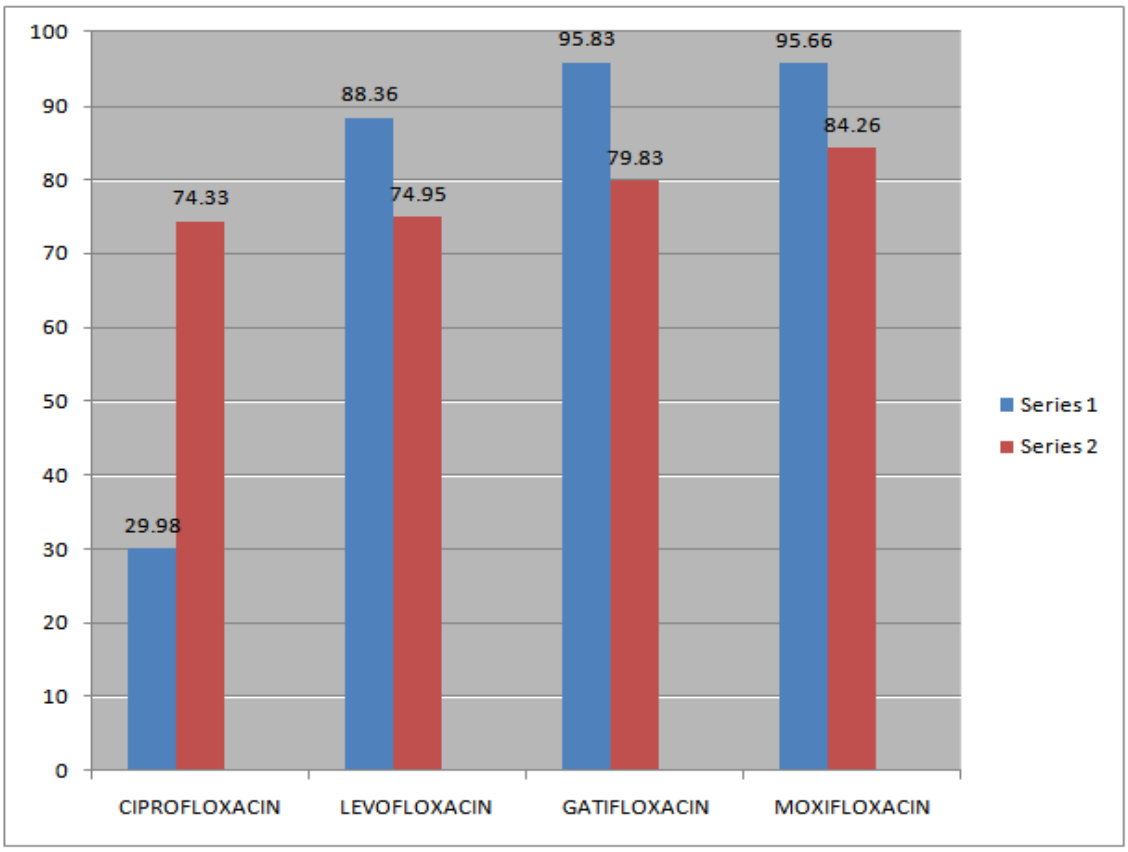



**Pie Chart: Microbial growth pattern in 50 cases of corneal ulcers**

The bacterial isolates showed varied susceptibility against selected 12 antibiotics. Overall, amikacin (92.06%) showed significantly highest sensitivity rate followed by gatifloxacin (88.77%) and gentamicin (87.3%). Gram-positives were 100% sensitive to vancomycin and aminoglycosides and gram-negatives to colistin. Gatifloxacin showed sensitivity of 94.43% and moxifloxacin sensitivity of 91.66% among gram-positive isolates. Gram-negative isolates were susceptible in highest percentage to amikacin, meropenem and moxifloxacin (84.26% each) followed by gatifloxacin (79.36%) .

Moxifloxacin showed highest sensitivity against P.aeruginosa. All yeast isolates were sensitive to tested antifungal drugs.

The Gram stain revealed bacteria in 19.04% (4/21), fungi in 66.7% (4/6) and neither bacteria nor fungi in case of mixed growth. The sensitivities of Gram smear for bacteria (16.67%) and fungi (37.50%) and that of KOH mount (33.33%) were significantly less compared to their specificities .




