

Original Research Article

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Bacterial and Fungal Profile of External Ocular Infections in a Tertiary Care Hospital

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ABSTRACT

The eye may be infected from external sources or through intraocular invasion of microorganisms carried by the blood stream. The common eye infections caused by bacterial and fungal pathogens are blepharitis, conjunctivitis, internal and external hordeolum, keratitis, dacryocystitis. Timely institution of appropriate therapy must be initiated to control the infections and thereby minimize the ocular morbidity. Objectives: To study the prevalence of Bacterial and Fungal etiology of External ocular infections and to study the antibiotic susceptibility Pattern of the isolated bacterial pathogens. This study was carried out at Chettinad Hospital & Research Institute. A total of 100 patients with ocular infection attending outpatient department of ophthalmology were included in the study. Conjunctival swabs and corneal scrapings were collected and sent to the microbiology laboratory. The organisms were identified by colony morphology and appropriate biochemical reactions. Among the 100 samples 38(63.3%) culture positive in which 21(55.2%) were bacterial isolates and 17(44.7%) fungal isolates. Coagulase negative *Staphylococcus* 14 (66.6%) was the commonest isolate that cause conjunctival infection followed by *Staphylococcus aureus* 2(9.5%), *Pseudomonas aeruginosa*, *Acanthobacter*, *Aeromonas*, *Streptococcus pneumoniae*, *Klebsiella* 1(4.7%) each. The split up of fungal isolates *Fusarium* 6 (35.2%) followed by *Aspergillus flavus* 4(23.5%), *Aspergillus niger* 4 (23.5%), *Aspergillus fumigatus* 2 (11.7%) and *Candida albicans* 1 (5.8%). The Gram positive isolates are susceptible to Cefazolin 94.11%, Vancomycin 100%, Ciprofloxacin 75.25% and the Gram negative isolates were susceptible to amikacin 66.6%, ciprofloxacin 66.6%. Coagulase negative *Staphylococci* frequently causes infection of the conjunctiva and eyelids followed by *Staphylococcus aureus*. Similarly, *Fusarium* spp frequently causes corneal infection.

Keywords

External ocular infection, Conjunctivitis, Keratitis, *Fusarium*, Coagulase negative *Staphylococci*

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Introduction

Ocular infections are common and their morbidity can vary from self-limiting, trivial infection to sight-threatening. Ocular infections can affect different eye structures; and their presentation and treatment vary accordingly. The causative agents of ocular infections can be bacteria, fungi, viruses and parasites. Pathogenic microorganisms cause diseases to the eyes due to their virulence and

host's reduced resistance from many factors such as personal hygiene, living conditions, socio-economic status, nutrition, genetics, physiology, fever and age (Vaughan *et al.*, 1992). The areas in the eye that are frequently infected are the conjunctiva, lid and cornea clinically external eye infections present as: conjunctivitis, keratitis, blepharitis, dacryocystitis and external hordeolum (Modarres *et al.*, 1998).

The conjunctiva and ocular adnexae are rapidly colonized by bacteria at birth and conjunctival bacterial micro flora undergo constant turnover. The flora isolated in healthy individuals consists primarily of Diphtheroids. Species of greater virulence, such as Coagulase negative *Staphylococci*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Neisseria meningitides*, *Neisseria gonorrhoeae*, *Haemophilus influenzae*, *Moraxella lacunata* and *Corynebacterium diphtheria* have also been reported (Frederic *et al.*, 2001).

Bacteria are the most common microorganisms that cause conjunctivitis. This is because the bacterial pathogens inhabit the ocular surface (i.e. mucous membrane of the conjunctiva), though the lysosomes and antibodies in tear and blinking mechanism keep their population in check (Idu *et al.*, 2003). Mycotic keratitis is usually caused by filamentous fungi and occurs in conjunction with trauma to the cornea with vegetation matter. In the tropics it is common in male agricultural workers. Eye trauma is the cause of fungal keratitis in temperate areas as well. The common fungal genera involved are *Fusarium*, *Alternaria* and *Aspergillus spp.* (Wong *et al.*, 1997). The ocular findings may be part of a widespread systematic infection. In deciding on appropriate treatment, both the causative pathogen and the structure affected must be considered. Differences in drug absorption, penetration, and availability to the various structures of the eye affect treatment decisions. Severity of infection, efficacy and safety of medication, and cost/ benefit ratios must be taken into consideration in choosing the proper pharmacologic management of various ocular infections.

This research, therefore, aims amongst others at evaluating the antibiotic sensitivity of the bacterial organisms isolated from the eye infections, studying the distribution of the

common bacterial and fungal isolates in the clinical features- conjunctivitis, blepharitis and keratitis, the distribution of these bacterial isolates amongst age groups and the distribution of these bacterial isolates between males and females.

