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Original Research Article

A retrospective study to analyse epidemiological profile of community acquired infectious keratitis in a tertiary care hospital located in Northern Mumbai

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ABSTRACT

Background: Epidemiology of infectious keratitis varies with geographical location. The aim of the study was to review the etiology of infectious keratitis in community that will help in management strategy.
Methods: A retrospective study was conducted at tertiary care hospital from November 2017 to December 2020. Corneal scrapings and corneal button were processed as per microbiological standards. Bacterial identification and antimicrobial sensitivity testing was done by automated Vitek-2 compact system. Significant fungal growth was confirmed by its colony morphology, pigment production and lacto phenol cotton blue (LPCB) mount examination.
Results: Of total 130 patients, culture positivity was 63 (48%), of these 59 (53%) were from corneal scrapping and 4 (21%) from corneal button. The most common bacterial isolates were *Streptococcus pneumoniae* 12 (38%), followed by *Pseudomonas aeruginosa* 6 (19%) and *Staphylococcus aureus* 4 (12%). The most common fungi isolated was *Fusarium spp* 14 (47%) followed by *Aspergillus spp* 9 (30%). One specimen showed *Acanthamoba spp* in wet mount preparation. Major associated factors were injury with vegetative matter 30 (48%) followed by contact lens use 12 (19%) and diabetes mellitus 11 (17%). Antibiotic susceptibility tests showed that all gram-positive cocci were susceptible to vancomycin (100%) and fluoroquinolones (100%) followed by third generation cephalosporins (80%). Whereas all gram-negative bacilli were susceptible to aminoglycosides (100%) followed by fluoroquinolones (90%).
Conclusions: Epidemiology of infectious keratitis vary with geographical location. Understanding local microbiological profile assist in empirical therapy when diagnostic facilities are not readily available.

Keywords: Antimicrobial susceptibility pattern, Bacterial, Corneal scrapping, Fungal, Keratitis, Parasitic

INTRODUCTION

Annually 4.2 million people developing corneal opacities worldwide that can be preventable if managed appropriately with proper diagnostic protocols.[1] Amongst them, infectious keratitis is one of the predominant causes of corneal morbidity.[2] Infectious keratitis can be caused by different groups of bacteria, fungi, viruses and parasites. These pathogens causes corneal damage directly or by release of toxins and enzymes or by activating the host immune system.[3] Many predisposing factors such as contact lens wear, dry eye, trauma, epithelial defect, systemic disease, and immunosuppressant may alter the defence mechanisms of the eye and allow entry of pathogens into cornea causing ulceration.[4] Furthermore, corneal ulceration might progress rapidly and lead to severe visual impairment.[5]

Because of diverse aetiology and overlapping features, clinical diagnosis can often be difficult.[6] As epidemiology and microbiological profile of microbial keratitis varies significantly with the type of patient population,

geographical location and weather. Besides, recent trend of increasing antibiotic resistance among ocular pathogens is a cause for concern, which drastically shifted the management strategy among ophthalmologists to believe on culture-driven treatment with effective formulations.[7]

The objective of this study was to review the prevalence and microbiological profile of keratitis that subsequently provide definitive information for ophthalmologists and policy makers to deal with ocular infections.

METHODS

A retrospective study was conducted at 250 bedded tertiary-care hospital from November 2017 to December 2020 after approval from institutional ethics committee with protocol reference number BMR/01/2020 on dated 20th February 2020. The hospital located in North Mumbai caters approximately 2500 patient's visit in ophthalmology department and has centralized electronic medical and laboratory record databases that use unique identification numbers. Socio-demographic variables, types of specimens and microbiological data of the patients were recorded using existed data entry system in the microbiology laboratory.

