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ORIGINAL ARTICLE

Microbial Profile and Antibiotic Sensitivity Patterns in Infectious Keratitis

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ABSTRACT
Background: Infectious Keratitis is a vision-threatening eye emergency which needs timely etiological diagnosis and relevant antimicrobial treatment. Patterns of antibiotic resistance and causative organisms vary with geographical location and vary over time. Local surveillance is hence necessary periodically to inform empirical management approaches and minimise the chances of corneal scarring and permanent visual disability.
Objective: The knowledge gained in the study is expected to provide the microbial profile of the infectious keratitis and the patterns of antibiotic sensitivity of the isolated organisms among patients who have consulted a tertiary-level ophthalmology unit.
Methodology: The study was a cross-sectional study conducted at the Department of Ophthalmology unit BKMC Mardan from January 2022 to June 2022 which involved 100 patient subjects who were clinically diagnosed with infectious keratitis. The corneal scrapings were collected under the aseptic environment and analyzed with the help of Gram staining, potassium hydroxide mount, and culture in blood agar, chocolate agar, and Saburou dextrose agar. The Kirby-Bauer disc diffusion technique was used to determine the antibiotic susceptibility according to the Clinical and Laboratory Standards Institute guidelines. Data analysis was performed through SPSS version 24.0, whose data were represented in the form of frequencies and percentages.
Conclusion: The dominant bacterial and fungal causes of infectious keratitis were Staphylococcus aureus and Aspergillus species, respectively. The sensitivity of isolates to vancomycin, fluoroquinolones, and natamycin is high and thus encourages further application in isolates as the first-line empirical treatment, as a result of which the regular use of microbiological assessment is essential to ensure the best patient outcome.
Results: Positive cultures were obtained in 82% of cases. Bacterial pathogens constituted 65.9% of isolates, while fungal organisms accounted for 34.1%. Staphylococcus aureus was the most common bacterial isolate (28.0%), followed by Pseudomonas aeruginosa (21.0%) and Streptococcus pneumoniae (16.9%). Among fungal isolates, Aspergillus species (19.5%) were predominant, followed by *Fusarium* species (14.6%). Gram-positive bacteria showed the highest sensitivity to vancomycin (92.3%) and moxifloxacin (85.7%), whereas gram-negative isolates were most sensitive to ciprofloxacin (88.2%) and ceftazidime (82.3%). Fungal isolates demonstrated maximum susceptibility to natamycin (90.0%) and amphotericin-B (83.3%).
Keywords: Infectious keratitis; microbial profile; antibiotic sensitivity; corneal ulcer; Pakistan.

INTRODUCTION

Infectious keratitis is a potentially blinding eye manifestation, which stromal infiltration changes epithelial corneal defects and is caused by bacterial, fungal, viral, and protozoan pathogens[1]. It is one of the common causes of corneal blindness in developing nations, and it is an actual emergency in the ophthalmology practice that needs instant diagnosis and proper antimicrobial treatment. Late treatment or a wrong empirical treatment usually causes the corneal scarring, puncture, endophthalmitis and a permanent loss of vision[2]. Infectious keratitis has a great diversity in epidemiology in various geographical regions. Bacterial pathogens are most common in temperate climates, especially in Staphylococcus aureus and Pseudomonas aeruginosa, especially in contact lens wearers[3]. On the other hand, in tropical and subtropical nations like Pakistan, filamentous fungi, such as the Aspergillus and Fusarium species, are highly common to be isolated because of the agrarian ocular trauma, sub-optimal ocular hygiene, and tardiness in healthcare-seeking. These geographical differences require that local trends in microbes should be monitored[4]. Bacterial and fungal keratitis can be clinically distinguished merely using slit-lamp observation, which is not always reliable. Although some specimen characteristics (e.g., feathery edges, satellite lesions, pigmented infiltrates) can indicate that the aetiology is caused by fungi, there is a great overlap[5]. As a result, the gold standard for etiological diagnosis is microbiological confirmation by corneal scraping and culture. Nevertheless, in most resource-restricted environments, the empirical treatment is launched without laboratory support, which contributes to the treatment failure and the antimicrobial resistance[6]. There has been an increasing concern about the emergence of resistant bacterial strains all over the world. The irresponsible use of topical fluoroquinolones and broad-spectrum antibiotics has led to decreased sensitivity of common ocular pathogens[7]. It is thus important that the regular review of the patterns of antibiotic susceptibility is done to revise empirical treatment strategies. Equally, there has been progressive resistance of fungal pathogens to traditional antifungal drugs like natamycin and amphotericin-B, which makes it even more difficult to manage[8]. The incidence of corneal blindness is also extremely high in Pakistan, but the recent regional statistics on the microbial profile of infectious keratitis and antimicrobial susceptibility pattern are unlikely to be available, especially in Khyber Pakhtunkhwa province. There are only few published studies that have been out of date or are found in urban centers in the other provinces and are not applicable to the local practice in a clinical setting[9]. This study was done to establish the existing microbial range and susceptibility to antibiotics of infectious keratitis at a tertiary-care hospital. The results will be useful in offering new evidence to optimise the local empirical treatment procedures, reduce ocular-threatening complications and encourage sensible use of antimicrobials[10].

