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Epidemiological and Microbiological Profile of Infective Keratitis in a Tertiary Care Centre, South India

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Abstract

Background: Infective keratitis is a potentially sight-threatening condition. 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 Kochi and analyze its aetiology, sensitivity patterns, risk factors and clinical outcome.

Materials and Methods: A prospective analysis of one and half year duration of clinical samples of 49 patients with keratitis was conducted at Amrita hospital, Kochi. These were subjected to standard microbiologic processing .Relevant information was recorded using standard proforma.

Results: Only patients with culture-proven infective keratitis (n=30, 61.2%) were included for analysis. The growth pattern showed pure bacterial (43%), pure fungal (12%) and mixed (6%) type of growth patterns. Majority of patients were urban and elderly. Pre-existing ocular disorders and topical steroid usage were the predominant risk factors. Coagulase-negative Staphylococcus (41.4%) 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. Graft failure was observed in 14% of patients and 4% cases required eye removal.

Conclusion: Our study found that pre-existing ocular diseases and topical steroid usage were the common risk factors for infective keratitis. The intriguing finding of resistance to fourth-generation fluoroquinolones in present study justifies judicious use of these drugs and a future study investigating the resistance patterns of gram-positive ocular pathogens against these would be very interesting and strongly recommended.

Keywords: infective keratitis; bacterial keratitis; coagulase-negative Staphylococcus; fourth-generation fluoroquinolones; 8-methoxyfluoroquinolones.

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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]

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[3] and that it is a ''silent epidemic'' happening unnoticed around the world.[4]

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 plus local organisms and resistance patterns help in rapid identification and appropriate selection of antimicrobial therapy.

Data on the burden of microbial keratitis in South Kerala, Kochi particularly is scarce compared to other parts of the country. Considering the fact that microbial aetiology is geographically dependent, the background of stating this study was to throw light on the various aspects of IK like its prevalence, microbial aetiology (Bacterial and fungal), antimicrobial susceptibility, risk factors and clinical outcome in a tertiary care set up in Kochi, South India.

MATERIALS AND METHODS:

Study Design: Prospective study.

Settings: Tertiary eye care center at Amrita Hospital in Kochi, Kerala, South India.

Study population: A total of 49 patients with infective keratitis were enrolled during the study period from August 2012 to January 2014.

Inclusion criteria: All patients with clinical findings of infective keratitis, presenting at AIMS hospital 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. The study was conducted after obtaining informed consent and approval by institutional ethical committee.

Clinical procedures:

After completing ocular examination under slit-lamp biomicroscope, corneal scrapings were collected under strict aseptic conditions using a sterile blade (No 15) by an ophthalmologist. Prior to the collection, 4% lignocaine without preservative was instilled. The entire procedure was performed under magnification of slit-lamp.

At first the material scraped from the base and edge of the corneal ulcer was inoculated directly onto the solid media in a row of C-shaped streaks and then into the liquid media. The media used were blood agar, chocolate agar, Sabouraud dextrose agar (SDA) and liquid media like brain heart infusion broth and thioglycollate medium. Subsequent scrapings were used for 10% potassium hydroxide (KOH) wet mount and Gram staining. Strict asepsis was observed during the sample collection on to the culture media and their transport to the laboratory.

Laboratory procedures:

The seeded media were incubated aerobically at 37°C except SDA which were incubated at 27°C. Blood and chocolate agar plates were incubated in 5% CO2 atmosphere. The isolates (bacteria and yeasts) were identified by Vitek 2 Compact system (Biomerieux, France). Yeast identification was further supplemented with germ tube test, chlamydospore formation on corn meal agar and sugar assimilation studies. Slide culture and lacto phenol cotton blue (LPCB) preparations were made to study the microscopic features. All laboratory methods followed standard protocols.[7] The susceptibility testing was done by both Kirby Bauer's disc diffusion and broth dilution methods as per Clinical and Laboratory Standards Institute guidelines.[8] The isolates were considered significant according to the criteria described by Bharathi et al.[9-11]

Data Analysis:

Statistical analysis of diagnostic tests was calculated with confidence intervals using "Medcalc" statistical software online.

RESULTS

The total number of samples processed during the study period was 49 and the number of positive samples (bacterial and fungal) was 30 (61.2%). Only the culture confirmed cases were selected for analysis.

Epidemiological findings:

The mean age of the study population was 49.46 years (range 1-80 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.

The predominant risk factor was pre-existing ocular disorders (n=13, 43.33%) followed by topical steroid usage (n=7, 23.33%) and trauma (n=4, 10%) (Fig1).