Specimen collection

All patients with suspected keratitis and clinically diagnosed suppurative corneal ulcer were included in the study. As per the department protocol, ophthalmologists examine all patients visiting hospital with complaint of corneal ulceration under slit lamp bio microscope. Corneal ulceration was defined as a defect in corneal epithelium with underlying stromal infiltration and suppuration along with signs of inflammation with or without hypopyon.[8]

Patients having very small infiltrates <2 mm, cases of suspected viral keratitis or typical viral keratitis and rescrapping of the same patients for follow up were excluded from the study. Other corneal ulcers excluded were healing ulcers, Mooren's ulcer, marginal keratitis, interstitial keratitis, atheromatous ulcer, neurotrophic ulcer and ulcer associated with systemic or autoimmune diseases. The size of the ulcer recorded in millimeters after staining with fluorescein. Any specific characteristics of ulcer, hypopyon and associated ocular conditions if present were recorded in patient's history sheet. An ophthalmologist under magnification of slit lamp did corneal scraping aseptically. Scrapings were carefully taken from the edge of the ulcer before administration of any antimicrobials using sterile 15 number blade (Bard Parkar) after instillation of topical proparacaine 0.5% solution.[9] After collection, material inoculated on the 5% sheep blood agar and Sabouraud's dextrose agar (SDA) (Biosmart, Mumbai) in multiple C shaped streaks and smeared onto two clean glass slides. One slide used for 10% potassium hydroxide (KOH) mount under direct microscopic evaluation and second slide for gram stain. Corneal buttons and corneal rim collected by ophthalmologist in operation theatres were directly inoculated on 5% sheep blood agar. All smears along with agar plates were kept in sterile box with tightly fitting lid and dispatched from collection area to the microbiology laboratory for further processing.

Diagnostic procedure

Standard microbiological procedures and interpretative criteria were employed for processing the corneal specimens.[10] Inoculated sheep blood agar was incubated aerobically at 37ºC for 24-48 hrs. Screening of agar plates were conducted at 24 hr and 48 hr.

Pure bacterial growth on plates were sub cultured and gram staining performed for morphology. Further bacterial identification and antibiotic susceptibility testing were done by Vitek-2 compact automated system (BioMerieux, Mary l'Etoile, France), interpretation was done according to the clinical and laboratory standards institute (CLSI) guidelines.[11] For suspected fungal keratitis, the scrapings were inoculated on Sabouraud dextrose agar (SDA) and incubated for 21 days at room temperature. Inoculated plates were examined every alternate days and declared as fungal culture negative thereafter. Fungal identification was done based on their colony morphology on obverse, pigment production on reverse and by observing lactophenol cotton blue stain microscopically. If amoebic cysts identified in wet mount, remaining material was inoculated onto non-nutrient agar overlaid with *Escherichia coli* and kept for incubation at 37ºC for 7 days. All microbiological data along with demographic profile obtained were maintained and analyzed using SPSS statistical software.

RESULTS

Out of total one hundred and thirty patients included in this study, 111 (85%) were corneal scrapings and 19 (17%) were corneal button. As per the gender distribution, 85 (65%) were males and 45 (35%) were females. Total culture positivity was 63 (48%), of these 59 (53%) were from corneal scrapping and 4 (21%) from corneal button. Amongst 63 culture positive specimens, 32 (51%) were bacterial and 30 (48%) were fungal isolates (Table 1). The most common bacterial isolates were *Streptococcus pneumoniae* 12 (38%), followed by *Pseudomonas aeruginosa* 6 (19%) and *Staphylococcus aureus* 4 (12%) (Table 2).

The most common fungi isolated was *Fusarium spp* 14 (47%) followed by *Aspergillus spp* 9 (30%) (Table 3). One specimen showed cysts of *Acanthamoeba* spp in wet mount preparation. Major associated factors were injury with vegetative matter 30 (48%) followed by contact lens use 12 (19%), diabetes mellitus 11 (17%) and injury with inorganic matter 7 (11%) (Table 4). Antibiotic susceptibility tests showed that all gram positive cocci were susceptible to vancomycin (100%) and fluoroquinolones (100%) followed by third generation

cephalosporins (80%). Whereas all gram negative bacilli were susceptible to aminoglycosides (100%) followed by fluoroquinolones (90%).

Table 1: Distribution of specimens based on rate of positivity in microbiological culture.

