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Original Research Article

Microbiological spectrum of ocular infections in patients of tertiary care eye hospital of Punjab

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ABSTRACT

Eye is a complex and sensitive organ and is therefore more vulnerable to trauma and various infections. Ocular infections are one of the most commonly encountered infections. Any part of the eye can be infected by bacteria, fungi, parasites or viruses. In order to minimize the ocular morbidity and mortality, timely antimicrobial treatment must be initiated on the basis of clinical and microbial evaluation. For specific antibacterial treatment, isolation and identification of bacterial pathogens along with antibiotic susceptibility spectrum is essential. Hence, this study was undertaken to isolate and identify the bacterial and fungal pathogens responsible for the development of ocular infections and to determine their in vitro susceptibilities to commonly used antibiotics. A total of 203 samples of suspected ocular infections comprising 117 conjunctival swabs, 34 corneal specimens, 24 lacrimal pus samples and 28 vitreous specimens were analysed. Out of them 102(50.1%) showed growth. 82(40%) were bacterial and 21(10.3%) were fungal isolates. Due to the emergence of drug resistance it is imperative that all ophthalmological samples must be tested for antibiotic resistance as far as possible. Indiscriminate use of over the counter antibiotic eye formulations should be avoided and also it is important to know the changing pattern of pathogens.

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1. Introduction

Eye is a unique organ and the most important sensory organs in the body.¹ It is a complex and sensitive organ and is therefore more vulnerable to trauma and various infections. Ocular infections are one of the most frequently encountered infections. Infection can occur exogenously due to penetrating injury to the eye or as a result of intraocular surgery. Infection may be acquired endogenously as a result of haematogenous spread of infection from other parts of the body. Indian population is vulnerable to ocular infections by virtue of subtropical

climate, trauma and surgical procedures. Even a minor infection elsewhere in the body, can be fatal to the eye in terms of visual compromise.

Any part of eye can be infected by bacteria, fungi, parasites or viruses.² Many opportunistic agents are frequently encountered in ocular infections due to widespread use of topical, systemic immunosuppressive agents and increasing number of patients with HIV.³ Bacteria are the predominant contributor of ocular infections worldwide. Infection can be mono or poly-microbial and is associated with various factors including contact lenses, trauma, surgery, dry eye state, chronic nasolacrimal duct obstruction and previous ocular infections.⁴⁻⁶

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The bacterial conjunctivitis is the most common ocular infection and it involves all ages and has a worldwide distribution.⁷ Normally, the conjunctiva harbours a population of bacteria that does not cause any disease, but however infections may occur when the microorganisms overwhelm local host defence mechanisms.¹

Mycotic keratitis is commonly seen in rural agricultural workers and has unfavourable prognosis due to its protracted course and it constitutes an important cause of blindness. Fungi gain access to the cornea due to a defect in corneal epithelium and cause tissue necrosis leading to the ulceration and subsequently corneal opacity. The predominant predisposing factors of mycotic keratitis are trauma by vegetative matter, indiscriminate use of topical corticosteroids, contact lens and rarely by retention of hair in the cornea.⁸ It is commonly caused by *Aspergillus* species, *Candida albicans*, *Fusarium*, etc.

Endophthalmitis and panophthalmitis are ocular infections which lead to a very severe sight threatening condition. Exogenous endophthalmitis is a complication of primary cataract, intraocular surgery and ocular trauma due to the introduction of pathogens like bacteria whereas the endogenous one is frequently due to systemic dissemination of the pathogens. Organisms causing these infections are mostly bacterial or fungal. Organisms causing bacterial endophthalmitis include *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococci*, *Pneumococci*, *Pseudomonas*, *Escherichia coli*. The common fungi causing fungal endophthalmitis are *Aspergillus*, *Fusarium*, *Penicillium* and *Candida*. Both keratitis and endophthalmitis are potentially devastating ocular conditions if not diagnosed early.^{9–11}

In order to reduce the ocular morbidity and mortality, timely antimicrobial treatment must be started on the basis of clinical and microbial evaluation.¹² For specific antimicrobial treatment, isolation and identification of bacterial pathogens along with the antibiotic susceptibility spectrum is essential.¹ The bacterial aetiology and their antimicrobial susceptibility must be updated to make a rational choice of initial antimicrobial therapy. Hence, this study was undertaken to isolate and identify the bacterial and fungal pathogens responsible for the development of ocular infections and to determine their *in vitro* susceptibilities to commonly used antibiotics.

