Bacterial Keratitis - A Study at a Tertiary Eye Care Hospital in Hyderabad – India

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ABSTRACT

Microbial keratitis is a sight threatening infection of the cornea and is a major cause of blindness in India. Though several risk factors such as corneal trauma, blepharitis, herpetic keratitis, xerophthalmia, keratopathies, and eyelid abnormalities, are cited, its incidence has been increased in the past few years, and the wear of contact lens emerging as one of the major risk factors. This retrospective study was undertaken at Sarojini Devi Eye Hospital- A Tertiary Eye Care Hospital in Hyderabad, India and is aimed to define the microbiological profile of bacterial keratitis, to identify the prevalence, its risk factors, and to test the in vitro antimicrobial resistance of the bacterial isolates. This is a retrospective study were a total of 102 patients presenting to the Sarojini Devi Eye Hospital - A Tertiary Eye Care Hospital in Hyderabad with infected corneal ulcer were included. Their socio-demographic data and risk factors were recorded. Corneal scrapings were collected from the edge of the ulcer and microbiologically processed using standard operating procedure. Bacteriological profile was determined by standard biochemical tests and the sensitivity/resistance of isolated strains was tested for on antimicrobial agents that are currently used in ocular infections (NCCLS disc diffusion test) according to the clinical and laboratory standards institute (CLSI) guidelines. In our study Corneal trauma followed by use of contact lens was found to be the major risk factors for microbial keratitis the age range of 41-60 years was the most affected group. The most commonly isolated bacteria were Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, klebsiella spp., serratia marcescens. Antimicrobial susceptibility pattern revealed that most of Gram-negative bacilli were susceptible to fourth generation cephalosporin -Cefepime, Lomefloxacin, Gatifloxacin, and Ciprofloxacin, while most of Gram-positive cocci were susceptible to vancomycin, Cefepime gatifloxacin and Ciprofloxacin Bacterial keratitis, the epidemiological data of which reveals its universal occurrence, and since it is a potentially sight-threatening corneal condition that can progress very rapidly, with complete corneal destruction occurring within 24-48 hours, particular attention should be given to predisposing risks like trauma, contact lens wear etc and to early diagnosis. While diagnosis is primarily clinical, it is substantiated largely by microbiological data and analysis and comparing the changing trends of the aetiology and their susceptibility patterns. To reduce the possibility of permanent visual loss and reduce structural damage to the cornea prompt treatment is needed. As fourth generation cephalosporin’s and Fluoroquinolones have shown promise in the treatment they should be used judiciously and since drug resistance among bacterial pathogens is an evolving process, routine surveillance and monitoring studies should be conducted to provide an update and to institute most effective empirical treatment for bacterial keratitis.

Keywords
Corneal trauma,
Contact lens wear,
Staphylococcus aureus,
Pseudomonas spp.,
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Introduction

Bacterial keratitis is a potentially devastating ocular condition presenting as an acute or chronic, slowly progressive or rapidly deteriorating infection involving any part of the cornea. It is one of the most common global causes of preventable monocular morbidity and irreversible blindness.

It is one of the leading causes of corneal blindness in developing countries (Assudani et al., 2013).

According to the World Health Organization reports Corneal ulceration is a major public health problem resulting in 1.5–2 million new cases of corneal blindness annually (Insan et al., 2013). Therefore, bacterial keratitis is an ophthalmic emergency that needs immediate treatment (Chittur et al., 2016).

The bacteriological profile and their susceptibility as well as resistant patterns vary from place to place and in the same place from time to time and are influenced by geographic and climatic factors; and habitat of the populations whether living in rural or urban sectors. (Ly et al., 2006; Leibovitch et al., 2005; Alexandrakis et al., 2000)

Till recently, most cases of bacterial keratitis were associated with ocular trauma or ocular surface diseases.

However, the widespread use of contact lenses has dramatically increased the incidence of contact lens related keratitis (Tananuvat et al., 2004; Al-Mujaini et al., 2009; Wong et al., 2012; Bourcier et al., 2003).