Type of growth (N=63)	Positivity rate (%)
Bacterial	32 (50.79)
Gram positive cocci	18 (56.25)
Gram negative bacilli	10 (31.25)
Gram positive bacilli	4 (12.5)
Fungal	30 (47.62)

Table 2: Percentage of microbial keratitis with bacterial etiology.

Isolates (N=32)	Positivity rate (%)
MSSA	3 (9)
MRSA	1 (3)
Staphylococcus epidermidis	2 (6)
S.pneumoniae	12 (38)
Pseudomonas aeruginosa	6 (19)
Diphtheroids	1 (3)
Lactococcus graviae	1 (3)
Elizabethkingia meningoseptica	1 (3)
Brevundimonas vesicularis	1 (3)
GNB	2 (6)
Corynebacterium mackinglei	1 (3)
GPB	1 (3)

Note: MSSA: Methicillin sensitive *Staphylococcus aureus*; MRSA: Methicillin resistant; GNB: Gram negative bacilli; GPB: Gram positive bacilli.

Table 3: Percentage of microbial keratitis with fungal isolates.

Isolates (N=30)	Positivity rate (%)
Fusarium spp	14 (47)
Aspergillus spp	9 (30)
Exophiala spp	2 (7)
Exserohilum rostratum	1 (3)
Curvularia spp	1 (3)
Scedosporium apiospermum	1 (3)
Trichophyton spp	1 (3)
Yeast cells	1 (3)

Table 4: Distribution of associated risk factors causing infectious keratitis.

Risk factors (N=30)	Rate (%)
Injury with vegetative matter	30 (48)
Contact lens use	12 (19)
Diabetes mellitus	11 (17)
Injury with inorganic matter	7 (11)
Corticosteroid therapy	3 (5)

DISCUSSION

Infectious keratitis is one of the leading cause of corneal blindness in developing countries.[12] In recent years, working adults and younger age groups using contact lenses and involved in recreational activities are developing infectious keratitis more.[13]

In the present study, rate of infectious keratitis were more in male patients (65%) as compared to female patients (35%). Similar findings observed in Bakshi et al and Saha et al study that concluded higher prevalence in males as compared to females.[14,15] Reason might be related to males being involved in more outdoor activities. While Yusuf et al and Cao et al found higher prevalence in females as compared to males.[13,16] This could be due to higher engagement of women particularly in the agricultural sector.

Considering the most common associated risk factors, present study observed that injury with vegetative matter 30 (48%) was commonest followed by contact lens use 12 (19%), diabetes mellitus 11 (17%) and injury with inorganic matter 7 (11%). Assudani et al reported similar observations, most common associated factors were trauma (44.45%) followed by diabetes mellitus (29.63%), contact lens (14.82%) and steroid (3.70%).[12] Similarly, Ranjini et al reported trauma in 54 (46%) followed by diabetes mellitus in 31 (26.5%), contact lens usage in 22 (19%) and corticosteroid therapy in 4 (3.5%) eyes.[17] Krishna et al and Bakshi et al both have reported ocular trauma as most common associated factor.[14,18] While Yusuf et al and Schafer et al have reported contact lens usage as most common associated factor.[13,19]

Microbiological profile showed that fungi are the most common etiological agents that account for 30-40% whereas bacteria account for 13-48% of all cases of suppurative keratitis, though etiology varies by geographical area.[20] In present study, out of 130 cases, 63 (48%) were culture positive, 32 (51%) were bacterial and 30 (48%) were fungal isolates. Ranjini et al reported 52 (44.5%) bacterial, 58 (49.5%) fungal and 7 (6%) mixed bacterial and fungal infection out of 117 positive cases.[17] Assudani et al noted that prevalence of microbial keratitis was 27%, among these 13% were bacterial and 14% were fungal origin.[12] While Srinivasan et al isolated equal numbers of bacterial (47.1%) and fungal (46.8%) agents causing infectious keratitis with 5.1% cases having mixed infections.[8] Bharati et al, Narsari et al, and Renato et al reported higher rates of microbial keratitis with prevalence varied from 22-71%.[21-13] On the other hand, Katara et al also reported culture positivity of 40%, of which 26% were fungal isolates and the remaining 14% of samples had bacterial etiology.[24] This diversity in prevalence of microbial keratitis might be due to variation in geographical area and seasonal differences throughout the year. In present study, most common fungi isolated was *Fusarium spp* 14 (47%) followed by *Aspergillus spp* 9 (30%). Similarly, Ranjini et al, Alkatan et al and Idiculla

