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Research Article

BACTERIOLOGICAL AND MYCOLOGICAL PROFILE OF OCULAR INFECTIONS IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Purpose: To identify the bacterial and fungal pathogens causing ocular infections in patients attending a tertiary care hospital.
Materials and Methods: All patients diagnosed with ocular infections presented between August 2013 to July 2014 were evaluated. The patients were examined under slit lamp biomicroscope and specimen was obtained using standard protocols.
Results: Out of 222 patients, 96 had conjunctivitis, 30 patients had keratitis, 53 patients as Dacryocystitis, 37 had eyelid infections and 6 patients had intraocular infections. Among 96 conjunctivitis, 41(42.7%) showed bacterial growth. *Staphylococcus aureus* (67.7%) was most commonly isolated among gram positive organisms *and Escherichia coli* (70%) among gram negative organisms. Among 30 keratitis patients, 8 showed fungal growth. *Fusarium spp*. was most commonly isolated. In Dacryocystitis patients, incidence was 28(52.8%). The commonest isolate was *Staphylococcus aureus* (59.1%) followed by *Escherichia coli* (66.7%). Among eyelid infections, incidence was (56.8%). *Coagulase Negative Staphylococcus* 12 (57.1%) was commonly isolated followed by *Staphylococcus aureus 9 (*42.9%).
Conclusion: In our study, Bacterial pathogens were more commonly isolated than fungal pathogens. The predominant bacteria isolated being *Staphylococcus aureus*. In keratitis, Fungal pathogens were more commonly isolated predominant *being Fusarium spp*.It is therefore important in culturing the samples in patients with these infections to prevent the emergence of resistant strains.

INTRODUCTION

Ocular infections are one of the most commonly encountered infections. Normally, there are various natural defence mechanisms that protect the eye against infections. These include the blink reflex, bioactive components of the tear film consisting of lysozyme, IgA and IgG and the surface epithelium of the cornea.[1] Infection results when these barriers are disrupted either due to exogenous or endogenous factors which facilitate intraocular invasion of the microorganisms.

The most frequently affected areas of the eye are the conjunctiva, cornea and the eyelids.[2] Eyelid margins harbours a variety of microorganisms and cause infections. These infections are usually localised but sometimes may spread to the adjacent tissues like conjunctiva and cornea.[3] Bacteria are the major causative agents that cause eyelid infections.

Dacryocystitis is inflammation of the lacrimal sac and occurs due to blockage of secretion of the tears. This causes accumulation of secretions and tears within the sac and causes infection. This is of particular importance since if left untreated it may lead to spread of infections to other parts of the eye.[4] Conjunctivitis refers to the inflammation of the conjunctiva which is mostly due to bacteria and virus and rarely fungus. The bacterial conjunctivitis is the most common ocular infection which involves all ages and has a worldwide distribution.[5]

Keratitis refers to inflammation of the cornea and the organisms commonly implicated are bacteria and fungi. Microbial keratitis is a potentially dreadful condition that requires prompt diagnosis and treatment to prevent further complications like endophthalmitis and panophthalmitis. Bacterial keratitis causes corneal ulceration which lead to corneal opacity and severe visual loss. Mycotic keratitis is common in rural agricultural workers and has an unfavourable prognosis due to its protracted course and constitutes an important cause of blindness.[6]

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Endophthalmitis and panophthalmitis are intra ocular infections which leads to a severe sight threatening condition. If these infections are left untreated it may lead to visual loss.

Hence, this study was undertaken to isolate and identify the bacterial and fungal pathogens responsible for the development of ocular infections.

MATERIALS AND METHODS

All the patients included in the study were examined by using slit lamp and the infections were diagnosed by the ophthalmologist using standard protocols. After detailed ocular examinations using standard techniques, specimens for smear and culture was obtained.

In case of blepharitis, specimen was obtained by swabbing the eyelid margin using a broth moistened sterile cotton swab.

In case of hordeolum externum, hordeolum internum and chalazion, the abscess were incised and swabs were taken. In case of lacrimal sac infections, pressure was applied over the lacrimal sac region and purulent material was collected from the punctum. Sometimes surgically excised lacrimal sac was also collected.

In case of conjunctivitis, a sterile cotton swab moistened with Brain heart infusion broth was used to collect the specimen. Patient was asked to look up and a moistened sterile cotton swab was wiped against the lower conjunctival sac of the affected eye from the nasal margin to the temporal margin and back again.

In all the above infections, two swabs were taken. Collected specimens were placed individually in two sterile dry test tubes and transported to the laboratory immediately.

Laboratory Procedures

Gram staining was performed for the received samples and examined under microscope for the presence of pus cells and the organisms Specimens obtained were inoculated onto the Blood agar plate, Mac conkey agar plate, chocolate agar plate and incubated aerobically for 18-24 hrs. and then observed the next day. The specimens were also inoculated onto Sabouraud's dextrose agar in duplicates, one incubated at room temperature and the other at 37 °C. SDA slopes were examined daily for the first week and twice weekly for the next three weeks.