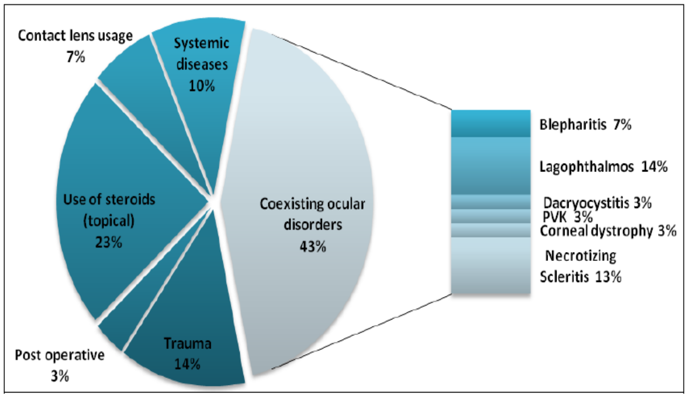

Figure 1: Risk factors associated with infective keratitis
PVK= Pre-existing viral keratitis

Among the ocular disorders, lid disorders and necrotizing scleritis were the primary causes. Diabetes mellitus and systemic hypertension were the leading causes among the systemic diseases. One patient had corneal ulceration due to surgical treatment of retinal detachment with silicon oil. Contact lens infection as a predisposing factor was seen in only two patients.

The cases occurred in all months throughout the year, but fungal keratitis peaked during the months of October-December and bacterial keratitis during July-September.

Microbiological findings:

Out of 30 culture positive samples, only 3 samples showed mixed microbial growth while the remaining were pure bacterial 21 (43%) and pure fungal 6 (12%) (Table 1).

Growth pattern	Cases	%
Pure bacterial growth	21	43
(single species of bacteria)	(18)	
(two species of bacteria)	(3)	
Pure fungal growth	6	12
(single species of fungi)		
Mixed microbial growth	3	6
(single species of fungi and single species of bacteria)		
Patients with positive cultures	30	61.2
Patients with negative cultures	19	38.8
Total number of corneal ulcers	49	100

Table 1: Microbial growth pattern in cultures from corneal ulcers (n=49)

A total of 29 bacterial isolates were obtained from 30 cases of culture proven IK. Gram-positive cocci [GPC] (n=17, 58.6%) were the predominant group among bacterial isolates followed by Gram-negative bacilli [GNB] (n=11, 38%). Coagulase-negative *Staphylococcus* [CONS] (n=12, 41.4%) was the most common gram positive isolate followed by *Staphylococcus aureus [S.aureus]* (n=4, 13.8%). The predominant isolate among GNB was *Pseudomonas aeruginosa* [*P.aeruginosa*] (n=7, 24.13%) A total of 9 fungal pathogens were cultured from patients with IK. The most common fungal isolate was *Candida spp* representing 44.44% (n=4) of all positive fungal cultures, followed by *Fusarium* spp (n=2, 22.22 %). One fungal isolate was not identified (Table 2).

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%) (Fig 2&3). 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 (Table 3).

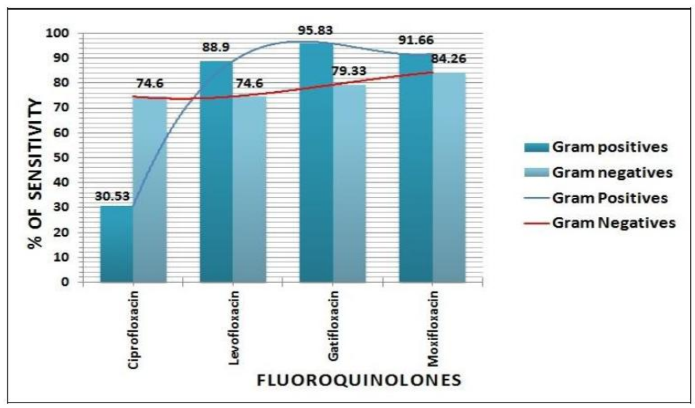

Figure 2: Sensitivity patterns of fluoroquinolones against gram-positive and gram-negative isolates.