**Series 1- Gram Positive**
**Series 2: Gram Negative**

**Chart 1: Sensitivity patterns of fluoroquinolones against gram-positive and gram-negative isolates.**

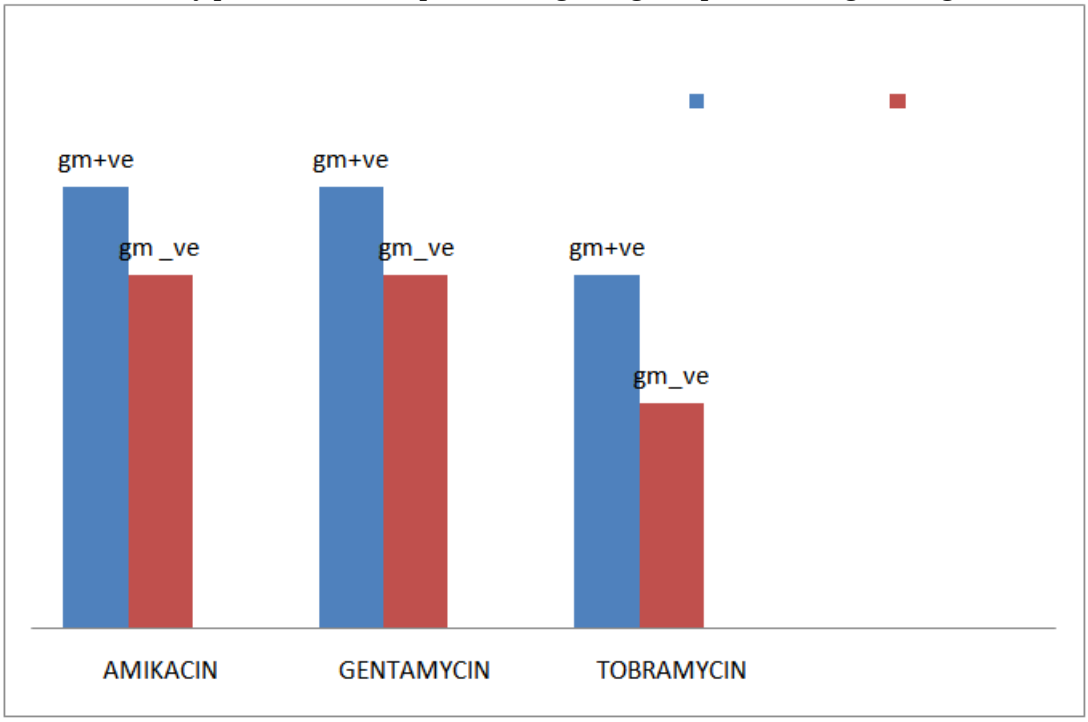



**Chart 2: Sensitivity Patterns Among Aminoglycosides In Gram Positive And Gram Negative Isolates.**

## IV. Discussion

Despite therapeutic advances in the treatment of IK, it continues to be a major cause of blindness, especially in developing nations.3,9 Its incidence rate changes significantly across regions and countries.3-9 As a result, corneal scarring is the second only to cataract as the most cause of visual disability in the world today 10 (Whitcher et al 1997; Srinivasan et al 1997; Norina et al 2008; Upadhyaya et al 1991; Chirambo et al 1986; Brilliant LB et al, 1985).

During analysis following data were deduced, 48%, 28%, 19% and 5% had bacterial, fungal, viral and mixed flora respectively. Overall culture was positive in 12% of bacterial and mixed infection whereas only 6% of fungal keratitis clinically diagnosed, had culture positivity, whereas viral keratitis was diagnosed clinically only, who showed adequate responses to antiviral treatment.

The male predominance in present study was attributed to their outdoor activities. Occupational analysis showed high prevalence rates among professionals and labourers due to similar reasons.

Gram stain showed sensitivity of 16.67% (95% CI: 4.84% to 37.40 %) and specificity of 83.33% (95% CI: 36.10 % to 97.24 %) in the case of bacterial infection. Similar findings were observed in other studies. 5, 20 The low sensitivity was attributed to the use of antibiotics prior to presentation. The sensitivity and specificity of KOH wet mount in fungal

detection was quite good. Parthasarathi et al.21 showed that use of CFW with KOH had a significant advantage while Sharma et al.22 reported that CFW was only marginally better than KOH. The predictive values for Gram stain in bacterial detection were significantly less when compared to KOH mount for fungal recognition. Thus KOH mount offers significant diagnostic potential in management of fungal keratitis. GPC (58.62%) represented the preponderance of bacterial isolates which is consistent with results from Gopinathan et al. (63.5%).5 Additionally Srinivasan et al**.**14 revealed that Gram-positive bacteria represented 65% of total isolates from IK patients. Moreover, predominance of Gram-positive BK was demonstrated in various other studies.24-27

Study shows bacterial keratitis is more common than fungal & viral with male predominance in younger age .trauma by vegetative material or injury is most common risk factor, final diagnosis based on risk factor, clinical feature, response to treatment .Viral keratitis diagnosed clinically. Bacterial keratitis has better prognosis than fungal and viral

Both gram-positive and gram-negative isolates showed varied susceptibilities to selected antibiotics. We found 100% coverage of aminoglycosides against gram-positive isolates except tobramycin (66%). Methicillin resistance was two times more frequent in CONS than in S.aureus. This finding highlights the need to consider appropriate empiric antibiotic therapy while treating Bacterial Keratitis. When we analysed overall sensitivity of fluoroquinolones (Fig 2), we found that gram-positive isolates were highly sensitive to gatifloxacin (95.83%) while gram-negative isolates to moxifloxacin (84.26%). However gatifloxacin, moxifloxacin and levofloxacin showed uniform activity only with little differences against gram-positive isolates.

Similarly we did not find much difference in sensitivity rates of older and newer generation fluoroquinolones and also between moxifloxacin and gatifloxacin (84.26% vs 79.33%) among gram-negative isolates. This reminds us the fact that ciprofloxacin and levofloxacin still remain active quinolones against gram-negative ocular pathogens. Abelson MB et al.37 described that continuous use of older fluoroquinolones can lead to selective pressure of pre-existing resistant mutants which may potentially increase the chance for second mutation thus conferring resistance to 8-methoxyfluoroquinolones. He further described that initial use of newer fluoroquinolones in place of older quinolones can solve this problem by dual-targeting mechanism which will avoid mutant selection.

Consistent with prior reports 34, 35**,** we also recorded low rates of resistance to moxifloxacin (25%) and gatifloxacin (16.7%). Finding of low resistance levels to these newer fluoroquinolones highlights the need to use them for first line monotherapy in BK.

Though clinical outcome was good in patients with BK in present study, 4% of patients required removal of eye due to bacterial infection. About 56% of patients developed one or more complications in spite of medical and surgical interventions. Corneal healed scar was achieved in 31.03% of the patients which is significantly less when compared to the findings of UshaGopinathan et al.5 (75%).

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