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Original Research Article

LABORATORY DIAGNOSIS, ETIOLOGICAL AGENTS, AND MICROBIOLOGICAL PROFILE OF OCULAR INFECTIONS WITH SPECIAL EMPHASIS ON KERATOMYCOSIS

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ABSTRACT

Background: Ocular infections are important causes of ocular morbidity and visual impairment, with considerable geographic variation in their microbiological spectrum and antimicrobial susceptibility. Local microbiological surveillance is therefore essential for timely diagnosis and appropriate therapy. **Objective:** To determine the laboratory diagnosis, etiological agents and microbiological profile of ocular infections, with special emphasis on keratomycosis, and to assess the antimicrobial susceptibility pattern of bacterial isolates.

Materials and Methods: A hospital-based observational study was conducted at a tertiary ophthalmic referral centre in western India from July 2018 to November 2020. A total of 400 patients with clinically suspected ocular infections were included. Clinical specimens were subjected to direct microscopy, including Gram staining and 10% potassium hydroxide (KOH) preparation, bacterial and fungal culture, organism identification and antimicrobial susceptibility testing using the Kirby–Bauer disc diffusion method according to CLSI guidelines.

Results: Of 400 patients, 65.5% were male, 57.5% were from rural areas and ocular trauma was the most common predisposing factor (48.25%). Culture positivity was observed in 250 (62.5%) specimens. Bacterial and fungal isolates accounted for 183 (45.75%) and 63 (15.75%) cases, respectively. *Pseudomonas aeruginosa* was the predominant bacterial isolate (42.08%), followed by *Escherichia coli* (19.67%), while *Aspergillus* spp. was the most common fungal isolate (63.49%), followed by *Fusarium* spp. (26.98%). Among 63 culture-positive fungal cases, 44 (69.84%) were KOH positive, with an additional eight KOH-positive/culture-negative cases. *P. aeruginosa* showed high susceptibility to amikacin (93.5%), gentamicin (94.8%), ciprofloxacin (89.6%), imipenem and meropenem (100% each).

Conclusion: Bacteria, particularly *P. aeruginosa*, predominated among ocular infections, while *Aspergillus* spp. was the leading fungal pathogen. Combined microscopy and culture, supported by local antimicrobial susceptibility surveillance, remains important for guiding effective therapy.

Keywords: Ocular infection; keratomycosis; *Pseudomonas aeruginosa*; *Aspergillus*; KOH microscopy; antimicrobial susceptibility.

INTRODUCTION

Ocular infections remain an important cause of ocular morbidity and preventable visual impairment worldwide. They encompass a broad spectrum of infections affecting the cornea, conjunctiva, lacrimal apparatus, and intraocular tissues, with bacterial, fungal, viral, and parasitic pathogens contributing to variable clinical presentations.[1,2] Delayed diagnosis or inappropriate antimicrobial therapy may result in corneal scarring, endophthalmitis, irreversible vision loss, and, in severe cases, loss of the eye. Rapid laboratory confirmation of the causative organism therefore plays a pivotal role in guiding appropriate management and improving visual outcomes.[2.3]

The microbiological profile of ocular infections varies considerably according to geographical location, climatic conditions, occupational exposure, socioeconomic factors, and local antimicrobial usage patterns.[4,5] In tropical and subtropical regions such as India, corneal trauma with vegetative matter and agricultural exposure predispose individuals to fungal keratitis, making filamentous fungi important ocular pathogens.[6,7] However, bacterial infections continue to account for a substantial proportion of ocular infections, highlighting the need for comprehensive microbiological evaluation to differentiate between bacterial and fungal etiologies and facilitate targeted therapy.[8,9]

Specific therapy of ocular infection often requires etiological diagnosis that is a combined effect of observation of characteristic clinical features and microbiological investigations. Clinical impression is central to guiding the laboratory investigation, and the aim of laboratory investigation is to confirm or rule out the clinical diagnosis.[9,10,11]

