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# A Study on Aerobic Bacterial Agents in Suppurative Keratitis in a Tertiary Care Centre in South Kerala

Authors
**Dr Jyothi. R, Dr Anna Cherian, Dr Honey Susan Paul, Dr Ramani Bai. J.T**

**Abstract**

**Background:** *Corneal ulcers are of everyday occurrence and the communities working in farms, quarries and mining areas, are the major sufferers of corneal blindness in our country. Objective - A study on aerobic bacterial etiological agents in suppurative keratitis.*

**Materials and *Methods*:** *The specimen was collected from suppurative keratitis cases irrespective of prior antibiotic treatment during the period of one year from March 2012 to February 2013. Those cases with loss of corneal epithelium with signs of inflammation, and stromal infiltration were taken as suppurative keratitis cases. Five or more corneal scrapings taken were used for Gram staining, inoculation on to Sheep Blood Agar (SBA), Chocolate Agar, Mac Conkey Agar. Extreme care is taken to keep the entire process sterile.*

**Results:** *During the one year period, out of the 100 patients with suppurative keratitis; there were 12 (17.64%) bacterial isolates. Staphylococcus aureus was the most common gram positive isolate and Psedomonas aeruginosa was the common gram negative one.*

**Conclusions:** *Although corneal ulceration is a very rare cause of mortality, it is one of the important causes of morbidity and disability in South Kerala, especially among lower socio-economic groups. The present study gives an insight about the bacterial aetiological agent of suppurative keratitis. The results of this study are similar to that in other parts of India. These findings have important public health implications for the treatment and prevention of corneal ulceration in the developing world.*

**Keywords:** *Suppurative keratitis, Corneal scrapings, SDA.*

## Introduction

Corneal ulcers pose a challenging problem to the ophthalmologists, as their etiology is of varied origin and their course unpredictable, prognosis uncertain and a specific therapy being still a confused issue. Corneal ulcers occur more frequently in elderly debilitated, arteriosclerotic and anemic persons. They occur among the lower classes exposed to injury and prone to neglect the infection. They occur specially during harvest season and by the time the patient seeks medical aid, the ulcers are much advanced and may recover without perforation but with opacities of varying density or end in perforation and its sequalae, leading to blindness. Bacterial keratitis is one of the most important causes of corneal pacifications, which is the second common cause of blindness world-wide after cataracts. The pattern of microbial keratitis varies with geographic region and according to the local climate. For example, USA has an incidence of 11 per 100,000 persons for microbial keratitis as compared to 799 per 100,000 persons in Nepal.[1] The bacteriological profile in keratitis shows huge

disparities amongst populations living in both western and in developing countries due to the fact that less industrialized countries have significantly lower number of contact lens users, hence fewer contact lenses related infections. Bacterial keratitis has been reported to account for 32.3% of all cases of corneal ulcer evaluated in Madurai (South India), 29.3% in Thiruchirapalli (South India), 35.6% in South Florida and 25% in Southern Ghana. In marked contrast, a study performed in Nepal documented the occurrence of a bacterial aetiology in 63.2% of all corneal ulcers. Bacteria are responsible for a larger proportion of corneal ulceration in temperate climates such as Britain and northern United States than in tropical regions such as South India, but in the subtropical urban climates of Hong Kong bacteria are a predominant cause for microbial keratitis.[2]

Retrospective analysis of data collected over a decade showed microbial keratitis 3563 (60.4%) out of 5897 suspected cases. They were culture-proven (bacterial 1849, 51.9%); According to the World Health Organization report, it is estimated that ocular trauma and corneal ulceration result in 1.5 to 2 million new patients of corneal blindness annually, posing a major public health problem for developing countries[3]