et al reported that filamentous fungi were more common compared to yeast and *Fusarium spp*.[17,25,26] was the most isolated species followed by *Aspergillus* spp. In contrast, Lack et al observed a higher incidence of *Aspergillus spp*.[20] Such differences in the isolation rates of these fungal pathogens can be due to differences in the climate and the natural environment of individual regions. Dry and hot weather conditions showed higher incidence of mycotic keratitis due to *Aspergillus spp* because their spores can tolerate tropical climate. *Fusarium spp* are common plant pathogens and are mostly found in soil.[20] Furthermore, indiscriminate usage of broad-spectrum antibiotics and corticosteroids also contributes to increasing trend of fungal keratitis.

Present study showed higher prevalence of bacterial keratitis, which was similar to other Indian studies done by Gopinathan et al and Das et al.[3,6] The most common bacterial isolate was *Streptococcus pneumoniae* 12 (38%), followed by *Pseudomonas aeruginosa* 6 (19%) and *Staphylococcus aureus* 4 (12%). Narsani et al also showed higher isolation of gram-positive organisms with *Staphylococcus aureus* being the commonest.[27] Reason might be the variation in climates as gram positive bacterial species are more frequently recovered in temperate zones and gram-negative species in tropical climates. As per the study conducted by Forbes et al 65-90% of corneal infections are having bacterial etiology with *Staphylococcus aureus*, *Streptococcus pneumonia* and *Pseudomonas aeruginosa* accounting for more than 80%.[28] While Assudani et al, Yusuf et al and Bharati et al have reported *Pseudomonas spp* as more common bacteria than *Staphylococcus aureus*.[12,13,21] Some studies showed coagulase-negative *Staphylococci* as the most common isolate. Schafer et al and Bharati et al have reported CONS affecting 40% and 18.39% respectively.[19,21]

Compared to bacterial and fungal etiology, the prevalence of parasitic keratitis is quite low. However, many case reports have being published so far. In present study, one case of *Acanthamoeba keratitis* reported based on wet mount preparation that showed cysts of *Acanthamoeba spp*. The scrapping material was inoculated onto non-nutrient agar overlaid with *Escherichia coli* and kept for incubation at 37ºC for 7 days but culture result was negative.

Usually, medical therapy consists of non-specific measures and the use of specific topical antimicrobial agents. As there are no standard guidelines for interpretation of topical ocular antibiotics, antibiotic sensitivity pattern along with clinical improvement is required to assess the efficacy of a particular antibiotic.

In present study, antibiotic susceptibility tests showed that all gram positive cocci were susceptible to vancomycin (100%) and fluoroquinolones (100%) followed by third generation cephalosporins (80%). Whereas all gram negative bacilli were susceptible to aminoglycosides (100%) followed by fluoroquinolones (90%).

Limitation

Present study had a limitation that only aerobic bacterial and fungal agents were identified and did not include anaerobic bacterial, amoebic and viral agents causing keratitis. The main reasons were cost and inadequate feasibility of maintaining anaerobic or viral cultures. Secondly, antimicrobial susceptibility pattern of commonest organisms were based on few isolates. Therefore, complete analysis regarding the microbial profile and their susceptibility pattern was not possible. Further studies inclusive of all pathogens would give a comprehensive picture of infectious keratitis in our region.

CONCLUSION

Epidemiology of corneal ulceration vary with geographical locations and patient population. Hence, routine microbiological pattern and their antimicrobial susceptibility will provide definitive information for ophthalmologists and policy makers to initiate appropriate empirical therapy of community acquired infectious keratitis.

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