Bacteria account for 65–90% of corneal infections with Staphylococcus aureus, S. pneumonia and Pseudomonas aeruginosa accounting for more than 80% of bacterial keratitis. (Forbes et al., 2007)

Ocular morbidity such as corneal scarring and subsequent visual loss can be significantly reduced by prompt institution of appropriate therapy guided by the knowledge of the causative agents.

In spite of advances in clinical diagnosis, molecular laboratory investigations, and the availability of potent antibiotics, visual morbidity continues to be high in underdeveloped countries. The antibiotic resistance among ocular pathogens is increasing worldwide, increasing the risk of treatment failure with potentially serious consequences (Brown, 2007; Sharma, 2011)

The Present study is to review the prevalence and to identify the, epidemiology pathogenesis, clinical presentations, diagnosis and treatment of microbial keratitis in this area and to test for the in vitro antimicrobial resistance.

Materials and Methods

This study was conducted at Sarojini Devi Eye Hospital a tertiary eye care hospital in Hyderabad, India for one year from May 2017 to April 2018.

All patients clinically and provisionally suspected with bacterial keratitis presenting to the OPD & IPD, at Sarojini Devi Eye hospital, Hyderabad, Telangana during the above period were included in the study. They were informed about the purpose of the study and then a standardized form was filled up for each patient which included - socio-demographic information as well as clinical information including duration of symptoms, previous treatment, predisposing ocular conditions, history of trauma and associated risk factors and visual acuity. The history and examination were focused on the following risk factors: corneal traumas, contact lens wear, Ocular surgery, ocular surface diseases,
lagophthalmos, steroid eye drops and corneal foreign bodies or sutures, use of contaminated medications, blepharitis as well as history of systemic diseases particularly diabetes mellitus.

**Clinical Signs & Symptoms**

The commonly presenting signs and symptoms with Patients of bacterial keratitis were: onset was acute with features relating to visual and sensory functions. Lid and conjunctival oedema, reduced vision, pain, redness, photophobia and discharge.

**Inclusion criteria**

All those cases presenting with a corneal infiltrate compatible with clinical diagnosis and sent by ophthalmologist with a provisional diagnosis of bacterial keratitis were included in this study.

**Exclusion criteria**

Corneal ulcers with negative cultures and not provisionally diagnosed as bacterial keratitis were excluded. All those microbial keratitis cases with typical features of viral infection, interstitial keratitis, fungal keratitis, and healing ulcers which includes Mooren’s ulcers, marginal ulcers, interstitial keratitis, sterile neurotrophic ulcers and other ulcers associated with autoimmune conditions were also excluded. (Packer et al., 2015; Ramsay and Lightman, 2001; Zegans and Srinivasan, 1998; Sharma and Aggarwal, 2015)

**Sample collection**

Visual acuity was tested by an ophthalmologist at the time of presentation and was recorded. After a detailed ocular examination using standard techniques, the ophthalmologist took corneal scrapings under aseptic conditions. These samples were labelled with the patient's name and the date of collection.

Corneal scrapings were taken after installation of 4% preservative-free lignocaine by an ophthalmologist using the no. 15 Bard-Parker blade with strict asepsis under slit-lamp. The collected scraping material from the leading edge and base of the ulcer was inoculated into solid/liquid culture medias and then smeared on 2 different slides in a thin, even manner for direct microscopy and Gram stain.

**Culturing**

Scrapings were inoculated directly on Blood agar, chocolate agar; these were placed into a candle jar for fastidious bacterial pathogens, which require CO2.

They were also inoculated into, brain-heart infusion broth and incubated aerobically.

After appropriate incubation, subcultures were made from brain heart infusion broth tube onto sheep blood agar (5%), chocolate agar, manitol salt agar and MacConkey agar in multiple C shaped streaks using the standard methods.

These subcultures were incubated overnight, and depending on the growth on blood agar, chocolate agar and MacConkey agar followed by morphology, cultural characteristics etc, bacterial cultures were confirmed by standard biochemical tests as per the clinical and laboratory standards institute (CLSI) guidelines.