2. Materials and Methods

The present prospective study was carried out from January 2019 to June 2020 in Microbiology department, Government Medical College, Amritsar. Various ocular samples were received from Ophthalmology department, Government Medical College, Amritsar.

All the patients included in the present study were examined by slit lamp bio-microscopy and the clinical conditions were diagnosed by the ophthalmologist using

standard protocols. After detailed ocular examinations using standard techniques, specimens for smear and culture was obtained from the various ocular tissues. Specimens were then subjected to direct microscopic examination i.e. Gram staining, 10% KOH wet mount, Calcofluor white staining, bacterial and fungal culture. Isolated bacteria & fungi were identified by the standard Microbiological procedures.¹³ Antimicrobial susceptibility testing of the bacterial isolates was performed as per the latest CLSI guidelines.¹⁴

3. Results

A total of 203 samples of suspected ocular infections comprising 117 conjunctival swabs, 34 corneal specimens, 24 lacrimal pus samples and 28 vitreous specimens were analysed. Out of them 102(50.1%) showed growth. 82(40%) were bacterial and 21(10.3%) were fungal isolates.

3.1. Epidemiological findings

Mean age of the patients was 46.7 years with standard deviation of 15.3 (46.7±15.3). The study showed slightly more preponderance for the males (n=104, 51%). 98 patients hailed from urban and 105 from rural area. The occupational group analysis showed significantly high incidence among farmers followed by labourers, it was observed that 57% (116) of the participants were illiterate. The predisposing factors associated with suspected keratitis patients were trauma, contact lens wear and post-operative.

3.2. Microbiological findings

The conjunctival swabs yielded 50(43%) bacterial isolates. Corneal scrapings grew 5(14%) bacterial isolates and 16(47.1%) fungal isolates and 1 mixed growth. Vitreous fluid yielded 10(36%) bacterial and 5(17.8%) fungal isolates. Among lacrimal pus samples bacterial growth obtained in 17(71%).

Among corneal and vitreous specimens, out of the 26 cases positive by direct microscopy (KOH mount and Calcofluor White staining), 18 cases showed growth and in 3 cases no growth was obtained on culture. Out of 36 cases negative on direct microscopy, growth was obtained in 3 cases and in remaining 33 cases no growth was obtained on culture. Sensitivity of the direct microscopy was 85.7% and specificity of the test was 80.9%. Amongst the 82 culture positive samples, 60(73%) gram negative bacterial isolates were identified while 22(27%) gram positive isolates were identified.

Out of 21 fungal isolates, 16 were from the corneal scrapings which yielded *Aspergillus* sp. (7), *Penicillium* sp. (4), *Candida albicans* (3) and *Fusarium* sp. (2). One corneal sample which yielded *Candida* showed the mixed growth along with Coagulase-negative staphylococcus. Among the 5 fungal isolates obtained from vitreous specimen 3 were *Aspergillus* sp. and 2 were *Candida*

albicans. There was no fungal isolate obtained from conjunctival swab and lacrimal pus specimen.

The predominant Gram-negative bacterium isolated was *Pseudomonas aeruginosa* which was 100% sensitive to polymyxin B, 87.5% sensitive to ceftazidime and 75% sensitive to amikacin. *Klebsiella pneumoniae* was 100% susceptible to amikacin, ceftazidime and polymyxin B, 87.5% sensitive to gentamicin and ofloxacin. The *E. coli* found to be 100% sensitive to amikacin, ceftazidime, ofloxacin and polymyxin B, 83.3% sensitive to gentamicin and ciprofloxacin.