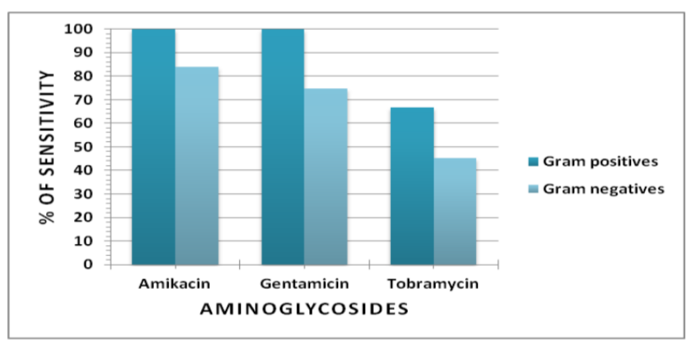

Figure 3: Sensitivity patterns of aminoglycosides against gram-positive and gram-negative isolates.

BACTERIA				
Bacteria	Pure isolate	Mixed with fungi	Total	%
Gram positive				
Coagulase Negative staphylococci	11	1	12	41.4
Staphylococcus aureus	4	0	4	13.8
Streptococcus viridians	1	0	1	3.4
GPB≈	1	0	1	3.4
Subtotal	17	1	18	62
Gram Negative				
Pseudomonas aeruginosa	6	1	7	24.13
Acinetobacter baumannii	1	2	3	10.3
Serratia marcescens	1	0	1	3.44
Subtotal	9	3	11	38
Total	25	4	29	100
FUNGI				
Fungi	Pure isolate	Mixed with bacteria	Total	%
Candida species	2	2	4	44.5
Acremonium spp.	1	0	1	20
Fusarium sp	1	1	2	40
Aspergillus sp	1	0	1	20
Unidentified hyaline fungus	1	0	1	20
Total	6	3	9	100

Table 2: List of Bacterial (n=29) and Fungal (n=9) Pathogens isolated from 30 culture positive cases of IK (GPB≈ = Gram positive bacilli)

	GRAMS STAIN		**KOH#**
(%)	**Bacteria**	**Fungi**	**Fungi**
Sensitivity	16.67 (95% CI: 4 to 84% to 37.40 %)	37.5 (95% CI: 8.97% to 75.30 %)	33.33 (95% CI: 7.88% to 69.93 %)
Specificity	83.33 (95% CI: 36.10% to 97.24 %)	100 (95% CI: 84.43% to 100.00 %)	100 (95% CI: 83.75% to 100.00 %)
PPV	80.00 (95% CI: 28.81% to 96.70 %)	100.00 (95% CI: 30.48% % to 100.00 %)	100.00 (95% CI: 30.48% to 100.00 %)
NPV	20.00 (95% CI: 6.91% to 40.71 %)	81.48 (95% CI: 61.90 % to 93.63 %)	77.78% (95% CI: 57.73% to 91.32 %)

Table 3: Sensitivity and specificity of corneal scraping smears in the detection of microorganisms with culture as gold standard method (KOH#– potassium hydroxide)

Clinical findings:

A total of 41% (n=13) of patients with IK required different forms of surgical interventions (Fig 4). Significantly more number of patients with BK (n=8, 61.53%) needed surgical treatment.

A total of 12 (41%) patients attained complete healing while in 3 (10%) patients the corneal graft was accepted successfully. Graft failure was observed in 4 (14%) patients and 4% (n=1) of patients required eye removal (Fig 5).

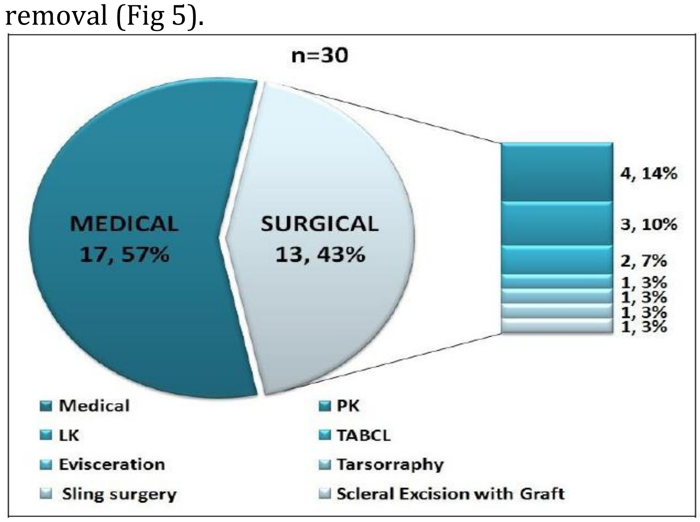

Figure 4: Percentages of culture positive cases requiring different types of surgical intervention
PK: Penetrating keratoplasty; **LK:** Lamellar keratoplasty; **TABCL** – Tissue adhesive with bandage contact lens