A culture was considered positive

When there is growth of the same organism on two or more media

Confluent growth of a known ocular pathogen at the site of inoculation on one solid medium
Growth in one medium with consistent direct microscopy findings

Growth of the same organism on repeated corneal scraping (Ubani, 2009; Tesfaye et al., 2013)

Optochin sensitivity test was also performed to identify Streptococcus pneumonia (Bertino, 2009)

In vitro antibiotic susceptibility testing of the bacterial isolates was performed by Kirby-Bauer disc diffusion method (Cheesbrough, 2006) against the mostly used ocular antibiotics.

The interpretation of the results was according to the Clinical Laboratory Standards Institute (CLSI) methodology as susceptible, intermediate and resistant.

**Results and Discussion**

A total of 102 patients with a corneal infiltrate that was compatible with a diagnosis of bacterial keratitis and clinically suspected of having bacterial keratitis were examined. Of the total 102 patients included in the study, the ages ranged from 0 to 80 years among which males were 78 (76.47%), and females were 24 (23.53%) (Table 1).

Out of the total 102 patients included in the study, 59 (57.85%) patients in the age group of 41 to 60 were the highest, followed by the age group 21 to 40 with 28 (27.45%), age group 0 to 20 were 06(05.88%) and 61 to 80 age group being 09 (08.82%) (Table 2).

Amongst the major risk factors related to bacterial keratitis, the most common risk factor was corneal trauma encountered in 34 (33.34%) patients.; followed by contact lens wear which was the second most common risk factor that was seen in 20(19.62%) cases;

ocular surgery constituted 11cases (10.78%)

Severe blepharitis was present in 10 (09.80%) of the patients, in 10 patients (09.80%) the keratitis was induced by foreign bodies—07 patients with organic material (vegetable or animal) and 03 patients with mineral particles. Dry eye syndrome was diagnosed in 09 (08.82%) patients and use of Contaminated medications was seen in 08 (07.84%) cases (Table 3).

We could yield bacteria (culture positivity) from the corneal smears of 89 (87%) patients. The corneal smears from 13 patients were culture negative, and this is due to use of ocular antibiotics before they were referred or visited the hospital (Table 4).

In our study, in almost all risk groups (except contact lens wearers), primarily Gram positive bacteria were isolated (59.56%), mostly Staphylococcus species (24) and in patients wearing contact lenses, Gram negative bacteria mostly Pseudomonas species were isolated

The predominant Gram positive bacteria were, mainly *Staphylococcus aureus* 24(26.97%), Coagulase negative *Staphylococcus* 18(20.23%) followed by S. pneumonia 11 cases (12.36%).

Among Gram negative bacteria Pseudomonas aeruginosa is the most predominant bacterium encountered. *Pseudomonas* species 20 (22.47%), followed by Klebsiella pneumoniae 12 cases (13.48) and *Serratia marcescens* 04 (04.49%) were the other bacteria isolated (Table 5).

The antimicrobial susceptibility patterns of isolated bacteria were tested on antimicrobial agents that are currently used in ocular infections. The new class of fluoroquinolones showed a good results against both Gram positive and Gram negative bacteria.
Among all fluoroquinolones, lomefloxacin has the best penetration of corneal tissues. The aqueous humour concentration after topical administration is highest for ciprofloxacin. Basing on all these factors and resistance rate and the penetration of the antibiotic at the level of infection the antibiotics were selected.

Among Gram positive cocci *Staphylococcus aureus* and CONS were 100% susceptible to vancomycin and to fourth generation cephalosporin Cefepime and showed maximum resistance to ciprofloxacin and Ofloxacin, while streptococcus pneumonia showed sensitivity to almost all the antibiotic tested except resistance to Gentamicin (Table 6).

Gram negative bacilli were most susceptible to Lomefloxacin (100%), Gatifloxacin (100%), Cefepime (100%) followed by Ciprofloxacin (90%). The highest resistance was seen to Gentamicin and Chloramphenicol (Table 7).

Bacterial keratitis and its complications constitute an important cause of ocular morbidity and is an ophthalmic emergency often leading to blindness, if early management is not instituted.

This case study includes 102 patients during a one year period at the Sarojini Devi Eye Hospital- A Tertiary Eye Care Hospital in Hyderabad India.