Microbial keratitis or infectious corneal ulcer is due to the proliferation of microorganisms and associated inflammation, and tissue destruction within the corneal tissue. It is a potentially sight threatening condition, and frequently presents as an ocular emergency. Bacterial keratitis is the most common cause of suppurative corneal ulceration. There are no specific clinical signs to confirm a definite bacterial cause, but clinicians should identify the risk factors for ocular infection, and assess the distinctive corneal findings to determine potential etiologies. Laboratory investigations are mandatory when there is a strong suspicion for a possible infectious keratitis. Based on the clinical and laboratory findings, a therapeutic plan can be initiated. Medical therapy with the appropriate antibiotics is the mainstay of treatment. The outcome usually depends on the preceding pathology, and extent of ulceration at the time of presentation.[4]External risk factors include bacterial keratitis following corneal trauma is more likely associated with an agricultural than with an industrial setting. Exposure to contaminated water, and other solutions such as tap water or eye wash solutions following corneal trauma can lead to bacterial keratitis. Nosocomial corneal infections have occurred in burn victims and unconscious patients during general anesthesia, or coma, as a result of lagophthalmos, and corneal exposure. Geographic and climatic factors also influence prevalence of bacterial keratitis.[4]

Microbial keratitis affects approximately 5 in 10,000 contact lens wearers. The use of contact lenses overnight is the single most common risk factor in the developed world.[5]

Systemic conditions such as malnutrition, diabetes, collagen vascular diseases, or chronic alcoholism also compromise ocular surface, and increase the risk of microbial keratitis caused by unusual organisms such as Moraxella. In patients with acquired immunodeficiency syndrome (AIDS), microbial keratitis does not appear to be more prevalent than in the normal population, however the keratitis tends to be more severe, and more resistant to therapy in these patients.[4]

Bacterial keratitis can be caused by multiple microorganisms like Staphylococcus, Pseudo-monas, and Streptococci. The smallest inoculum that can produce infection in the human cornea remains undetermined. A single viable organism can theoretically initiate corneal infection.

A more recent, although smaller, survey isolated an organism in 68% of 300 eyes suspected to have bacterial keratitis; 83% were gram-positive, 17% were gram-negative, and 2% were polymicrobial. Most bacterial keratitis is caused by five major groups: Staphylococcus spp., Streptococcus spp. (Streptococcus pneumoniae, groups A through G streptococci), other gram-positive organisms (Bacillus and Propionibacterium spp.), gram-negative organisms (e.g., Pseudomonas,

Haemophilus, Moraxella), and the Enterobacteriaceae (Proteus, Serratia, Klebsiella, Enterobacter, Citrobacter).[6]

The prominence of certain organisms responsible for bacterial keratitis has been changing over many years. *S. pneumoniae* was the most common responsible agent in the past, but other gram-positive organisms, opportunistic commensals, *Pseudomonas,* anaerobes, and protozoa are now increasingly being reported. With the advent of refractive surgery, especially laser-assisted in-situ keratomileusis (LASIK), more unusual organisms, such as *Nocardia* and *Mycobacterium* spp., are causing keratitis. The apparent changes in causal organisms could be the result of numerous factors, such as improved isolation techniques; less frequent culturing as highly effective broad-spectrum single agents (i.e., fluoroquinolones) have been developed; increased use of topical corticosteroids (i.e., refractive and cataract surgery); increased population of systemically immunodeficient patients; and an expansion in the use of soft contact lenses, especially extended-wear and cosmetic lenses.[6]

Gram-positive organisms, particularly *Staphylo-coccus aureus,* continue to be the most common agents causing bacterial keratitis in most series. The order of prevalence in one series from New York City was *S. aureus, Moraxella, Pseudomonas,* and *S. pneumoniae*. A different order—*S. aureus, S. pneumoniae, Pseudomonas,* and *Moraxella*—was seen in a similar series from London. *Pseudomonas* and *Streptococcus* spp. are the most common bacterial pathogens isolated in previously healthy eyes in the southern United States.[6]The purpose of the study is to evaluate the current pathogens causing bacterial keratitis at tertiary care centre.

**Materials and Methods**

Specimens were collected from both OP and IP patients with suppurative keratitis in a minor theatre in front of the microbiology lab, and inoculations done immediately. After properly cleaning the eye with normal saline, a drop of local anaesthetic (proparacaine 0.5%) was instilled, then eyes focused on a slit lamp and the corneal ulcer cleaned with sterile cotton swab saline soaked in saline. Scrapings are taken from edges, and base of the ulcer with a No. 26 G needle or a sterile blade.