Materials and Methods

This study was carried out at Chettinad Hospital and Research Institute from March 2012 to March 2013. A total of 100 patients with ocular infection attending outpatient department of ophthalmology were included in the study. The samples were collected and submitted for microbiological evaluation from patients clinically diagnosed with ocular infections such as blepharitis, conjunctivitis, external and internal hordeolum, corneal ulcer, dacryocystitis. All the patients were examined on the slit-lamp biomicroscope. After detail examination samples were collected for smear and culture.

Sample collection

Collection of sample is done by ophthalmic surgeon⁽²³⁾. A collection kit must readily be available and it includes:

Collection of conjunctival material

Sterile moistened cotton swab or calcium alginate swab are used. Bacterial culture medium such as BHIB or normal saline may be used for moistening the swab.

Patient is requested to look up, the lower eye lid is pulled down using thumb with an absorbing tissue paper and moistened swab is rubbed over the lower conjunctival sac from medial to lateral side and back again.

The procedure is often slightly painful.

Sterile plastic (soft) bacteriological loop may be used for collection of material

Avoid collection of tears only.

Biochemical test - Catalase, Oxidase,

Coagulase, -Indole, Methyl red, Voges Proskauer, Triple sugar Iron, citrate, Urease, Mannitol motility medium

Procedure for processing conjunctival sample

The specimens were inoculated directly onto the blood agar, chocolate agar, Sabouraud's dextrose agar. The plates are inoculated and are incubated at 37°C overnight. Based on the colony morphology the pathogens were identified and biochemical test were done.

A part of the collected specimens was subjected to gram staining. A standardized protocol was followed for each ocular specimen for the evaluation of significant microbiological features (Mackey-McCartney; practical medical microbiology⁽²⁵⁾).

Collection of corneal scrapings

Corneal scrapping was taken in ophthalmology department under local anaesthesia i.e. 4% paracaine eye drops without preservative. Corneal scrapping is done from the leading edge and the base of the ulcer by using kimura spatula or 15 no sterile Bard Parker Surgical Blade with the help of slit lamp under aseptic conditions

Procedure for processing keratitis sample

Gram staining was performed. Culture on Blood agar in the form of 'c' shape streak. Incubate at 37°C for 24 hrs. Based on colony morphology bacterial pathogens were identified by biochemical reactions. Antibiotic Sensitivity is done by Kirby Bauer's Disc diffusion method.

Result and Discussion

During the study period of one year a total of 100 samples were collected from patients who

are clinically diagnosed with external ocular infections. Out of 100 samples 68 samples shows positive result and the remaining 32 was culture negative. In this study, Male patients are affected more when compared to the female patients. Off the 100 patients 44% were female patients and 56% were male patients. Both male and Female patients of age group >60 were highly affected with External ocular infections (Table 1).

Among the 100 samples, 65% of samples were collected from patients with conjunctival infection such as Conjunctivitis, Blepharitis, Dacryocystitis and 35% of the sample were isolated from patients with infection of the Cornea most commonly Keratitis. The most common external ocular infection is conjunctivitis 43 followed by Corneal ulcer 23, Blepharitis 1, Dacryocystitis 1 (Table 2).

Systemic disease related to eye disease:

1. Diabetic patients - 17
2. Non-Diabetic patients - 83

The Corneal ulcer is mainly due to infection with agents such as foreign body / sand, thorn, paddy husk, infection with finger. In this study, most of the corneal ulceration is due to infection with paddy husk. About 8 patients were infected with Paddy husk his clearly shows that these patients were mainly from Agriculture fields (Table 3). Among the 68 culture positive samples 37(54.4%) were Bacterial isolates and 23(33.3%) were fungal isolates (Table 4). The predominant bacterial isolate is Coagulase negative *Staphylococci* 18(48.8%) followed by *Staphylococcus aureus* 10(27%), *Pseudomonas aeruginosa* 5(13%), *Klebsiella* 2(5.5%), *Acinetobacter* 1(2.7%), *Streptococcus pneumoniae* 1(2.7%), *Citrobacter* (3%), *Enterobacter* (3%) (Table 5). The Gram positive isolates are susceptible to Cefazolin 94.11%, Vancomycin 100%, Ciprofloxacin 75.25%, Erythromycin 76.4%

and Clindamycin 82.35% (Figure 1). The Gram negative organisms were mostly sensitive to amikacin, Third generation cephalosporins, gentamicin, piperacillin tazobactam, carbencillin and fluroquinolones like ciprofloxacin (Figure 2).

Off the 23(33.3%) fungal isolates *Fusarium* sps 11(37%) is the predominant organism followed by *Aspergillus flavus* (21.1%), *Aspergillus niger* 4(17%), *Aspergillus fumigatus* 2(18.6%) and *Candida albicans* 1(4.3%) respectively (Table 6).