For bacterial identification, colony characteristics were identified by observing the plates, gram staining done and appropriate biochemical reactions were performed. The commonly performed biochemical reactions were Catalase test, Coagulase test, Motility test, Indole test, Citrate utilisation test, Urea Hydrolysis test, and sugar fermentation tests.

In case of Keratitis, Corneal scrapings were obtained from edge and base of the ulcer and bed side inoculation was done onto Blood agar plate, Mac conkey agar plate and chocolate agar plate and Sabourauds dextrose agar with antibiotics in duplicates ,one kept at room temperature and the other at 37* C. The material obtained by next scraping was spread onto labelled slides in a thin even manner for 10% KOH wet mount and Gram staining.

SDA slopes were observed daily for the first week and then twice weekly for the next three weeks and discarded if there is no growth. The specimens inoculated onto the Blood agar plate, Mac conkey agar plate, chocolate agar plate were incubated aerobically for 18-24 hrs. and then observed the next day and discarded if there is no growth.

RESULTS

A total of 222 cases of ocular infections were analysed over a period of one year from August 2013 to July 2014.

Among them, 96 cases were clinically diagnosed as conjunctivitis, keratitis constituted 30 cases, lacrimal sac infections constituted 53 cases, eyelid infections comprised of 37 cases and intraocular infections (endophthalmitis and panophthalmitis) constituted 6 cases. The statistical analysis was done by using SPSS version 17 and P values was obtained by Pearson Chi-Square test.

Out of 96 conjunctivitis cases, 41 (42.7%) yielded bacterial growth**.** Among the 41 positive cases in conjunctivitis, gram positive cocci accounted for majority of the cases 31 (75.6%) followed by gram negative bacilli 10 (24.4%)**.**The predominant isolate among gram positive isolates was *Staphylococcus .aureus* 21 (67.7%), followed by *Coagulase Negative Staphylococcus 8* (25.8%) and *Streptococcus pneumoniae* 2 (6.5%) [Table-1].

Table 1 Distribution of conjunctivitis cases according to spectrum of gram positive organisms

S.No	Organism	Total No.(%)
1.	Staph.aureus	21(67.7)
2.	CONS	8(25.8)
3.	Strep.pneumoniae	2(6.5)
	Total	31(100)

The predominate isolate among gram negative bacilli was *Escherichia coli* 7 (70%) and next isolated was *Klebsiella pneumoniae* 3(30%).

Among 30 cases with Keratitis, 15 cases (50%) were positive by direct microscopy with 10% KOH mount and another 15 cases (50%) were negative**.**

Out of 15 cases positive by Direct microscopy with 10% KOH mount, 7 cases (23.3%) showed growth on culture and 8 cases (26.7%) were negative for culture in Sabourauds dextrose agar.

Out of 15 cases negative on direct microscopy with 10% KOH mount, only 1 case (3.3%) was positive by culture and the remaining 14 cases were negative on culture on Sabourauds dextrose agar**.** The sensitivity of KOH mount was 87.5% and the specificity of the test was 63.6%. P-value was 0.013 which was statistically significant.[Table-2]

Table 2 Microscopy (10% KOH) versus culture among keratitis cases

10% KOH mount	Culture		Total
	Positive	Negative	
Positive	7(23.3%)	8(26.7%)	15(50%)
Negative	1(3.3%)	14(46.7%)	15(50%)
Total	8(26.7%)	22(73.3%)	30(100%)

Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Pearson chi-square test		
				Value	Degree of freedom	p-value

87.5	63.6	93.3	46.7	6.136	1	0.013

Hence p-value is 0.013 (p-value should be less than 0.05). Thus it is statistically significant. So there is no significant difference between the microscopy and Culture

Out of 8 cases positive for fungal aetiology, 6 cases (75%) were found to be *Fusarium sp*. and 2 cases (25%) were found to be *Penicillium sp.*

Among the total of 53 cases of Dacryocystitis, bacterial growth was seen in 28 cases (52.8%). The common organisms isolated were gram positive cocci 22 (78.6%) followed by the gram negative bacilli 6 (21.4%).

Out of 22 isolates of gram positive cocci, *Staphylococcus aureus* constituted 13 (59.1%) and *Coagulase Negative Staphylococcus* constituted 9 (40.9%).[Table-3]

Table 3 Distribution of gram positive isolates in Dacryocystitis cases

S.No.	Organism	No. of Cases	Percentage
1.	Staph.aureus	13	59.1%
2.	CoNS	9	40.9%
	Total	22	100.0%

Out of 6 isolates of gram negative organisms, majority were *Escherichia coli* 4 (66.7%) followed by *Klebsiella pneumoniae* 2 (33.3%).

Out of 37 cases of eyelid infections, the bacterial growth was seen in 21 cases (56.8%). Out of 21 cases, *Staphylococcus aureus* constituted 9 (42.9%) and CONS constituted 12 (57.1%).

Six intraocular samples (5 vitreous samples and 1 aqueous sample) were received from post-operative endophthalmitis patients. These samples were subjected to direct microscopy by gram staining and culture .The samples were negative by culture.