The corneal scrapings taken are used for wet film examination, Gram staining, inoculated on to Sheep Blood Agar (SBA), Chocolate Agar, Mac Conkey Agar and Glucose broth. Extreme care is taken to keep the entire process sterile. In some patients who are at risk of penetration, that is, those with corneal thinning, only few scrapings could be taken, and they were inoculated accordingly. Antibiotic sensitivity testing was done according to the nature of isolating organisms, and the availability of ophthalmic preparations. Ampicillin, Gentamicin, Cephal-exin, Chloramphenicol, Ceftriaxone, Amikacin, and Ciprofloxacin were generally used for Gram-negative bacilli. Pencillin, Gentamicin, Cephalexin, Cloxacillin, and Erythromycin were the antibiotics tested generally for Gram-positive cocci (Staphylococcus) Gentamicin, Amikacin, Ceftazidime, and Ciprofloxacin were tested for Pseudomonas aeruginosa. All sensitivity tests were done on MHA. Sensitivity testing for Streptococcus pneumoniae was done in SBA.

**Results**

Out of 100 patients included in the study, 68 were males, and 32 were females. Male to female ratio is 2.125: 1. Age of patients included in the study ranged from 10-82 years, and keratitis cases were maximum in 41-50 year age group (29%), followed by 51-60 years (19%). Minimum incidence was found to be in extremes of age. Occupational profile of the patients included in this study showed a maximum incidence among manual labourers, followed by housewives and students. 66% of patients included in this study were from rural areas, and 22% from semi-urban areas, 12% from urban areas. 82% of patients included in this study belonged to the low socio-economic group. Among the 56 fungal isolates, 39

cases occurred in the rainy season (May – July). Prior treatment was taken by 63% of patients included in this study. Topical antibiotics alone were taken by 55.5% of patients, and57% of patients gave history of ocular trauma. Particulate material constituted 50.87% of the traumatic agents. Both wooden stick and building materials constituted 17.54% each. 8 patients included in this study had Diabetes mellitus. 4 patients gave history of foreign body fall and keratitis in the past. Of the 100 cases of corneal ulcers, 68 were culture positive, and 32 were culture negative. Out of the 68 culture positive 12 were bacterial isolates (17.64%). *Staphylococcus aureus* was the most common gram positive isolate, and *Psedomonas aeruginosa* was the common gram negative isolate. All the gram positive isolates were sensitive strains. All the *Staphylococcus spp*. were resistant to Pencillin. All four gram negative isolates were sensitive. One *Pseudomonas aeruginosa* isolate was resistant to Gentamicin. *Acinetobacter* and *E coli* were resistant only to Ampicillin. Klebsiella spp. was resistant to Ampicillin, Gentamicin, and Cephalosporin- first generation..

**Table 1:** Sex Distribution of Cases

| Gender | No | % |
|---|---|---|
| Males | 68 | 68 % |
| Females | 32 | 32 % |

**Table 2:** Incidence according to age groups - Male and Female

| Sl. no | Age Group | Male | Female | Total | % |
|---|---|---|---|---|---|
| 1 | 0-10 | 1 | 0 | 1 | 1% |
| 2 | 11-20 | 6 | 3 | 9 | 9% |
| 3 | 21-30 | 7 | 3 | 10 | 10% |
| 4 | 31-40 | 11 | 4 | 15 | 15% |
| 5 | 41-50 | 19 | 10 | 29 | 29% |
| 6 | 51-60 | 14 | 5 | 19 | 19% |
| 7 | 61-70 | 8 | 5 | 13 | 13% |
| 8 | > 70 | 2 | 2 | 4 | 4% |
| | Total | 68 | 32 | 100 | |

**Table 2:** Distribution according to occupation

| Sl. No | Occupation | Total No | % |
|---|---|---|---|
| 1 | Manual Labourers | 62 | 62% |
| 2 | Farmers | 7 | 7% |
| 3 | Housewives | 12 | 12% |
| 4 | Students | 9 | 9% |
| 5 | Office Workers | 4 | 4% |
| 6 | Others | 6 | 6% |