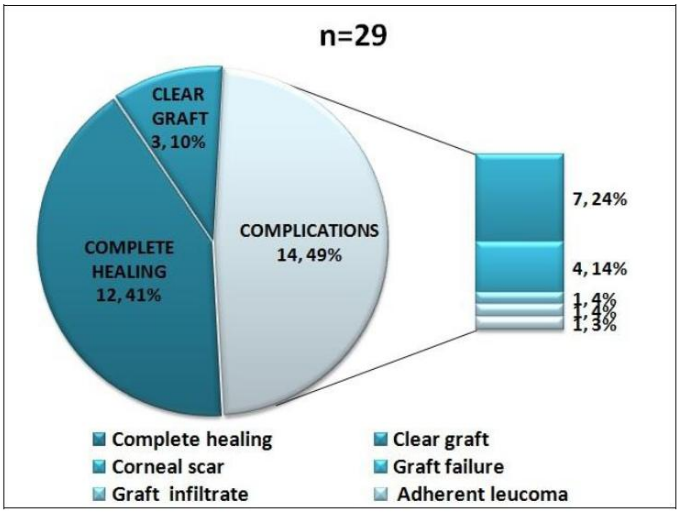

Figure 5: Treatment outcome in patients with microbial keratitis **(*n*=29)**
AL= Adherent Leucoma, *one patient could not be followed up

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. [5]Kochi being a coastal city situated in South Kerala, India features a tropical monsoon climate with high levels of humidity.[12] Looking at the socio-economic and local weather conditions of Kochi, the current study had been carried out to find the impact of geographical differences and risk factors on the frequency and aetiology of IK.

In our study the age of the culture-positive patients ranged from 1-80 years with mean age of 49.46 years, the contributing factor being outdoor occupation. These results were in concurrence with the earlier reports.[13] Majority of the patients were men with men to women ratio of 1.33:1 which matches previous studies.[14] However Ai-Yousuf N et al.[15] and Kotigadde S et al.[13] reported higher incidence among women. 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.

Majority of the publications[5, 14] recorded trauma as major risk factor in India. But we found pre-existing ocular diseases and topical steroid usage as predominant risk factors and trauma was accounted for 13.33% only. However Bharathi et al.[16] from South India reported similar finding which matches with our study. This discrepancy is related to relatively less farming and field activities in Kochi, high percentage of its residents living in urban areas and rapid urbanisation over the last decade.[17] The same reason may be attributed to low prevalence of fungal keratitis in present paper.

Contact lenses (CL) predisposed to7% of all corneal ulcers and all had a bacterial infection. However this finding varies with the reports from South India (0–2%) [18] and is related to small volume of study population in our survey.

Silicone oil (SO) is being used with increasing frequency as an effective intraocular tamponade in treatment of complex retinal detachments.[19] We found one referred patient with similar history. Long term presence of SO in posterior segment may lead to its prolapse into anterior segment causing anatomical changes in cornea and make it more vulnerable to infections.

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.

Microbiology etiology was established in 61% of the study population which is similar to the reports from other investigators[23] but close to the reports from Hyderabad[5] (60.4%) and Madurai[10] (68.4%) from South India and West Bengal[24], Eastern India (67.7%). However the prevalence rates were rather high in Nepal[25] (80%) and relatively lower in South Kerala[26] (21.4%) and Gujarat[27] (27%). Prevalence of microbial keratitis varies from 22-82%. The low isolation rate may reflect extensive use of topical medications as explained by Srinivasan et al. [10].

Monomicrobial infection was seen in 55% of the cases, the most common being bacterial (43%). This is similar to earlier reports.[28] However Upadhyay MP et al.[25] from Nepal reported high prevalence rate of BK (63%) which is almost thrice the value (22.7%) recorded by Basak SK et al. [24] from West Bengal.

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.[10] 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]

In contrast to other studies from Asia [5, 25], where *Streptococcus pneumoniae* were more common, our study demonstrated predominance of CoNS related BK similar to study by Kaliamurthy et al. [29] (44.6%). Gopinathan et al. [5] believed that underreporting of CONS may be due to tendency to consider it as normal commensal in conjunctiva by some of the studies. However, the criteria to establish the importance of positive cultures looked alike in majority of these studies. Whatsoever, being recognized as normal ocular flora it is no wonder that CONS can invade the corneal tissues whenever external ocular microenvironment is compromised. The reasons for regional variation in the relative etiologies appeared to be connected to local ecological conditions.

