

To Study the Spectrum of Bacterial Keratitis in Western India

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Keywords

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Abstract

Aim: To study the spectrum of bacterial keratitis in western India.

Material and methods: The study, which received ethical board approval from the authors' institution, involved a retrospective analysis of all subjects who presented to ophthalmic microbiology having a diagnosed infectious corneal ulceration. Following topical anaesthesia, an ophthalmologist scraped the base as well as edges of the ulcerated portion of the cornea with a sterile Kimura platinum spatula or a sterile Bard-Parker knife while using a slit lamp to magnify the area. Standard microbiological examinations were now possible.

Results: From the 250 corneal lesions that were scraped, 100 were found to have bacteria as the single isolating organism. Fifty patients showed considerable development of several bacterial species or growth of multiple bacterial species and fungi ("mixed growth") in the scraped ulcer. Eighty (80%) of the bacterial culture isolates were Gram-positive, whereas just twenty (20%) were Gram-negative. Staphylococcus spp. were the most frequently identified bacterial pathogens (61%), followed by Streptococcus spp. (13%), Pseudomonas aeruginosa (10%), Bacillus spp. (5%), Acinetobacter spp. (3%) and Aeromonas spp. (2%). The least common bacterial isolates were from species including Enterobacter, Klebsiella, Serratia marcescens, and Flavobacterium.

Conclusion: In this scenario, Staphylococcus spp. were the most often identified bacterial pathogens from keratitis patients. Both gatifloxacin and moxifloxacin showed excellent susceptibility against a wide range of Gram-positive and Gram-negative bacteria.

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1. Introduction

Bacterial keratitis seems to be a potentially fatal corneal disease because a few of the highly threatening bacterial aetiological pathogens may fully destroy the cornea within twenty four to forty eight hours. The notion that the range of bacterial corneal diseases is mainly controlled by the local microbial ecosystem as well as by geographical & climatic factors is probably the cause of the varying frequencies of numerous microbes documented in series from multiple locations^{1 2} Numerous high-virulence organisms have been discovered, namely *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, as well as *Staphylococcus aureus*³. Coagulase-negative staphylococci have been linked to an increase in the incidence of bacterial keratitis in several geographical areas^{1 4 6}

People having ophthalmic infections are progressively being reported to develop multidrug-resistant CoNS^{4 7} All of the risk factors as well as microorganisms found play a part in the visual outcome after bacterial keratitis⁸. Bacterial keratitis must be treated immediately because it is an ophthalmic crisis. Because there exists no laboratory diagnosis initially, intervention is typically aggressive and broad-based. In cases of keratitis produced by bacteria sensitive to antibiotics, empirically guided management might be adequate in the absence of a microbiological diagnosis, however there remains a risk that resistant pathogens may lead to an abnormally bad visual prognosis.⁹ Focused therapy must be based on laboratory information pinpointing the causative organisms and presenting antibacterial susceptibility findings¹⁰ Knowing the microbiological trend of bacterial keratitis might

help in the effective management of the disease if resources are scarce.

2. Material and methods

The study, which received ethical board approval from the authors' institution, involved a retrospective analysis of all subjects who presented to ophthalmic microbiology having a diagnosed infectious corneal ulceration. Following topical anaesthesia, an ophthalmologist scraped the base as well as edges of the ulcerated portion of the cornea with a sterile Kimura platinum spatula or a sterile Bard-Parker knife while using a slit lamp to magnify the area. Standard microbiological examinations were now possible.¹¹

Numerous scrapings were necessary to gather the material essential for direct microscopy as well as culture. The leftover corneal scrape material from every individual was inoculated straight onto media which endorse the development of bacteria, fungi, and *Acanthamoeba*. A segment of every individual's scrape substance had been utilised for direct microscopy. Such media comprised brain-heart infusion agar as well as broth, Sabouraud dextrose agar as well as broth, and sheep blood agar. In the earlier days, *Acanthamoeba* ulcers were also cultivated on nonnutrient agar with an overlay of *Escherichia coli*. Corneal scrapings were utilised to label plates of solid media with rows of "C" streaks after they were inoculated. The broth-blood agar as well as the brain-heart infusion agar were cultured at thirty seven degrees Celsius for one week prior to getting monitored everyday. For 4 weeks, the Sabouraud dextrose agar plates as well as the broth were cultured at room temperature with daily checks.

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If any of the following circumstances occurred, there was considered to be significant bacterial growth:

This could imply that (a) the same bacteria was isolated on various solid media, (b) the appearance of the bacteria formed in culture was consistent with the actual observations obtained through microscopy, or (c) the similar organism was cultivated on several aqueous as well as solid media.