**Table 4:** Habitat distribution of study population

| Habitat | No | % |
|---|---|---|
| Rural | 66 | 66% |
| Semi-urban | 22 | 22% |
| Urban | 12 | 12% |
| Total | 100 | 100% |

**Table 5:** Distribution of cases based on socio-economic status

| Socio-economic status | No | % |
|---|---|---|
| Low | 82 | 82% |
| Middle | 16 | 16% |
| High | 2 | 2% |

**Table 6:** Corneal Ulcers: Seasonal variation

| Sl. No | Months | Fungal isolation | Bacterial isolation | Total no of cases |
|---|---|---|---|---|
| 1 | Dec, Jan, Feb | 12 | 2 | 21 |
| 2 | Mar, Apr, May | 4 | 1 | 7 |
| 3 | Jun, Jul, Aug | 32 | 7 | 58 |
| 4 | Sep, Oct, Nov | 8 | 2 | 14 |
| Total | | 56 | 12 | 100 |

**Table 7:** Corneal ulcers - history of prior treatment

| Treatment History | No | % |
|---|---|---|
| Treated | 63 | 63% |
| No Treatment | 37 | 37% |

**Table 9:** Corneal Ulceration & Ocular Trauma

| Ocular Trauma | No | % |
|---|---|---|
| Present | 57 | 57% |
| Absent | 43 | 43% |

**Table 10:** Ocular Trauma: Causes of injury

| Sl. No. | Traumatic Agents | No | % |
|---|---|---|---|
| 1 | Particulate material (Sand, Dust) | 29 | 50.88 |
| 2 | Wooden Stick | 10 | 17.54 |
| 3 | Building Material | 10 | 17.54 |
| 4 | Insects | 1 | 1.75 |
| 5 | Fingernails | 1 | 1.75 |
| 6 | Dirty Water | 1 | 1.75 |
| 7 | Vegetative matter | 5 | 8.77 |
| Total | | 57 | |

**Table 11:** Corneal Ulcers: Other predisposing factors

| Sl. No | Predisposing factors | No | % |
|---|---|---|---|
| 1 | DM | 8 | 50.00% |
| 2 | History of fall and keratitis in the past | 4 | 25.00% |
| 3 | Facial Palsy | 2 | 12.50% |
| 4 | Dry eye | 1 | 6.25% |
| 5 | Lid abnormality | 1 | 6.25% |
| Total | | 16 | |

**Table 12:** Corneal ulcers - Culture positivity

| Positivity | No | % |
|---|---|---|
| Culture Positive | 68 | 68% |
| Culture Negative | 32 | 32% |

**Table 13:** Bacterial Pathogens Isolated

| Species | No. of isolates | % |
|---|---|---|
| Staphylococcus aureus | 3 | 25 |
| Staphylococcus epidermidis | 1 | 8.33 |
| Streptococcus pneumoniae | 1 | 8.33 |
| Pseudomonas aeruginosa | 3 | 25 |
| Acinetobacter spp. | 2 | 16.67 |
| Escherichia Coli | 1 | 8.33 |
| Klebsiella spp. | 1 | 8.33 |
| Total | 12 | |

**Table 14:** Antibiotic sensitivity of gram positive organisms (% sensitivity)

| Antibiotic | Staphylococcus aureus (3) | | Staphylococcus epidermidis (1) | | Streptococcus pneumoniae (1) | |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % |
| Pencillin | 0 | 0 | 0 | 0 | 1 | 100 |
| Gentamicin | 3 | 100 | 1 | 100 | NT | |
| Cephalexin | 3 | 100 | 1 | 100 | NT | |
| Erythromycin | 3 | 100 | 1 | 100 | NT | |
| Chloramphenicol | 3 | 100 | 1 | 100 | NT | |
| Ceftriaxone | NT | | NT | | 1 | 100 |
| Amikacin | 3 | 100 | 1 | 100 | NT | |
| Vancomycin | 3 | 100 | 1 | 100 | 1 | 100 |
| Cloxacillin | 3 | 100 | 1 | 100 | NT | |
| NT - Not Tested | | | | | | |