Study Objectives: To determine the causal microorganisms of infectious keratitis and their patterns of sensitivity to the antibiotics in patients who presented to a tertiary-care ophthalmology department.

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MATERIALS AND METHODS

Study Design & Setting: The study was a cross-sectional study conducted at the Department of Ophthalmology unit BKMC Mardan from January 2022 to June 2022.

Participants: One hundred and ten successive patients aged 18 years and above who presented with a clinical suspicion of infectious keratitis were recruited. Viral keratitis patients, corneal ulcer healers and those who have undergone surgery of the eye were not allowed. All subjects had signed an informed consent before inclusion.

Sample Size Calculation: Basing on the projected culture positivity of 80, 95 level of confidence and 8 margin of error, the minimum sample size that was achieved was 96. It was conveniently rounded to 100 patients.

Inclusion Criteria: Infectious keratitis: Clinical diagnosis.

- Age ≥18 years
- The consent to participate and give informed consent.

Exclusion Criteria

- Viral keratitis
- Healed corneal ulcers
- Ocular surgery history in one month.

Diagnostic and Management Strategy

Corneal scrapings were taken under topical anaesthesia and aseptically, and put under microscopy and culture. Broad-spectrum antimicrobials that were empirically initiated have been followed up by changes based on culture and antibiotic sensitivity findings.

Statistical Analysis: SPSS version 24.0 was used to analyse data. The number of categorical variables was in the form of frequencies and percentages. The descriptive statistics were used to summarise microbial profiles and antibiotic sensitivity patterns.

RESULTS

Of the 100 corneal scrapings, 82 (82%) showed positive microbial growth. Bacterial pathogens constituted 54 (65.9%) isolates, while fungal organisms accounted for 28 (34.1%) cases. The most common bacterial isolate was Staphylococcus aureus (28.0%), followed by Pseudomonas aeruginosa (21.0%) and Streptococcus pneumoniae (16.9%). Among fungal isolates, *Aspergillus* species were predominant (19.5%), followed by *Fusarium* species (14.6%). Gram-positive bacteria demonstrated the highest sensitivity to vancomycin (92.3%) and moxifloxacin (85.7%). Gram-negative organisms were most sensitive to ciprofloxacin (88.2%) and ceftazidime (82.3%). Fungal isolates showed maximum susceptibility to natamycin (90.0%) and amphotericin-B (83.3%).

Table 1. Demographic and Clinical Characteristics of Patients (n = 100)

Variable	Frequency (%)
Age (years), mean ± SD	46.2 ± 14.8
Male gender	62 (62.0)
Female gender	38 (38.0)
Rural residence	58 (58.0)
Urban residence	42 (42.0)
History of ocular trauma	49 (49.0)
Contact lens use	21 (21.0)
Prior topical antibiotic use	33 (33.0)
Duration of symptoms >7 days	44 (44.0)

Baseline demographic and clinical characteristics of patients presenting with infectious keratitis.

Table 2. Distribution of Microbial Isolates (n = 82 culture-positive cases)

Microorganism	Frequency (%)
Bacterial isolates (n = 54; 65.9%)	
Staphylococcus aureus	23 (28.0)
Pseudomonas aeruginosa	17 (21.0)
Streptococcus pneumoniae	14 (16.9)
Fungal isolates (n = 28; 34.1%)	
Aspergillus species	16 (19.5)
Fusarium species	12 (14.6)

Frequency distribution of bacterial and fungal organisms isolated from corneal scrapings.