The specific identification of bacterial pathogens was based on staining characteristics, microscopic morphology, culture and biochemical properties using standard laboratory criteria. Fungi was identified by direct microscopic examination using potassium hydroxide (KOH) preparation, their colony characteristics on SDA and by the morphological appearance of the spores in lactophenol cotton blue stain.[10,11,12] Antimicrobial susceptibility testing further assists in selecting appropriate antimicrobial agents and monitoring emerging resistance patterns among bacterial pathogens.[13,14]

Although the microbiological profile of ocular infections has been reported from various parts of India, significant regional variation exists in the spectrum of pathogens and their antimicrobial susceptibility. Data from western India, particularly on laboratory diagnosis with special emphasis on keratomycosis, remain limited. Therefore, this study was undertaken to evaluate the laboratory diagnosis, etiological agents, microbiological profile of ocular infections, and antimicrobial susceptibility patterns of bacterial isolates at a tertiary care ophthalmic centre in western India.

MATERIALS AND METHODS

Study design, setting and period

This hospital-based observational study was conducted at the Western Regional Ophthalmic Institute, a tertiary care teaching hospital in western India, between July 2018 and November 2020. The study aimed to determine the laboratory diagnosis, etiological agents, and microbiological profile of ocular infections, with particular emphasis on keratomycosis. Consecutive patients presenting with clinically suspected ocular infections during the study period were enrolled according to predefined eligibility criteria.

Study participants

Patients presenting with clinically diagnosed ocular infections, including keratitis, conjunctivitis, blepharitis, dacryocystitis, uveitis, endophthalmitis, orbital cellulitis, and other ocular infections, were considered eligible. Patients with corneal ulcers showing epithelial defects with stromal inflammation and those with evidence of filamentous fungi on direct smear (10% potassium hydroxide [KOH], Gram stain, or Giemsa stain) were also included where applicable. Patients with a history of penetrating keratoplasty in the affected eye or those with no light perception were excluded. A total of 400 patients fulfilling the eligibility criteria were included in the final analysis after written informed consent was obtained.

Sample size and sampling technique

A total of 400 patients were included during the study period using a consecutive sampling technique. No formal sample size calculation was documented in the study protocol.

Study tool and data collection

Clinical and demographic information was recorded using a standardized predesigned proforma that included sociodemographic characteristics, duration of symptoms, predisposing ocular conditions, associated systemic risk factors, and relevant clinical findings. All patients underwent ophthalmic examination, including assessment of visual acuity and slit-lamp biomicroscopy performed by an ophthalmologist. Ocular specimens were collected under strict aseptic precautions according to the site of infection. Corneal scrapings were obtained from the leading edge and base of corneal ulcers using a sterile Bard-Parker blade(No.15) after topical anesthesia with preservative-free 2% lignocaine hydrochloride. Conjunctival swabs, aqueous humour, vitreous humour, contact lens and corneal rim, corneal button specimens were collected whenever clinically indicated.

Specimens were subjected to direct microscopic examination using gram stain and 10% KOH wet mount preparation. Culture was performed on nutrient agar, blood agar, MacConkey agar and

incubated for 48 hours at 37 ºC in the incubator. Bacterial isolates were identified by colony morphology, Gram staining characteristics, motility, and standard biochemical reactions. For isolation of fungi the material was directly inoculated into the Sabouraud's Dextrose Agar (SDA) making multiple 'C' or 'S' shape streak. Fungal Cultures were incubated at room temperature and 37 ºC over a period of four weeks. Fungi were identified by their microscopy appearance in KOH mount preparation, colony characteristics on SDA and by the morphological appearance of the spores in lactophenol cotton blue stain, and in some cases by slide culture method. Antimicrobial susceptibility testing of bacterial isolates was performed using the modified Kirby–Bauer disk diffusion method on Mueller–Hinton agar, and results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Additional special tests, including detection of methicillin-resistant *Staphylococcus aureus* (MRSA), inducible clindamycin resistance (D-test), and extended-spectrum β-lactamase (ESBL) production, were performed when indicated. Internal quality control procedures were maintained using standard American Type Culture Collection (ATCC) reference strains.