Table.1 Age and Sex distribution

s.no	Age	Male	Female	Total
1.	0-15	6	9	15
2.	15-30	8	3	11
3.	30-45	10	8	18
4.	45-60	11	4	15
5.	>60	21	20	41

Table.2 Clinical conditions

s.no	Infections	Number of samples
1.	Conjunctivitis	43
2	Blepharitis	1
3.	Dacryocystitis	1
4.	Cornel ulcer	23

Table.3 Injuring agent in case of cornel ulcer

s.no	Nature of agent	Number
1.	foreign body/sand	5
2.	paddy husk	7
3.	thorn	2
4.	others	2
5.	no agent	8

Table.4 Total number of bacterial isolates

s.no	Organisms	Number (%)
1	CONS	16(43.24%)
2	<i>Staphylococcus aureus</i>	10(27%)
3	<i>Pseudomonas aeruginosa</i>	5(13%)
4	<i>Acinetobacter</i>	1(2.7%)
5	<i>Streptococcus pneumoniae</i>	1(2.7%)
6	<i>Klebsiella</i>	2(5.4%)
7	<i>Citrobacter</i>	1(2.7%)
8	<i>Enterobacter</i>	1(2.7%)

Table.5 Total number of fungal isolates

S.no	Organisms	Number (%)
1	<i>Fusarium sps</i>	11(37%)
2	<i>Aspergillus flavus</i>	5(21%)
3	<i>Aspergillus niger</i>	4(17%)
4	<i>Aspergillus fumigatus</i>	2(18.6%)
5	<i>Candida albicans</i>	1(4.3%)

Fig.1 Antibiotic sensitive pattern of gram positive cocci

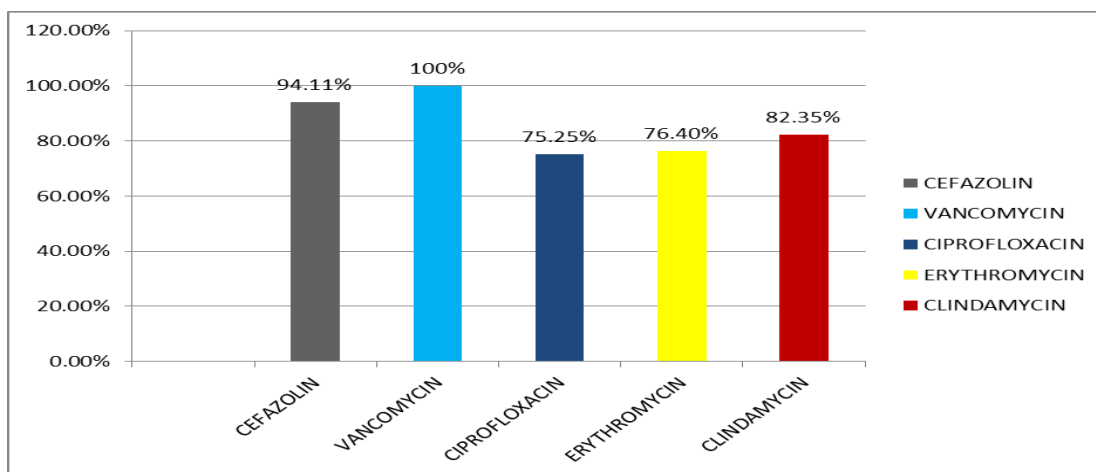
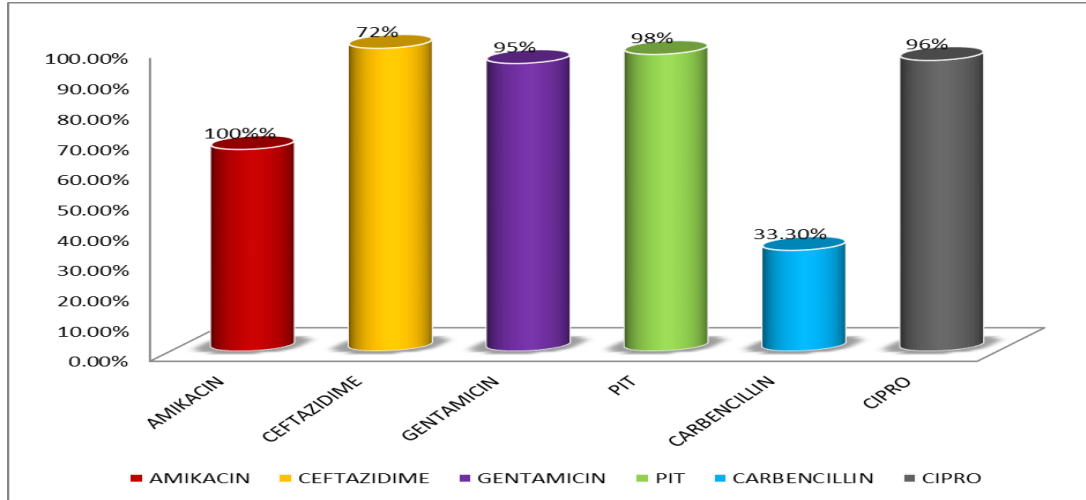