The discs were gathered from Hi-Media, Mumbai, India, and Mueller Hinton agar; the bacteria had been detected using conventional biochemical test procedures [12]; as well as their sensitivity to amikacin (30 g), chloramphenicol (30 g), ciprofloxacin (5 g), gatifloxacin (5 g), gentamicin

(10 g), moxifloxacin (5 g), ofloxacin (5 g), as well as tobramycin (10g)

3. Results

The ocular ulcers of 250 individuals have been scraped for microbial analysis. Averaging 44.74 ± 6.25 years, the patients' ages ranged widely. Fifty-fifty (60%) of the patients were male, and one hundred (40%) were female. Out of the total of 250 ulcers, microorganisms (bacteria and fungus) were cultured from 200 (80%). (Table 1). Sixty percent of those who had culture-proven microbial keratitis had a history of ocular trauma from both living and nonliving sources (Table 2). Table 2 provides a full breakdown of the reported predisposing and risk variables, classified according to the microbiological agent detected.

Table 1: Aetiological spectrum of microbial keratitis

| Bacterial only | Fungal only | Mixed (bacterial + fungal) | No growth | Total |
|----------------|-------------|----------------------------|-----------|-------|
| 100 | 50 | 50 | 50 | 250 |
| 40 | 20 | 20 | 20 | 100 |

Table 2: Putative risk factors for culture positive microbial keratitis

| | Number =250 | Percentage |
|-------------------------|-------------|------------|
| Trauma | 150 | 60 |
| Mud, dust, and soil | 59 | 39.33 |
| Leaf & vegetable matter | 40 | 26.67 |
| Stick | 18 | 12 |
| Stone | 9 | 6 |
| Insect | 7 | 4.67 |
| Finger nail | 5 | 3.33 |
| Wood piece | 4 | 2.67 |

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| | | |
|---------------------------------|----|------|
| Metal (iron) | 3 | 2 |
| Animal tail, horn | 2 | 1.33 |
| Glass piece | 2 | 1.33 |
| Ball | 1 | 0.67 |
| Contact lens wear | 3 | 1.2 |
| Use of eye drops (antibiotics) | 55 | 22 |
| Use of traditional eye medicine | 47 | 18.8 |

Table 3: Bacterial isolates recovered from patients with corneal ulceration

| Bacteria | Number | Percentage |
|--|-----------|------------|
| Staphylococcus epidermidis | 40 | 40 |
| Staphylococcus aureus | 20 | 20 |
| Staphylococcus saprophyticus | 1 | 1 |
| Staphylococci total | 61 | 61 |
| Streptococcus pneumoniae | 10 | 10 |
| Viridans streptococci | 3 | 3 |
| Streptococci total | 13 | 13 |
| Total Gram-positive cocci total | 74 | 74 |
| Bacillus species | 5 | 5 |
| Corynebacterium diphtheriae. | 1 | 1 |
| Gram-positive bacilli total | 6 | 6 |
| Gram positive organisms total | 80 | 80 |
| Pseudomonas aeruginosa | 10 | 10 |
| Acinetobacter species | 3 | 3 |
| Aeromonas species | 2 | 2 |
| Enterobacter species | 1 | 1 |
| Klebsiella pneumoniae | 2 | 2 |
| Serratia species | 1 | 1 |
| Flavobacterium species | 1 | 1 |
| Gram negative total | 20 | 20 |

Table 4: Frequent bacterial isolates and percentage of strains susceptible to antibacterial agents in bacterial keratitis

| Organism (number of isolates) | AK | C | CF | G | GF | MO* | OF | TB |
|-------------------------------|----|---|----|---|----|-----|----|----|
| | | | | | | | | |

| | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) |
|---------------------------------|---------|----------|---------|----------|----------|--------|----------|----------|
| Staphylococcus epidermidis (40) | 36 (90) | 32(80) | 28(70) | 32 (80) | 38 (95) | 28(70) | 32(80) | 32 (80) |
| Staphylococcus aureus (20) | 19(95) | 17(85) | 15(75) | 17 (85) | 20(100) | 14(70) | 17 (85) | 18 (90) |
| Streptococcus pneumoniae (10) | 4 (40) | 8 (80) | 7 (70) | 3(30) | 10 (100) | 9(90) | 9(90) | 4(40) |
| Bacillus spp. (5) | 5 (100) | 5(100) | 3(60) | 5(100) | 5(100) | 4(80) | 5(100) | 4(80) |
| Pseudomonas aeruginosa (10) | 9(90) | 4(40) | 8 (80) | 9(90) | 7 (70) | 8(80) | 7(70) | 7(70) |
| Acinetobacter sp. (3) | 3(100) | 2(66.67) | 2(85.7) | 2(89.28) | 3(100) | 3(100) | 2(66.67) | 2(66.67) |

From the 250 corneal lesions that were scraped, 100 were found to have bacteria as the single isolating organism. Fifty patients showed considerable development of several bacterial species or growth of multiple bacterial species and fungi ("mixed growth") in the scraped ulcer (Table 1).

