ORIGINAL ARTICLE

An evaluation on Microbiological spectrum of ocular infections in patients

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ABSTRACT

Introduction: 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. **Methods:** All the samples were inoculated on to Brain heart infusion broth, Blood agar, Macconkey agar and Sabouraud's dextrose agar. Multiple C shaped streaks were performed on solid media for the corneal scrapings. The growth was identified based on standard laboratory procedures. **Results:** A total of 223 samples of suspected ocular infections comprising 122 conjunctival swabs, 39 corneal specimens, 29 lacrimal pus samples and 33 vitreous specimens were analysed. Out of them 108(51.3%) showed growth. 88(42.2%) were bacterial and 29(12.1%) were fungal isolates. **Conclusions:** Due to emergence of drug resistance it is imperative that all the ophthalmological samples must be tested for antimicrobial resistance as far as possible.

Keywords: Microbiological spectrum, ocular infections, various factors

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Introduction

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 Infection encountered infections. 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. Many opportunistic agents are frequently encountered in ocular infections due to widespread use of topical, systemic immunosup pressive agents and increasing number of patients with HIV.² 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.³⁻⁵

The bacterial conjunctivitis is the most common ocular infection and it involves all ages and has a worldwide

distribution.⁶ 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.⁷

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 defectin 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}

Materials and Methods:

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.

Microbiological processing Direct Gram's staining and KOH mount was performed for all samples. All the samples were inoculated on to Brain heart infusion broth, Blood agar, Macconkey agar and Sabouraud's dextrose agar. Multiple C shaped streaks were performed on solid media for the corneal scrapings. The growth was identified based on standard laboratory procedures. (Cheesbrough M, 2006) Antibiotic sensitivity testing Antibiotic sensitivity was done for bacterial isolates using kirby bauer disk diffusion method using discs of standard potency. The results were interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines. (Clinical and Laboratory Standards Institute (CLSI), M100-S22) Special stains Giemsa staining, acid fast staining and modified acid fast staining were done where ever required.

Results:

A total of 223 samples of suspected ocular infections comprising 122 conjunctival swabs, 39 corneal specimens, 29 lacrimal pus samples and 33 vitreous specimens were analysed. Out of them 108(51.3%) showed growth. 88(42.2%)were bacterial and 29(12.1%) were fungal isolates.

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.

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 in3 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 scrappings 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 Coagulasenegative staphylococcus. Among the 5 fungal isolates obtained from vitreous specimen 3 were Aspergillus sp. and 2 were Candida

Specimen Organism	Conjunctival	Corneal	Vitreous	Lacrimal	Total
	swab	scrapping	specimen	pus	
Staphylococcus aureus	28(53%)	1(20%)	5(31%)	5(23.5%)	39(54.1%)
Coagulase-negative staphylococcus	12(22%)	4(61%)	5(41%)	5(23.5%)	26(21.2%)
Streptococcus pneumoniae	4(4%)	-	1(10%)	1(5.9%)	6(5.8%)
Pseudomonas aeruginosa	5(8%)	1(20%)	3(21%)	3(11.8%)	12(8.2%)
Klebsiella pneumoniae	7(12%)	-	-	3(11.8%)	10(7.1%)
Escherichia coli	5(8%)	-	-	4(17.6%)	9(6.1%)
Proteus mirabilis	-	-	-	1(5.9%)	1(0.6%)
Total	53(100%)	6(100%)	14(100%)	22(100%)	103(100%)

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

Discussion:

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.¹²

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.^{13,14} Male predominance in our study was attributed to their outdoor activities.¹⁵ 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.¹⁶ 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.^{17,18} 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.¹⁹ 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.²⁰ 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%.²¹ 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 culturealso has its own limitations and a fungal element is unlikely to be misinterpreted during microscopic examination.²² 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.^{23,24} A study conducted by A.O. Okesola et al also showed Coagulase negative Staphylococci as second common isolate.²⁵ In a study by Dagnachew et al, in 2014 from North west Ethiopia, Klebsiella pneumoniae was the commonest isolate among the gram negative organisms.²⁶

Coagulase Negative Staphylococci 3(60%) was the major bacterial isolate identified from corneal specimens followed by 1(20%) each of S.aureus and Pseudomonas. Similarlyin another study about bacterial keratitis by Bourcier T, Coagulase negative staphylococcus was the most frequent organism isolated on corneal scrapings.²⁷

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%).²⁸

The unique structure of the human eye, the use of contact lenses and the constant exposure of the eye directly to the environment renders it vulnerable to a number of uncommon infectious diseases caused by parasites, and bacteria. Some of these infectious eye diseases, prior to the invention of contact lenses were rare; in the present study bacteria were isolated from the contact lens wearers as described above.²⁹ Drug resistance was encountered in the bacterial isolates vancomycin, except for fluroquinolones, chloramphenicol and tobramycin. Resistance trend was evidently seen in other antibiotics tested. Genotypic methods such as Quantitative PCR using real-time PCR and loop-mediated isothermal amplification (LAMP) assays can be used as the final confirmatory test for detection of intraocular infections. The lack of a confirmatory test is a limitation of the present study.

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 knowthe 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.

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