Fig.2 Antibiotic sensitive pattern of gram negative bacilli



A combination of mechanical, anatomic, immunologic and microbiologic factor prevents Ocular infections and do not allow the survival of pathogenic species in eye. However in certain circumstances they gain accesses to the eye and cause infection. Prompt and specific therapy can be instituted if the microbes can be isolated and their susceptibility to the antimicrobials is known. However, the ability to isolate the causative organism depends on a variety of factors including the amount of inoculums, the site from which it is taken, the media used for culture and also on the empirical treatment received before collection of the samples. Hence, the culture-positivity varies from Centre to Centre (*Bharati et al., Bacteriology of Ocular infections.*).

During the study period of one year out of 68 culture positive cases, 56% were in male sex and the remaining 44% were in the female sex. This obvious preponderance of male sex is due to their outdoor activities and is prone for injury. Most of them belong to low socioeconomic group. (*Srinivasan et al., (29)*). In this study a total of 68 cases show culture positivity out of which 54.4% were bacterial isolate and 33.3% were fungal isolates. The most common Bacterial Ocular infection is

Conjunctivitis, Blepharitis and Dacryocystitis. Fungi were identified as the predominant aetiological agent for corneal ulceration (*Sundaram et al.,*). Bacterial conjunctivitis is the most commonly seen external ocular infection as seen in other studies (*Vaughan et al.,*). Out of 37 bacterial isolates, Coagulase negative Staphylococci (48.8%) is the predominant organism causing Conjunctivitis i.e. *Staphylococci epidermidis* causing this infection (*Das et al.,*). The second most organism causing bacterial conjunctivitis is *Staphylococcus aureus* (27%) followed by *Pseudomonas aeruginosa* (13%), *Streptococcus pneumoniae* (2%), *Klebsiella*, *Acinetobacter*, *Citrobacter*, *Enterobacter* 3% each. The causes of bacterial conjunctivitis is the alteration in the normal flora, which can occur by external contamination, by spread from adjacent sites or via blood-born path way and disruption of epithelial layer covering the conjunctiva (*Bauman, 2010*).

The Antimicrobial sensitivity pattern of Gram positive cocci and Gram negative bacilli shows similar results with other studies (*Bharati et al.,*). The Gram positive isolates are susceptible to Cefazolin 94.11%, Vancomycin 100%, Ciprofloxacin 75.25%, Erythromycin 76.4%, Clindamycin 82.3% and

the Gram negative isolates were susceptible to Amikacin 100%, ceftazidime 66.6%, Gentamicin 95%, Ciprofloxacin 96%, piperacillin-tazobactam 98%, carbencillin 33.3%. Resistance and sensitivity based on *in vitro* testing may not reflect the true clinical resistance and response to an antibiotic because of the host factors and penetration of the drug. Vancomycin revealed a highest efficacy against Gram positive cocci isolates compared with other antibacterial agents. Vancomycin is a glycopeptide; it inhibits early stages in the cell wall mucopeptide synthesis and it exhibits greatest potency against Gram positive Ocular isolates. Out of 23 fungal isolates, *Fusarium* sps (37%) is the common organism causing corneal ulceration followed by *Aspergillus flavus* (22%) but in most of the studies *Aspergillus flavus* was isolated as the predominant organism causing corneal ulcer. The prevalence rate from other studies are 25-35% in Pondicherry, 50% in Hyderabad, 64% in Chennai (Venugopal *et al.*,⁽³⁰⁾). The other organisms for corneal ulcers are similar when compared with other studies as *Aspergillus niger* (17%), *Aspergillus fumigatus* (18%) and *Candida albicans* (4%). Venugopal *et al.*, reported that men are more susceptible to corneal ulcer than female. In a study with 3528 cases in Delhi Mycotic Keratitis seems to be prevalent in males, in farmers and the most common predisposing factor remains trauma to the cornea (Chander J Sharma⁽³¹⁾).

In conclusion, coagulase negative Staphylococci frequently causes infection of the conjunctiva and eyelids followed by *Staphylococcus aureus*. Similarly, *Fusarium* sps frequently causes infection of the cornea most commonly corneal ulcer. Infections of the cornea due to filamentous fungi are a frequent cause of corneal damage in developing countries in the tropics and are difficult to treat. Microscopy is an essential tool in the diagnosis of these infections.

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