Eighty (80%) of the bacterial culture isolates were Gram-positive, whereas just twenty (20%) were Gram-negative (Table 3). Staphylococcus spp. were the most frequently identified bacterial pathogens (61%), followed by Streptococcus spp. (13%), Pseudomonas aeruginosa (10%), Bacillus spp. (5%), Acinetobacter spp. (3%) and Aeromonas spp. (2%). The least common bacterial isolates were from species including Enterobacter, Klebsiella, Serratia marcescens, and Flavobacterium (Table 3).

The susceptibility profile of the most often isolated bacteria is shown in Table 4. Since these drugs are often used to treat eye infections in the area, only the susceptibility test findings for amikacin, chloramphenicol, ciprofloxacin, gentamicin, gatifloxacin, moxifloxacin, ofloxacin, and

tobramycin were analysed. The antimicrobial gatifloxacin was shown to be effective against more than 95% of Gram-positive bacteria, with ofloxacin and moxifloxacin also showing good results. Amikacin and gentamicin resistance was seen in almost 92% of Pseudomonas aeruginosa isolates, whereas ciprofloxacin resistance was observed in 84% and moxifloxacin resistance was observed in 80%.

4. Discussion

Trauma or injury to the corneal epithelial barrier can cause ulceration as well as the invasion of inflammatory cells, which can lead to bacterial keratitis [13]. The majority of the infection is caused by Gram-positive bacteria notably Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, as well as different Streptococcus and Bacillus species. With quick identification as well as management, eyesight consequences including corneal scarring or perforation can be prevented.

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We found that trauma accounted for sixty percent of the contributing variables in our subjects, similar to other data from Iran¹⁴ Qatar¹⁵ as well as Sudan¹⁶. Mud, dirt, as well as dust had been most frequently noted preceded by vegetable as well as leaf waste.

Subjects in the study reported injuries from dirt as well as dust grains about half as often as they reported injuries from plant matter. Dust injuries might have occurred more frequently during windy seasons, while vegetable debris injuries might have occurred more frequently during this site's busiest farming season. Contact lens usage is a major risk element for bacterial keratitis, as per studies from Saudi Arabia, France, as well as Australia. Compared to percentages recorded in the aforementioned studies, just twelve percent of subjects in the present series acknowledged ever utilised contact lenses. In this study, eighty percent of the corneal lesions scraped produced bacteria that could be cultured. Optimism for cultural norms varied between forty and seventy three percent in other studies from Australia¹⁹, France¹⁸ and other countries.

Despite strong host defences, organisms including *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa* might infiltrate the stroma²⁴ as well as their propensity to adhere to the base or edge of an epithelial lesion is a sign of their pathogenicity^{25 26}. The majority of the microbes in this collection were Gram-positive cocci. Other Indian authors also observed 69.1 percent²⁷ and 65.65 percent², hence this result is compatible with them. In other countries, eighty three percent of corneal diseases were caused by Gram-positive bacteria, according to Bourcier et al.¹⁸ as well as Green et al.²⁰

It was discovered that *S. epidermidis* had been the most prevalent gram-positive microorganism. *S. epidermidis* was the bacteria that was most frequently secluded in a study conducted in Switzerland, making up forty percent of the isolates³ Previously performed studies in India^{5 27 29} Australia⁶ the United States of America, Israel, Canada, France, and New Zealand recognised *S. epidermidis* or coagulase-negative staphylococci as the most prevalent isolate.^{18 30 33} Thirty eight percent of corneal ulcer isolates had been CoNS, as demonstrated by Ly et al. Butler et al.¹⁹ discovered that twenty three percent of isolates belonged to CoNS, marking it the most typical isolate collected through corneal ulcers, in a research on elderly people in Australia. This shows that *S. epidermidis* continues to be a significant cause of keratitis.