In our study male subjects 78 (76.47%) were more affected than female patients 24 (23.53%). This is similar to the study done by Chittur Y. Ranjini *et al.*, (2016) and also study done by Tityal *et al.*, (2006). This could be attributed to the increased outdoor activity of men especially in the working age group. In contrast, in the study done in China, women were more affected and most of them were over the age of 60 (Cao *et al.*, 2014). This could be due to higher employability of women particularly in the agricultural sector in China.

In this study, the commonest age group affected were 41–60 years 59 (57.85%), followed by 21-40 years 28 (27.45%), 61- 80 years 09 (08.82%), and 0-20 years 06 (05.88%)

Which is in agreement with the results of Cameron *et al.*, (2006) in Sydney and Das *et al.*, (2013) in Kolkata. Similar age group was also most commonly affected in south India and western Orissa by Srinivasan *et al.*, and Samir Mahapatra *et al.*, (2009) While in the eastern study and Bangladesh study, by Sharmeen Ahmed *et al.*, (2010); Basak *et al.*, (2005) the commonest age group was 21-40 years.

The most common age group who are affected (41-60 years) impact the socio-economic conditions as they usually support families financially.

Table 1 Showing Sex distribution

| Sl.no | SEX      | No. of cases | Percent  |
|-------|----------|--------------|----------|
| 1     | Males    | 78           | 76.47%   |
| 2     | Females  | 24           | 23.53%   |
**Table.2** Showing Age distribution

| Sl.no | AGE      | No.of cases | Percent  |
|-------|----------|-------------|----------|
| 1     | 0 - 20   | 06          | 05.88%   |
| 2     | 21 - 40  | 28          | 27.45%   |
| 3     | 41 -60   | 59          | 57.85%   |
| 4     | 61 - 80  | 09          | 08.82%   |

**Table.3** Showing Risk factors distribution

| RISK FACTORS                              | No. of Cases | Percent  |
|-------------------------------------------|--------------|----------|
| Corneal trauma                            | 34           | 33.34%   |
| Contact lens wear                         | 20           | 19.62%   |
| Occular surgery                           | 11           | 10.78%   |
| Blepharitis                               | 10           | 09.80%   |
| Organic material (vegetable or animal)    | 10           | 09.80%   |
| Dry eye syndrome                          | 09           | 08.82%   |
| Use of Contaminated medications           | 08           | 07.84%   |

**Table.4** Showing culture positivity among suspected bacterial Keratitis samples

| Total No. of samples | Culture positive (percent) | Culture Negative (percent) |
|----------------------|----------------------------|---------------------------|
| 102                  | 89 (87.25%)                | 13(12.75%)                |

**Table.5** Showing Bacteria Isolates distribution

| Bacteria Isolated                          | Number | Percent  |
|-------------------------------------------|--------|----------|
| Staphylococcus aureus                     | 24     | 26.97%   |
| Coagulase negative Staphylococcus         | 18     | 20.23%   |
| Streptococcus pneumoniae                  | 11     | 12.36%   |
| Pseudomonas spp.                          | 20     | 22.47%   |
| Klebsiella spp.                           | 12     | 13.48%   |
| Serratia marcessens                       | 04     | 04.49%   |

**Table.6** Showing Antibiotic sensitivity of Gram Positive Isolates

| ANTIBIOTIC                        | BACTERIAL ISOLATE | St.aureus (24) | CONS (18) | Str.pneu (11) |
|-----------------------------------|-------------------|----------------|-----------|---------------|
| Gentamicin (10mcg)                | S R               | 18 06          | 14 04     | 03 08         |
| Tobramycin (10 mcg)               | S R               | 14 10          | 14 04     | 11 0          |
| Gentamicin (5mcg)                 | S R               | 17 07          | 18 0      | 11 0          |
| Moxifloxacin (5 mcg)              | S R               | 15 09          | 18 0      | 11 0          |
| Ofloxacin (5 mcg)                 | S R               | 10 14          | 10 08     | 11 0          |
| Ciprofloxac (5mg)                 | S R               | 10 14          | 10 08     | 11 0          |
| Chloramphenicol (30 mcg)          | S R               | 20 04          | 13 05     | 11 0          |
| Vancomycin (30 mcg)               | S R               | 24 0           | 18 0      | 11 0          |
| Cefepime                          | S R               | 24 0           | 18 0      | 11 0          |
Table 7 Showing antibiotic sensitivity of Gram negative isolates