**Table 15:** Antibiotic sensitivity of *Pseudomonas aeruginosa* (% sensitivity)

| Antibiotic | Pseudomonas aeruginosa (3) | |
|---|---|---|
| | No. | % |
| Ampicillin | NT | |
| Gentamicin | 2 | 66.67 |
| Cephalexin | NT | |
| Chloramphenicol | NT | |
| Ciprofloxacin | 3 | 100 |
| Amikacin | 3 | 100 |
| Ceftazidime | 3 | 100 |

**Table 16:** Antibiotic sensitivity of *Acinetobacter*, *E coli*, and *Klebsiella spp.* (% sensitivity)

| Antibiotic | Acinetobacter (2) | | Escherichia Coli (1) | | Klebsiella spp. (1) | |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % |
| Ampicillin | 0 | 0 | 0 | 0 | 0 | 0 |
| Gentamicin | 1 | 50 | 1 | 100 | 0 | 0 |
| Cephalexin | 1 | 50 | 1 | 100 | 0 | 0 |
| Chloramphenicol | 1 | 50 | 1 | 100 | 1 | 100 |
| Ciprofloxacin | 2 | 100 | 1 | 100 | 1 | 100 |
| Amikacin | 2 | 100 | 1 | 100 | 1 | 100 |
| Ceftriaxone | 2 | 100 | 1 | 100 | 1 | 100 |

**Discussion**

Corneal blindness is a major public health problem worldwide and infectious keratitis is one of the predominant causes. In view of the importance in timely diagnosis and treatment of keratitis which would otherwise lead to serious complications like loss of vision, hypopyon formation, endopthalamitis, etc, this study was intended to bring into light bacterial etiological agents and their susceptibility pattern.

Of the 100 patients included in the study 68 were males and 32 were females. Male: Female ratio is 2.125:1. This finding is in accordance with the results got in studies done by Punia et. al. [48],

Bharati et. al. [47] in India. In this study, age of patients ranged from 10-82 years, and keratitis was maximum in 41-50 year age group (29%), followed by 51-60 years (19%), and 31-40 years (15%). Minimum incidence was found to be in extremes of age groups. In the age group with more than 70 years, it was found to be 4%, and 1% in the less than 10 years age group. The incidence increased steadily through age groups up to age of 60 years, and decreased thereafter. Thus the maximum number of cases occurred in the most productive years of life. High incidence of keratitis in men in 41-50 year age group can be attributed to the fact that men in this age are more actively involved in outdoor activities. Corneal ulcers showed a higher incidence in the economically active age group (21-50 years) representing 58.53% of the isolates according to a study done in Vishakapatnam.[9]

Majority of the patients included in this study were manual laborers which included construction workers, factory workers, and non-specific agriculture related workers. Farmers constituted less than 10% of the cases. This may be due to the decreasing areas of agricultural land, and a dwindling agricultural workforce in South Kerala. The other significant groups were housewives (12%), students (9%), and office-workers (4%). But, farmers were mostly affected in studies done in Vishakaptnam[9].

In majority of the studies, keratitis cases occurred commonly in low income groups, and a similar occurrence was noted in rural population. In this study, about 66% cases were rural population, 22% semi-urban, and 12% belonged to urban population. Similar finding was got in a study done in Gujarat[10] and Bhopal.[11]82% of patients who participated in the study belonged to low socioeconomic group, 16% to middle socioeconomic group and 2% to high socioeconomic group. In the study major predisposing factor for keratitis was found to be the history of corneal injury. This could be attributed to the fact that majority of patients were rural residents, belonging to low socioeconomic group and majority were manual labourers. So their working and living environment exposed them to the threat of foreign body fall and the resulting corneal ulcer. Geographical location and climate were reported to influence the clinical presentation and outcome of fungal diseases, probably through changes in the causative agent.[12]