Streptococci accounted for only thirteen percent of the specimens in our study. *S. pneumoniae* had been the most commonly recognised type of bacteria in cases of bacterial keratitis, according to earlier research from India^{2 34} as well as other impoverished countries, such as Bangladesh as well as Saudi Arabia^{35 36} In an Australian investigation, just eight percent of the isolates seemed to be streptococci⁶ Due to an obstruction in the lacrimal drainage, streptococci are frequently discovered in corneal abscesses in developing nations^{6 37}. Additionally, people residing in rural locations might have a separate etiology for corneal ulcers than those residing in urban regions. The patient population was not examined in the present study or the studies mentioned above in order to distinguish between rural as well as urban individuals.

Ps. aeruginosa had been the most frequently isolated type of bacteria, according to studies from

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South Florida as well as Hong Kong [38, 39], but in our investigation, it accounted for only ten percent of the samples. Our findings differ from those obtained by scientists in Beijing, China [41], Malaysia [40], as well as India [29]. The differences could be attributed to a variety of factors, including climate, the prevalence of contact lens-related keratitis, as well as the intensity of the instances in every study. Eye bacteria are expanding concurrently with the emergence in antibiotic resistance among systemic pathogens, that might have devastating consequences such as the escalation of already hazardous illnesses such as keratitis, endophthalmitis, orbital cellulitis, as well as panophthalmitis, that can result in irreversible visual impairment [42–44].

In the latest series, just a minor percentage of *S. epidermidis* samples seemed to be susceptible to the antibiotic, and just eighty percent to eighty five percent of Gram-positive bacteria were in agreement with a prior finding from our lab [45]. Moxifloxacin, a fluoroquinolone of the fourth generation, was efficient against seventy percent of *S. epidermidis* and *S. aureus* isolates and ninety percent of *S. pneumoniae* isolates. Nevertheless, gatifloxacin was efficient against *S. pneumoniae* as well as ninety five to hundred percent of staphylococci. These results support those from other studies [46, 47], that showed that fluoroquinolones of the more recent generation were sensitive to eighty percent of CoNS.

According to Jhanji et al. [48] of India, the isolating pathogens in a case of keratitis brought about by CoNS seemed to be resistant to moxifloxacin, gatifloxacin, ciprofloxacin, as well as cefazolin in vitro. They were also immune to

moxifloxacin in clinical setups. According to the findings of the present study, gatifloxacin as well as ofloxacin might be recommended as 1st treatments for bacterial keratitis brought forward by Gram-positive microorganisms because they displayed the least rates of resistance amongst fluoroquinolones analysed. As per study by Parmar et al. [45], gatifloxacin might be a favoured substitute for ciprofloxacin as first-line monotherapy in bacterial keratitis. Corneal ulcer recovery rates with gatifloxacin seemed to be considerably greater in diseases prompted by Gram-positive bacteria compared to those precipitated by Gram-negative microbes.

Older fluoroquinolones such as ofloxacin, ciprofloxacin, as well as levofloxacin preferentially impede topoisomerase IV of Gram-positive microbes, whereas more recent fluoroquinolones including moxifloxacin, gatifloxacin, as well as besifloxacin exhibit more evenly aligned suppression of both DNA gyrase as well as topoisomerase IV⁴⁹ ⁵⁰ These most recent fluoroquinolones have greater MIC numbers in organisms as well as greater quantities in ophthalmic tissue than older fluoroquinolones⁴⁹. 8-methoxy fluoroquinolones notably gatifloxacin as well as moxifloxacin have been reported to preserve their effectiveness in the presence of single-step resistance mutations in staphylococcal as well as streptococcal strains⁵⁰ The standard Kirby-Bauer disc diffusion technique of in vitro antibacterial susceptibility testing may not apply to corneal microbes because the antibacterial level achieved in the ophthalmologic tissue via topical regime may be considerably larger than the level achieved in the ophthalmologic tissue via systemic regime. On the basis of conventional in vitro

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antibacterial susceptibility testing, multiple researches have actually identified sensitive as well as resistant trends of corneal diseases [4, 27, 47], as well as these in vitro susceptible as well as resistant trends have successfully directed in vivo treatment by these antibiotics. Such outcomes give clinicians the information they need to decide on the best principal treatment plan for curing the most common corneal diseases.

5. Conclusion

Staphylococcus spp. had been the most frequently discovered bacterial microbes in this case from keratitis patients. With regard to a variety of Gram-positive as well as Gram-negative bacteria, gatifloxacin as well as moxifloxacin both exhibited excellent susceptibility.

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