Study variables and outcome measures

The independent variables included age, sex, clinical diagnosis, type of ocular specimen, duration of symptoms, predisposing ocular conditions, and associated systemic risk factors. The primary outcome was the laboratory-confirmed microbiological diagnosis of ocular infection, including identification of bacterial and fungal etiological agents. Secondary outcomes included the distribution of microbial isolates, direct microscopy findings, culture positivity, and antimicrobial susceptibility patterns of bacterial isolates. Keratomycosis was analysed as a major subgroup with emphasis on the spectrum of fungal pathogens identified by direct microscopy and culture.

Ethical considerations

Written informed consent was obtained from all participants before enrolment. Patient information was collected using a standardized proforma, and confidentiality of study data was maintained throughout the study.

Statistical Analysis

Data were compiled and analysed using Epi Info CDC 7. Categorical variables were summarized as frequencies and percentages. The statistical methods applied corresponded to the predefined study objectives and reported outcome measures, and statistical significance was determined using a two-sided *P* value of <0.05.

RESULTS

Table 1 shows that ocular infections were most frequent among patients aged 41–60 years (37.25%), with a male predominance (65.5%) and greater representation from rural areas (57.5%); ocular trauma was the leading predisposing factor (48.25%), and corneal scraping constituted the commonest specimen (52.5%).

Table 1: Clinico-demographic characteristics of patients with ocular infections (N=400)

Characteristic	n	%
Age (years)		
• 0–20	45	11.25
• 21–40	110	27.50
• 41–60	149	37.25
• 61–80	96	24.00
Sex		
• Male	262	65.50
• Female	138	34.50
Residence		
• Rural	230	57.50
• Urban	170	42.50
Major predisposing factors		
• Ocular trauma	193	48.25
• Pre-existing ocular disease	50	12.50
• Contact lens use	23	5.75
• Ocular surgery	21	5.25
• Diabetes mellitus	20	5.00
• Corticosteroid therapy	2	0.50
• HIV infection	2	0.50
• No predisposing factor	89	22.25
Clinical specimen		
• Corneal scraping	210	52.50
• Conjunctival swab	63	15.75
• Contact lens	39	9.75
• Corneal rim	29	7.25
• Anterior chamber tap	22	5.50
• Vitreous tap	20	5.00
• Bandage contact lens	11	2.75

• Corneal button	6	1.50

Table 2 demonstrates an overall culture positivity of 62.5%, with bacterial isolates (45.75%) exceeding fungal isolates (15.75%); Pseudomonas aeruginosa was the predominant bacterial pathogen (42.08%), whereas Aspergillus spp. was the leading fungal isolate (63.49%), followed by Fusarium spp. (26.98%).

Table 2: Laboratory diagnosis and microbiological profile of ocular infections

Laboratory diagnosis	**n**	**%**
• Culture positive	250	62.50
• Culture negative	150	37.50
Microbiological diagnosis		
• Bacterial isolates	183	45.75
• Fungal isolates	63	15.75
• Parasitic isolates	4	1.00
Bacterial isolates (n=183)	**n**	**%**
• *Pseudomonas aeruginosa*	77	42.08
• *Escherichia coli*	36	19.67
• *Staphylococcus epidermidis*	26	14.21
• *Staphylococcus aureus*	20	10.93
• *Klebsiella pneumoniae*	17	9.29
• *Streptococcus pneumoniae*	2	1.09
• *Bacillus cereus*	2	1.09
• *Proteus mirabilis*	1	0.55
• *Actinomycetes* spp.	1	0.55
• *Chlamydia trachomatis*	1	0.55
Fungal isolates (n=63)	**n**	**%**
• *Aspergillus* spp.	40	63.49
• *Fusarium* spp.	17	26.98
• *Curvularia* spp.	4	6.35
• *Candida albicans*	2	3.17

Table 3: Comparison of KOH microscopy with fungal culture among cases with laboratory evidence of fungal infection (n=71)

KOH preparation	**Culture positive n (%)**	**Culture negative n (%)**	**Total n (%)**
KOH positive	44 (61.97)	8 (11.27)	52 (73.24)
KOH negative	19 (26.76)	0 (0.00)	19 (26.76)
Total	**63 (88.73)**	**8 (11.27)**	**71 (100.00)**

Table 3 shows that among 71 cases with laboratory evidence of fungal infection, 63 (88.73%) were culture positive and 52 (73.24%) were KOH positive; 44 cases were positive by both methods, while eight were KOH positive despite negative culture.