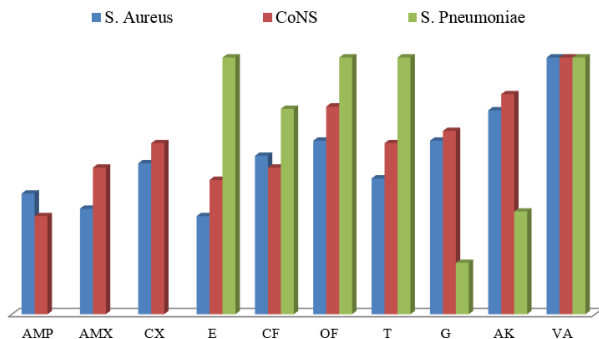


Fig. 1: Antibiotic sensitivity pattern of the gram positive isolates in percentage

4. Discussion

Currently, ocular infections are considered as one of the major contributors of nonfatal disabling conditions in both high and low income countries.¹⁵ Up to 5% of all the blindness may be attributed to consequences of ocular trauma and resulting infection.¹⁶ Global estimates of the ocular infections as a cause of blindness range from 1.5 to 2 million cases per year.¹⁷ In parts of the world with difficulties about the access to healthcare, poorer health indices and a higher proportion of workers within high risk occupations such as farming and agriculture, incidence of ocular infections is more.¹⁸

In our study majority of patients were from age group 41-50 with a mean age of 46.7 years and male to female ratio was 1.05:1. These results were in concurrence with the earlier reports.^{19,20} Male predominance in our study was attributed to their outdoor activities.²¹ Occupational analysis indicated high prevalence rates among farmers and labourers due to their work environment. The farmers are usually exposed to trauma by some organic matter (such as dried rice stems or maize) which facilitates invasion of fungi.²² A higher prevalence of ocular infections in rural population (52%) and illiterate individuals (57%) can be explained by lower awareness of health & hygiene practices and local medical conditions which makes them more prone to infections.^{23,24}

Predisposing factors associated with suspected keratitis patients were trauma (68%), contact lens wear (12%) and post-operative cases (6%). These findings are in concordance with the study conducted by Hitesh J et al on the etiological diagnosis of microbial keratitis in a tertiary care hospital in Gujarat.²⁵ Among suspected endophthalmitis cases 85.7% were post-operative, 7.2% were post-traumatic and 3.5% of endogenous endophthalmitis. Similar findings were observed in study conducted at Bangalore, India by Banu A et al.²⁶

Sensitivity of direct microscopy for identification of fungal elements in various ocular specimens was 85.7% and it was comparable to a study conducted by Sharma et al. where the sensitivity was 81.2%.²⁷ So, the performance of direct microscopy in identification of fungal elements in our study were in good accordance with the various studies and it clearly establish its high diagnostic sensitivity which can be compared with culture. Moreover, culture is a time-consuming laboratory method which is not commonly available in clinical practice. Sharma et al. have recommended the introduction of anti-fungal therapy whenever a KOH+CFW+gram stained smear is positive for fungus because they believe that the gold standard of culture also has its own limitations and a fungal element is unlikely to be misinterpreted during microscopic examination.²⁸

Predominant isolate identified among conjunctival specimens was *Staphylococcus aureus* 26(52%) and followed by 10(20%) Coagulase Negative Staphylococci (CoNS), 3(6%) *Streptococcus pneumoniae*, 5(10%) *Klebsiella sp.*, 3(6%) each of *Pseudomonas aeruginosa* and *Escherichia coli*. Similar studies done by Samuel S O et al and Ra'ad Al-Dorri AZ et al, have reported *Staphylococcus aureus* as the predominant isolate in conjunctivitis.^{29,30} A study conducted by A.O. Okesola et al also showed Coagulase negative Staphylococci as second common isolate.³¹ In a study by Dagnachew et al, in 2014 from North west Ethiopia, *Klebsiella pneumoniae* was the commonest isolate among the gram negative organisms.³²

Coagulase Negative Staphylococci 3(60%) was the major bacterial isolate identified from corneal specimens followed by 1(20%) each of *S.aureus* and *Pseudomonas*. Similarly in another study about bacterial keratitis by Bourcier T, Coagulase negative staphylococcus was the most frequent organism isolated on corneal scrapings.³³

Bacterial isolate most commonly identified among vitreous specimen was CoNS 4(40%), followed by 3(30%) *S.aureus*, 2(20%) *Pseudomonas*. This data is consistent with study done by Kodati S et al which showed Coagulase negative Staphylococci (CoNS) as the most common isolate (54.6%).³⁴

Most common bacterial isolate observed among lacrimal pus specimens were 4(23.5%) each of *S.aureus* and CoNS, which correlated with study done by C.P. Shah et al from Nepal.³⁵ Among the Gram negative organism, *E.coli*