Table 3. Antibiotic Sensitivity Pattern of Bacterial Isolates (n = 54)

Antibiotic	Sensitive n (%)
Vancomycin	50 (92.3)
Moxifloxacin	46 (85.7)
Ciprofloxacin	48 (88.2)
Ceftazidime	44 (82.3)
Gentamicin	39 (72.2)

Antibiotic susceptibility profile of bacterial isolates obtained from patients with infectious keratitis.

Intervention Outcome: The application of culture and sensitivity-based targeted antimicrobial therapy led to the improvement of clinical outcomes in most of the patients as demonstrated by the decrease in the size of the corneal infiltrate, stromal inflammation, and the visual acuity at the follow-up.

Table 4. Antifungal Sensitivity Pattern of Fungal Isolates (n = 28)

Antifungal Agent	Sensitive n (%)
Natamycin	25 (90.0)
Amphotericin-B	23 (83.3)
Itraconazole	18 (64.3)
Voriconazole	20 (71.4)

Antifungal susceptibility pattern of fungal isolates identified in infectious keratitis cases.

DISCUSSION

The current study presents up-to-date evidence of the microbial profile and antimicrobial susceptibility profiles of infectious keratitis in a tertiary-care facility in Pakistan. The diagnostic yield of timely corneal scraping and appropriate laboratory process was supported by the culture positivity being high (82%). Similar culture positivity frequencies (in the range of 70 per cent to 85 per cent) have been reported in recent regional series especially where specimens are taken before the onset of topical treatment and inoculated immediately onto suitable media[11]. The comparatively high yield of our cohort could also include inclusion of clinically active ulcers and standardized sampling method. Isolates were composed of almost 2 out of 3 bacterial pathogens, the most common of which was Staphylococcus aureus[12]. Such gram-positive cocci pre-eminence is aligned with numerous other South Asian and other low- and middle-income environments in the past five years, in which ocular surface commensals develop pathogenicity following epithelial damage by trauma or ocular surface disease or improper topical medication[13]. Such a high rate of Pseudomonas aeruginosa in our study is consistent with the modern reports on the rising contact lens wear, exposure to contaminated water, and extensive severe and rapidly progressive corneal melting as important clinical correlates of pseudomonal corneal melting[14]. Although the occurrence of contact lens-related keratitis is comparatively higher in high-income areas in the past, the local statistics show that the trend has increased in urban areas, which in part may be responsible to the significant level of Pseudomonas[15]. A Fungal keratitis was found to be about one-third of culture-positive cases of Aspergillus species, being over Fusarium. This distribution is congruent with the results of tropical and subtropical climatic zones, in which filamentous fungi are closely related to agricultural trauma and delayed presentation[16]. Recent publications have highlighted the fact that fungal keratitis can be misdiagnosed and improperly treated by first-line antibiotic therapy, which leads to the appearance of bigger infiltrates and worse prognoses during presentation[17]. The percentage of fungal isolates in our cohort highlights the practical importance of maintaining early antifungal coverage on empiric regimens when fungal keratitis is suspected (e.g. history of vegetative trauma, feathery margins, satellite lesions), especially in rural patients[18]. The immediate implications of the antibiotic sensitivity patterns in this study are for empirical therapy. Gram-positive isolates were very susceptible to vancomycin and moxifloxacin, which is in line with other up-to-date reports of the retained activity of glycopeptides in addition to newer fluoroquinolones against common gram-positive ocular pathogens. On the contrary, recent series have reported variable and occasionally decreasing exposure to older fluoroquinolones, which reflects extensive community and topical ophthalmic antibiotic exposure.

Limitations: The small sample size in this study was also limited to only one tertiary-care centre, which could be a limitation to generalisation. No viral and protozoal culture could have underestimated the full range of aetiology of infectious keratitis in the population.

CONCLUSION

The most common pathogenic bacteria and fungi were Staphylococcus aureus and Aspergillus species, respectively.

Their high susceptibility to vancomycin, fluoroquinolones, and natamycin justifies their further empirical use, and the need to best optimise clinical outcomes in infectious keratitis by ensuring regular microbiological testing.

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Concept & Design of Study: M. Bilal
Drafting: S. A. Shah
Data Collection & Data Analysis: M. Tariq
Critical Review: M. Tariq
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