Table 4: Antimicrobial susceptibility pattern of major bacterial isolates

Antimicrobial agent	***P. aeruginosa* (n=77)**	***E. coli* (n=36)**	***K. pneumoniae* (n=17)**	***S. aureus* (n=20)**	***S. epidermidis* (n=26)**
Amikacin	72 (93.5)	36 (100.0)	17 (100.0)	—	—
Gentamicin	73 (94.8)	36 (100.0)	17 (100.0)	20 (100.0)	26 (100.0)
Ciprofloxacin	69 (89.6)	34 (94.4)	7 (41.2)	11 (55.0)	15 (57.7)
Imipenem	77 (100.0)	36 (100.0)	17 (100.0)	—	—
Meropenem	77 (100.0)	36 (100.0)	17 (100.0)	—	—
Vancomycin	—	—	—	20 (100.0)	26 (100.0)
Linezolid	—	—	—	20 (100.0)	26 (100.0)

Values are presented as n (% susceptible). "—" indicates that the antimicrobial was not part of the relevant organism-specific panel reported in the thesis.

Methicillin-resistant *S. aureus*: 2/20 (10.0%); methicillin-resistant *S. epidermidis*: 7/26 (26.9%).

Table 4 indicates high antimicrobial susceptibility of *P. aeruginosa* to gentamicin (94.8%), amikacin (93.5%) and ciprofloxacin (89.6%), with complete susceptibility to imipenem and meropenem; all *S. aureus* and *S. epidermidis* isolates were susceptible to vancomycin and linezolid, while methicillin resistance was observed in 10.0% and 26.9% of these isolates, respectively.

DISCUSSION

The present study demonstrated that ocular infections predominantly affected individuals in the 41–60-year age group (37.25%), with male predominance (65.5%) and greater representation from rural areas (57.5%). This demographic profile

is comparable with Das et al,[8] who reported 68.1% males in a large Indian ophthalmology network, and with Ranjini et al,[15] in whom males constituted 61.5% and the 41–60-year age group was most frequently affected. Similar male predominance was also observed by Deorukhkar et al,[16] (68.31%), Kumar et al,[17] (61.5%), Kumari et al,[18] (70.96%), and Sunov et al,[19] (66%). Ocular trauma was the commonest predisposing factor in the present study (48.25%). This was close to the 46% reported by Ranjini et al,[15] but lower than the 58.06% reported by Kumari et al,[18] 60.15% by Deorukhkar et al,[16] 78.5% by Kumar et al,[17] and 90% by Tewari et al.20 The lower proportion in the present study may partly be related to inclusion of a broader spectrum of ocular infections, whereas several comparison studies were restricted to corneal ulcers or keratomycosis.

The overall culture positivity in the present study was 62.5%, closely resembling the 63.02% reported by Deorukhkar et al,[16] and 59.3% reported by Tewari et al.[15] In comparison, previous studies,[17,19,21] reported culture positivity from 37 to 56%. Bacterial isolates constituted 45.75% of all specimens and were more frequent than fungal isolates (15.75%). A similar bacterial predominance was reported by Tewari et al,[20] with bacteria constituting 65.1% and fungi 34.9% of positive isolates, and by Das et al,[8] who reported bacterial and fungal etiologies in 51.06% and 38.27%, respectively. In contrast, Deorukhkar et al,[16] reported fungal isolates more frequently than bacterial isolates (57.91% versus 42.08%), while Ranjini et al,[15] also observed a slight predominance of fungi over bacteria, highlighting the regional and clinical variation in ocular microbiology.