| ANTIBIOTIC                | BACTERIAL ISOLATE |  
|---------------------------|-------------------|
|                           | Pseudomonas aeruginosa (20) | Klebsiella sp (12) | Serratia marcescens (04) |
| Gentamicin (10 mcg)       | S 11 R 09 S 09 R 03 S 03 R 01 |
| Tobramycin (10 mcg)       | S 12 R 08 S 10 R 02 S 00 R 04 |
| Gatifloxacin (5 mcg)      | S 20 R 00 S 12 R 00 S 04 R 00 |
| Moxifloxacin (5 mcg)      | S 18 R 02 S 12 R 00 S 03 R 01 |
| Ofloxacin (5 mcg)         | S 16 R 04 S 12 R 00 S NT R NT |
| Lomefloxacin              | S 20 R 00 S 12 R 00 S 04 R 00 |
| Ciprofloxacin (5 mcg)     | S 18 R 02 S 11 R 01 S 03 R 01 |
| Chloramphenicol (30 mcg)  | S 09 R 11 S 12 R 00 S 02 R 02 |
| Vancomycin (30 mcg)       | S NT R NT S NT R NT S NT R NT |
| Cefepime                  | S 20 R 00 S 12 R 00 S 04 R 00 |

As per our study we found that corneal trauma is the commonest predisposing or risk factor in our patients as seen in 34 cases (33.34%) followed by Contact lens wear in 20 cases (19.62%), Ocular surgery 11 cases (10.78%) blepharitis 10 cases (09.80%), organic material (vegetable or animal) 10 cases (09.80%) followed by co-existing ocular disorders like Dry eye syndrome in 09 cases (08.82%) and Use of Contaminated medications in 08 cases (07.84%).

As per our study we found that corneal trauma is the commonest predisposing or risk factor in our patients as seen in 34 cases (33.34%) followed by Contact lens wear in 20 cases (19.62%), Ocular surgery 11 cases (10.78%) blepharitis 10 cases (09.80%), organic material (vegetable or animal) 10 cases (09.80%) followed by co-existing ocular disorders like Dry eye syndrome in 09 cases (08.82%) and Use of Contaminated medications in 08 cases (07.84%).

This is similar to other studies by Bataineh et al., (2008) Shoja et al., (2004) Tabbara et al., (2000) and also Indian studies by Chittur Y. Ranjin et al., (2016) Bangalore, Karnataka, Assudani et al., (2013) Sethi et al., (2010) who all demonstrated injury to eye as the predominant risk factor followed by the foreign body induced microbial keratitis.

Trauma is a more common predisposing factor for bacterial keratitis in low income countries, where it accounts for up to 77.5% of cases. (Vajpayee et al., 2000) the presence of organic materials within the wound represents a much higher risk than does the presence of nonorganic substances. Ocular surface diseases such as dry eye syndrome, chronic blepharitis, and eyelid pathologies also predispose to bacterial keratitis.

Contact lens related keratitis is the major risk factor in advanced countries and most of the cases are young patients.

The annual incidence of ulcerative keratitis in contact lens wearers is 4-21 per 10,000 daily wear Extended wear soft contact lens users have a 10–15 times higher annual incidence of ulcerative keratitis than daily wear soft contact lenses.

This may be due to inappropriate lens wear and absence of awareness of lens usage and care among contact lens wearers

However, other studies by Frédéric Schaefer et al., (2001) at Switzerland Tewelde, Erice et al., (1993) in Minnesota, USA, Yousuf et al., (2009) reported contact lens wear as most important risk factor.

This difference may be attributed to low prevalence of contact lens usage in under developed, and developing countries and
more of contact lens use in developed countries.