Table 1: Distribution of the different bacterial isolates from various specimens

Specimen Organism	Conjunctival swab	Corneal scrapping	Vitreous specimen	Lacrimal pus	Total
Staphylococcus aureus	26(52%)	1(20%)	3(30%)	4(23.5%)	44(53.6%)
Coagulase-negative staphylococcus	10(20%)	3(60%)	4(40%)	4(23.5%)	21(20.5%)
Streptococcus pneumoniae	3(6%)	-	1(10%)	1(5.9%)	5(4.9%)
Pseudomonas aeruginosa	3(6%)	1(20%)	2(20%)	2(11.8%)	8(7.8%)
Klebsiella pneumoniae	5(10%)	-	-	2(11.8%)	7(6.9%)
Escherichia coli	3(6%)	-	-	3(17.6%)	6(5.9%)
Proteus mirabilis	-	-	-	1(5.9%)	1(0.9%)
Total	50(100%)	5(100%)	10(100%)	17(100%)	82(100%)

2(17.6%) was the frequent isolate followed by *Klebsiella pneumoniae* 2(11.8%) and it correlated well with the study by Patel K et al.³⁶

Most common pathogen causing keratomycosis was *Aspergillus* sp. (7) followed by *Penicillium* sp. (4), *Candida albicans* (3) and *Fusarium* sp. (2). The corneal sample which yielded *Candida albicans*, showed mixed growth along with Coagulase-negative staphylococcus.

Similarly in the study by Arora U et al from India, also showed *Aspergillus* sp. As most commonly isolated fungi in Keratomycosis.³⁷ In contrast to our findings studies conducted by Srinivasan M et al from South India in 1994 and Das S et al from India in 2014 had reported *Fusarium* as the predominant fungal isolate in Keratitis.^{18,38}

Predominant fungal isolate obtained from vitreous specimen were *Aspergillus* sp. (3) followed by *Candida albicans* (2). Similar results were observed in a study by Satpthy G in which *Aspergillus* spp. were the most common isolates followed by *Candida albicans*.³⁹

In our study, among the Gram positive isolates the most frequent isolate was *Staphylococcus aureus* which was 100% susceptible to vancomycin, amikacin, (79.4%), gentamicin (67.6%) and ofloxacin (67.6%). Coagulase negative Staphylococci showed 100% susceptibility to vancomycin, (85.7%) amikacin, (80.9%) ofloxacin, (71.4%) gentamicin.

This is in concordance with the study conducted by Rajesh S et al in which, all Gram positive isolates were susceptible to vancomycin (100%), *Staphylococcus aureus* showing maximum susceptibility to aminoglycosides followed by fluoroquinolones.²³

In present study Methicillin resistance was seen in 41.2% of *Staphylococcus aureus* isolates and 33.3% of Coagulase negative *Staphylococcus* isolates. In study done by Chuang C C et al and Deguchi H et al methicillin resistance was observed in 52.8% strains of *S.aureus* and 47% Coagulase negative *Staphylococcus* respectively.^{40,41}

Streptococcus pneumoniae isolates found to be 100% susceptible to vancomycin, erythromycin, tetracycline and ofloxacin followed by (80%) ciprofloxacin and (40%) amikacin. Similar study done by Tewelde et al in South west

Ethiopia which reported the same.⁴²

Gram negative bacteria isolates found to be 100% sensitive polymyxin B and ceftazidime followed by amikacin (93.7%), ofloxacin (87%), gentamycin (82.8%). Gram negative isolates showed maximum sensitivity to Amikacin, ceftazidime, ofloxacin and polymyxin B and Gentamycin which was similar to the study done by Whitcher JP et al.⁴³

Sensitivity and resistance pattern based on in vitro testing may not truly reflect the clinical resistance and response to an antimicrobial because of the host factors and the penetration of drug.⁴⁴ High resistance is usually due to overuse and empirical treatment given to the patients that ultimately leads to emergence of drug resistant strains.^{45,46}

5. Conclusion

Due to emergence of drug resistance it is imperative that all the ophthalmological samples must be tested for antimicrobial resistance as far as possible. Indiscriminate use of various over the counter available antibiotic eye formulations should be avoided and it is important to know the changing profile of pathogens. To mitigate the burden of ocular infections, ophthalmologist should regard on risk reduction and must comply with the etiologic approach of diagnosis.

6. Conflict of Interest

The authors declare that there are no conflicts of interest in this paper.

7. Source of Funding

None.

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