Among bacterial pathogens, Pseudomonas aeruginosa was the predominant isolate in the present study (42.08%), followed by Escherichia coli (19.67%), Staphylococcus epidermidis (14.21%), S. aureus (10.93%) and Klebsiella pneumoniae (9.29%). The proportion of Pseudomonas was considerably higher than that reported by Tewari et al.20 (18.9%), Kumar et al,[17] (17.14%), Deorukhkar et al,[16] (7.96%), and Das et al.8 (12.53%). Conversely, S. aureus was the predominant bacterial pathogen in the previous studies,[15,19,20] whereas Deorukhkar et al,[16] identified Streptococcus pneumoniae as the commonest bacterial isolate. These differences support the existence of substantial geographical and institutional variation in bacterial ocular pathogens and emphasize the importance of locally generated microbiological data.

Among fungal isolates, Aspergillus spp. predominated in the present study (63.49%), followed by Fusarium spp. (26.98%), Curvularia spp. (6.35%) and Candida albicans (3.17%). This predominance of Aspergillus was consistent with Kumari et al,[18] who reported Aspergillus in 51.61% of fungal isolates, Tewari et al,[20] (35.4%), and Sunov et al,[19] (90.9%). In contrast, Baral et al,[21] reported Fusarium as the commonest fungus (44.4%), followed by Aspergillus (27.8%), while Deorukhkar et al,[16] reported corresponding proportions of 35.04% and 18.0%; Kumar et al,[17] and Ranjini et al,[15] also identified Fusarium as the predominant fungal pathogen. Such differences may reflect climatic conditions, environmental fungal reservoirs and patterns of agricultural exposure. KOH microscopy detected fungal elements in 44 of the 63 culture-positive cases (69.84%), while eight additional cases were KOH positive despite negative culture. This was comparable with Tewari et al,[20] in whom 21 of 31 culture-positive fungal cases were smear positive, whereas Kumari et al,[18] reported a higher KOH sensitivity of 90.32%. These findings support the complementary role of direct KOH microscopy and culture in laboratory diagnosis of keratomycosis.

The antimicrobial susceptibility profile showed high susceptibility of P. aeruginosa to amikacin (93.5%), gentamicin (94.8%) and ciprofloxacin (89.6%), with complete susceptibility to imipenem and meropenem. Tewari et al,[20] similarly reported complete susceptibility of Pseudomonas isolates to meropenem and amikacin, although ciprofloxacin susceptibility was lower in their series. All S. aureus and S. epidermidis isolates in the present study were susceptible to vancomycin and linezolid, whereas ciprofloxacin susceptibility was lower. This pattern was comparable with Ranjini et al,[15] who observed complete susceptibility of Gram-positive cocci to vancomycin but greater resistance to ciprofloxacin and gentamicin. Sunov et al,[19] likewise reported preserved vancomycin activity and discussed increasing fluoroquinolone resistance among ocular bacterial pathogens. Overall, the findings indicate that ocular infections in this setting are characterized by a relatively high culture yield, predominance of P. aeruginosa among bacterial isolates and Aspergillus among fungal isolates, with generally preserved activity of aminoglycosides, carbapenems, vancomycin and linezolid. The variability observed across Indian studies reinforces the need for periodic local surveillance to guide empirical antimicrobial therapy.

CONCLUSION

The study demonstrates that ocular infections were predominantly observed among middle-aged males and rural residents, with ocular trauma emerging as the leading predisposing factor. A substantial proportion of specimens yielded positive cultures, with bacterial isolates predominating over fungal isolates. Pseudomonas aeruginosa was the most frequent bacterial pathogen, whereas Aspergillus spp. was the predominant fungal isolate. KOH microscopy provided useful rapid evidence of fungal infection and complemented culture, including in culture-negative cases. Most major bacterial isolates retained good susceptibility to

aminoglycosides, carbapenems, vancomycin, and linezolid, although reduced fluoroquinolone susceptibility was observed in selected organisms. These findings highlight the importance of prompt microbiological evaluation, combined use of direct microscopy and culture, and periodic local surveillance of pathogen distribution and antimicrobial susceptibility to support appropriate empirical and targeted therapy for ocular infections.

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