The overall success rate of bacterial isolation was high in the present study, with 89 (87.25%) of smears being positive. This is similar to the study done by Frédéric Schaefer et al., (2001)

In our study the most predominant bacterial isolates causing keratitis were Gram positive bacteria *Staphylococcus aureus* 24(26.97%) and followed by gram negative bacteria *Pseudomonas* 20 (22.47%). This is similar to other Indian studies by Gopinathan et al., (2009) and Das et al., (2013) A study conducted in Pakistan by Narsani et al., (2009) also showed higher isolation of Gram positive organisms with *S. aureus* being the most common (60%). Assudani et al., (2013) and Yusuf et al., (2009) in their studies have reported *Pseudomonas* as more common bacteria than *S. aureus*. Sethi et al., (2010) Gopinathan et al., (2009) Bharati et al., (2007) in their studies showed coagulase negative Staphylococci as the most common isolate

*Pseudomonas aeruginosa* was mostly isolated from tropical places with higher maximum and minimum temperatures (Green et al., 2008), whereas lower rates are found in cooler climates (Hedayati et al., 2015)

Even though the main bacteria known to cause severe keratitis are *S. aureus* and *P. aeruginosa*, the prevalence and degree of occurrence of corneal pathogens over others are dependent on the variation with the patient local population, health of the cornea, geographic location, climatic zone variation as Gram positive bacterial species are more frequently recovered in temperate zones and Gram negative species in tropical climates.

In our study of the antimicrobial susceptibility pattern, the predominant Gram positive bacteria *Staphylococcus aureus* and CONS were 100% sensitive to vancomycin and fourth generation cephalosporin cefepime and more than 75% sensitivity was seen in *S.aureus* isolates to gatifloxacin, gentamicin and chloramphenicol and showed maximum resistance to ciprofloxacin and Ofloxacin, while streptococcus pneumonia showed sensitivity to almost all the antibiotic tested except resistance to Gentamicin.

Gram negative bacilli were most susceptible to Lomefloxacin, Gatifloxacin, Cefepime followed by Ciprofloxacin and showed the highest resistance to Gentamicin and Tobramycin.

Among the Gram negative isolates, *Pseudomonas aeruginosa* exhibited good sensitivity to gatifloxacin, Cefepime and lomefloxacin (100%) followed by ciprofloxacin (90%) and moxifloxacin (90%) and was the least sensitive to chloramphenicol.

This is similar to several other studies including an Indian study by Chittur Y. Ranjini et al., (2016)

The standard protocol for treatment of bacterial corneal ulcer in our patients was topical instillation of antibiotics.

As there are no standard CLSI guidelines yet for topical ocular antibiotics, proper interpretation of the drug sensitivity, antibiotic sensitivity pattern coupled with clinical improvement is needed to assess the efficacy of a particular antibiotic.

In conclusion, Bacterial keratitis despite all the advances in the diagnosis and treatment is the most aggressive and destructive pathogen invading the cornea and is responsible for ocular morbidity and vision threatening (corneal scarring and vascularisation) ophthalmic emergency and often leading to blindness and is a therapeutic challenge that
needs immediate institution of treatment. As expansion of contact lenses wear has increased the worldwide incidence of bacterial keratitis, Contact lens wearers and extended wear contact lenses in particular should be informed of the potential risk, and should be carefully instructed on how to care for their lenses.

A proper examination of patients, there clinical history coupled with detailed clinical examination and use of newer methods of diagnosis, aided by more specific laboratory microbiological investigations is necessary in order to analyze and compare the changing trends of the aetiology and, to identify the predisposing factors, their susceptibility patterns which would be beneficial in applying an appropriate antimicrobial treatment.

An early diagnosis, repetitive cultures and smears, and professional management, proper interpretation of the drug sensitivity pattern routine surveillance and monitoring studies for drug resistance and use of new classes of fluoroquinolones, such as lomefloxacin, and fourth generation cephalosporin’s over the current gold standard of therapy, which consists of a combination of cefazolin and gentamicin provide a broad spectrum of antibacterial activity, better tolerance, and good tissue penetration, and will go a long way towards preventing the complications and advocates the most effective empirical treatment for bacterial keratitis.

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