Table 4 Distribution of organisms in Eyelid infections

S.No.	Organism	No. of Cases	Percentage
1.	Staph aureus	9	42.9%
2.	CoNS	12	57.1%
	Total	21	100.0%

DISCUSSION

Ocular infections are one of the most common infections in our country due to virtue of subtropical climate. The anterior segment of the eye is infected by direct invasion by the anterior route while the blood borne infections may reach the posterior segment of the eye. Even a minor infection elsewhere in the body may be fatal to the eye in terms of visual compromise.

The incidence of bacterial conjunctivitis in this study was 42.7%. This was in accordance with a study done by Agaba *et al* [7]., in 2014 from South Western Uganda , where the incidence rate was 44.4%. On the other hand in a study done by S.O. Samuel *et al*., [8] from Nigeria in 2012, a higher incidence of 59.6% was reported. Among the 41 isolated pathogens in conjunctivitis, gram positive organisms 31 (75.6%) were more commonly isolated than the gram negative organisms 10 (24.4%). A study conducted by Ramesh *et al*.,[3] from South India in 2010, also showed that gram positive organisms were commoner than gram negative organisms in bacterial conjunctivitis.

Staphylococcus aureus 21 (67.7%) was the commonest organism isolated among gram positive organisms in this study. Similar studies done by S.O. Samuel *et al*.[8], A.O.Okesola *et al*.[,9] and Alaa Zanzal *et al*.[10],in 2005 from Tikrit Hospital in Ibadan, have reported *Staphylococcus aureus* as the commonest isolate in conjunctivitis. In our study, among the gram negative organisms, *Escherichia coli* 7(70%) was the commonly isolated organism followed by *Klebsiella pneumoniae* 3(30%) whereas in a study by Dagnachew *et al* [11]., in 2014 from North west Ethiopia, *Klebsiella pneumoniae* was the commonest organism isolated among the gram negative organisms.

In case of Keratitis, the sensitivity of KOH mount versus culture was 87.5% and it was slightly higher when compared to a study by Sharma *et al* [12] where the sensitivity was 81.2%. In a study by Ramakrishnan *et al*, [13] the sensitivity of KOH mount was 99.3% which was higher when compared with our study. Hence, KOH mount has a definite value in diagnosis of fungal keratitis.

In the present study, the most commonly isolated fungi was *Fusarium sp.* 6(75%) followed *by Penicillium sp.*2 (25%). Similar studies conducted by M Srinivasan *et al*.,[14] from South India in 1994 and Das S *et al*.,[15] from India in 2014 had reported *Fusarium* as the most common fungal isolate in Keratitis. In a study by Usha *et al*.,[16] in 2006 from India, had showed *Aspergillus* sp. as the most commonly isolated fungi in Keratomycosis.

In the present study, 53 clinically diagnosed cases of Dacryocystitis of all ages and both sexes were studied. Our study showed an incidence rate of 52.8% which was comparable with a study by Mandal *et al*.,[17] in 2008 from India. The gram positive organisms 22(78.6%) were more commonly isolated than gram negative organisms 6(21.4%) in this study. This correlated well with the studies conducted by Mandal *et al*.,[17] and Madhusudan *et al*.,[18] *Staphylococcus aureus* 13(59.1%) was the commonest isolate in this study followed by *CoNS* 9(40.1%) which correlated with the study done by C.P.Shah *et al*.,[19] in 2011 from Nepal.

In the present study, the incidence was (56.8%) among eyelid infection cases. Among the 21 positive cases ,*CoNS* 12 (57.1%) was more often isolated than *Staphylococcus aureus* 9(42.9%).This was comparable to the study by Parima *et al*.,[20] in 2012 where *CoNS* was the most common isolate followed by *Staphylococcus aureus.*

Six samples were received from post operative endophthalmitis patients. The samples received were 5 vitreous samples and 1 aqueous sample. The patients were already on treatment with topical and systemic antibiotics and the visual outcome was deteriorating inspite of the treatment. The samples were collected for microbiological evaluation to find out the organism. Direct microscopy with gram staining was performed for these samples and then culture was performed. However, the results the direct microscopy by gram staining and culture was negative.

In short, bacterial conjunctivitis though self limited appropriate antimicrobial treatment accelerates resolution and reduces complications. Hence culturing the conjunctiva before starting therapy is warranted. In our study, majority of corneal ulcers are due to *Fusarium sp*. which is one of the most virulent ocular pathogens that underscores the need for more effective methods of diagnosis and treatment to reduce the burden of unavoidable blindness.

KOH mount has a definite place in diagnosis of keratomycosis and hence meticulous examination of corneal scrapings by KOH mount and early institution of antifungal therapy may limit ocular morbidity.

Dacryocystitis and eyelid infections are one among the predisposing factors for post operative endophthalmitis and is therefore important in culturing the samples in the patients .The microbiological diagnosis of endophthalmitis is based on microscopy and culture of the organisms from the intraocular fluids. Despite best microbiological techniques and immediate processing of the samples, the sensitivity of the conventional methods in detecting the organisms is low. Highly sensitive techniques like Real time PCR has a extraordinary role in diagnosis of intraocular infections.

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