P. aeruginosa is the predominant gram-negative bacterium that causes corneal ulceration, trauma and contact lens wear being common risk factors. Overall *P.aeruginosa* accounted for 24.13% of bacterial isolates which is in agreement with the results of G. Singh et al. [30] (28.1%) but in variance with results of Kaliamurthy et al. [31] (9.7%).

Excess and misuse use of both systemic and topical antibiotics, inappropriate dosing and prolonged duration of therapy continue to fuel an increase in antibiotic resistant ocular pathogens.

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 BK.

Fluoroquinolones act by blocking bacterial DNA synthesis by inhibiting either DNA gyrase for gram-positive or topoisomerase IV for gram-negative bacteria.[32] The fourth generation fluoroquinolones (8-methoxyfluoroquinolones), gatifloxacin and moxifloxacin are different from the older generation in having an 8-methoxy group attached to them. This structural change has led to increased binding affinity to topoisomerase IV, thus enhancing their potency against gram-positive organisms without changing its activity against gram-negative organisms. Besides this, both the drugs exhibit dual-binding mechanism that involves targeting both DNA gyrase and topoisomerase IV which confers less resistance potential by inhibiting two simultaneous mutations at both the target sites.[33]

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. This finding underscores the fact that both gatifloxacin and moxifloxacin are equally potent in combating ocular infections due to gram-positive pathogens.

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.

Though newer fluoroquinolones have been added to therapeutic armamentarium to combat ocular infections, their emerging resistance is of major concern for ophthalmologists. Researchers have documented significantly increasing resistance rates to gatifloxacin and moxifloxacin among ocular *Staphylococci*, most commonly CONS and *S.aureus*.[34, 35] A recent study by Bispo et al.[36] identified double point mutations in quinolone resistance determining region of the topoisomerase subunits and considered it to be a major cause of resistance to 8-methoxyfluoroquinolones. Frequent and wide spread use of newer fluoroquinolones in ophthalmology is acknowledged as a driver for selection of these mutant strains.

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.

According to Mark Dunbar[38], moxifloxacin has significantly high ocular bioavailability compared to gatifloxacin. And also found that minimum inhibitory concentration (MIC) values of moxifloxacin against gram-positive organisms were low compared to MIC values of gatifloxacin against gram-negative organisms. However Moss et al. [35] reported 100% sensitivity of moxifloxacin and gatifloxacin against both gram-positive and gram-negative bacteria. In present literature also, we found MICs of moxifloxacin either lower than or equivocal to gatifloxacin among gram-positive and gram-negative ocular isolates. This discrepancy may be related to several factors like presence of endemic resistance, regional regional differences, type and virulence of the isolates and host factors.

We noted that 36.36% of gram-negative isolates were multidrug resistant of which 27.3% was *P.aeruginosa*. As opposed to gram-positive susceptibility results, we observed gram-negative isolates showing low sensitivity to aminoglycosides (Fig 3).

When we analyzed susceptibility patterns of quinolones against *P.aeruginosa,* we found that moxifloxacin had shown highest sensitivity compared to gatifloxacin (87.51% vs 71.42%) while ciprofloxacin and levofloxacin showed similar sensitivity rates (57.14%). The above findings were in agreement with the sensitivity rates quoted by Kaliamurthy J et al [29]**.**

A significantly larger number of patients (58%) with BK needed surgical intervention suggesting poor response to medical treatment compared to fungal keratitis. In contrast Usha Gopinathan et al.[5] from south India reported more number of surgical interventions among patients with fungal keratitis. With advent of new antifungal drugs and development of new antifungal strategies, the response to treatment has become much better than older times.

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 Usha Gopinathan et al.[5] (75%). This difference may be related to two reasons: firstly, the investigation involved a large number of patients over a period of 10 years and secondly the sample size in our study is relatively small to give any statistical significance.

CONCLUSION

Though the current investigation was limited by small sample size, it makes several significant contributions to current literature. First, finding of pre-existing ocular diseases and topical steroid usage as common risk factors in our region helps us to implement key risk management methods and practices like avoidance of injudicious use of topical steroids and patient education about awareness of risk factors. Second, knowledge of local prevalence of etiological agents of IK and their susceptibility patterns helps in guiding ophthalmologists to select appropriate antibiotic for empirical therapy. Finally, the intriguing finding of resistance to newer fluoroquinolones in present study justifies judicious use of these drugs and a future study investigating the resistance patterns of gram-positive ocular pathogens against these would be very interesting and strongly recommended.

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