In this study 58% of cases presented in monsoon season..Next higher incidence occurred in winter. Minimum cases presented in summer. Microbial keratitis is an important cause of ocular morbidity in India. It can lead to severe visual and structural damage to eyes. Various factors like occupational hazards, compromised hygiene state, nutritional & immune status, self-medication and many more play an important role in predisposition of microbial keratitis.[11]

In this study, 57% gave history of ocular trauma prior to corneal ulceration, while 43% did not give any obvious history of injury. Corneal injury is the most important predisposing cause (and often the precipitating cause) of corneal ulceration, especially in the developing countries where most of the population were daily wage manual laborers. Corneal injury was caused by substances like sand dust, which contributed to 29%. Other particles which caused injury were wooden piece, building material, vegetative matter, insects, fingernail, and dirty water. None of the injuries reported in this study were caused by paddy husk which was one of the major causes in Central Kerala in the past where rice cultivation was common.

Contact lens usage, although the commonest predisposing factor in developed countries[13], was conspicuously absent in this study. Not even a single patient presented with history of contact lens usage. This can be attributed to the virtual absence of contact lens usage among the low income group. It is also prudent to think that the contact lens users would prefer private hospitals for treatment.8 patients in the study were suffering from Diabetes mellitus. Similar finding was obtained in a study done in Gujarat.[14]

Out of the 68 culture positive cases, 12 cases yielded pure bacterial isolates. The possible reason for reduced bacterial corneal ulcers might be more successful treatment of bacterial corneal ulcers in the peripheral centers and/or by general practitioners.

17.6% of the total isolates were bacterial. *Staphylococcus aureus* which constituted 25% of bacterial isolates was the predominant Gram positive organism in this study, others being *Staphylococcus epidermidis,* and *Streptococcus pneumoniae* constituting one case each in the study. This finding is supported by a number of other studies[1]. Leck AK, Thomas PA, *et al,* in a multi-centric study in 2002 reported Streptococci (including *Pneumococci*) as the commonest bacterial pathogen associated with keratitis in South India[15]. However, in the Indian subcontinent, most of the studies have shown *Strptococcus pneumoniae* as the commonest bacteria, and *Pseudomonas aeruginosa* as the commonest Gram negative isolate from corneal ulcers both in the general population, as well as in contact-lens users [(107)]. Pseudomonas species was a predominant bacterial isolate (55%) in a study done in Thailand, and in Peshawar [(52)]

In this study, *Pseudomonas* was the commonest Gram negative isolate (25%). Other Gram negative isolates were *Acinetobacter spp.*, *Escherichia Coli,* and *Klebsiella spp. Pseudomonas aeruginosa* have emerged as a predominant Gram negative isolate in many of the studies done in India[14]and abroad[1].

All the gram positive organisms isolated in this study were sensitive strains. But the four *Staphylococcus spp.* (3 *Staphylococcus aureus* and 1 *Staphylococcus epidermidis*) were resistant to Penicillin.

All the gram negative isolates were also sensitive. Out of the 3 *Psedomonas aeruginosa* isolated, one was resistant to Gentamicin. *Acinetobacter* and *E coli* were resistant only to Ampicillin, but *Klebsiella* was resistant to Ampicillin, Gentamicin, and Cephalexin.

Antibiotic susceptibility testing showed most of bacterial isolates sensitive to Amikacin and Gentamicin.[16] The Gram-negative and Gram-positive bacteria were all sensitive to Chloramphenicol.[17] Though majority of cases healed well following aggressive treatment, few cases ended up in corneal opacification, vascularization, and perforation.

**Conclusion**

Although corneal ulceration is a very rare cause of mortality, it is one of the important causes of morbidity and disability in South Kerala, especially among lower socio-economic groups. The present study gives an insight about the bacterial etiological agents of suppurative keratitis .The results of this study are similar to that in other parts of India. .Bacterial ulcers, though less common, are still significant, and sensitivity testing of bacteria is important, as drug resistance is very common, especially in a referral care context. A complete microbiological investi-gation, including antibiotic sensitivity testing becomes mandatory and sight-saving in such a situation.

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