Original Article

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Microbiology Section

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

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ABSTRACT

Aim: To identify the etiology, incidence and prevalence of External ocular bacterial and fungal infections, and to assess the in-vitro antimicrobial susceptibility to the bacterial isolates.

Material & Methods: This study include 125 patients with external ocular infections treated in the tertiary care hospital-Chettinad Hospital and Research Institute, Chennai, India, between March 2011 to April 2012. The patients were examined by slit-lamp biomicroscopy, and then corneal scrapings, conjunctival swabs and purulent material were collected for cultures, smears and antibiotic sensitivity test by using standard protocols.

Results: Out of 125 patients with external ocular infection, culture positivity was found in 80(64%) patients and rest of 45(36%) patients were culture negative. Among the 80(64%) culture positive patients, 45(56%) patients had conjunctival infections and 35(44%) had keratitis. From conjunctival infections - 49 bacterial isolates were recovered. The predominant bacterial isolate was found to be Coagulase negative *Staphylococci* 21(43%) followed by *Staphylococcus aureus* 12(24%). Among keratitis patients, 10(29%) patients had bacterial infection, 23(66%) patients had fungal infections and 2(5%)patients had mixed infections with bacteria and fungi. The predominant fungus was Fusarium species 12(48%) followed by *Aspergillus flavus* 6(24%). The gram positive isolates were susceptible to Vancomycin 100% followed by Ciprofloxacin 75%. Gram negative isolates were susceptible to Imipenam 100%, Amikacin 100% and Ciprofloxacin 96%.

Conclusion: Coagulase negative *Staphylococci* frequently causes infection of the conjunctiva. 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.

Key Words: External ocular infections, Conjunctivitis, Keratitis, Antibiotic profile

INTRODUCTION

The eye is a unique organ that is almost impermeable to almost all external agents. The defence mechanisms of the eye are the tears which contain several substances (e.g. lysozymes and interferon), the eyelids and eye lashes. 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 [1]. Ocular infections are common and their morbidity can vary from self-limiting, trivial infection to sight- threatening. 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, external hordeolum [2].

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 & blinking mechanism keep their population in check [3]. Microbial keratitis is a potentially vision threatening condition that requires prompt diagnosis and treatment to prevent untoward outcomes. The common fungal genera in Mycotic keratitis are fusarium, Alternaria and Aspergillus spp [4]. Eye trauma is the cause of fungal keratitis.

The bacterial etiology and their susceptibility as well as resistance patterns may vary with geographic location according to the local population [5,6]. To make a rational choice of initial antibiotic therapy, the current trends in the etiology of bacterial ocular infections and their susceptibilities must be updated. The purpose of this study was to identify the etiology, incidence and prevalence of ocular bacterial and fungal infections, and to assess the in-vitro susceptibility of these ocular bacterial isolates to commonly used antibiotics.

MATERIALS AND METHODS

The study included 125 patients with clinically diagnosed external ocular infections such as blepharitis, conjunctivitis, keratitis and dacryocystitis treated in the tertiary care hospital-Chettinad Hospital and Research Institute, Chennai, India, for the period of one year from March 2011 to April 2012. All patients included in this study were examined on the slit-lamp bio-microscope by the ophthalmologist using standard protocols [7]. After detailed ocular examinations, using standard techniques, specimens for culture and smear were obtained by scraping and swabbing the eyelid margin using sterile blade (# 15) on Bard-Parker handle and sterile broth-moistened cotton swabs in case of blepharitis [8,9]. Similarly, specimens were also obtained from scraping the corneal ulcers. Conjuctival cultures were obtained by wiping a broth-moistened swab across the lower conjunctival cul-de-sac in conjunctivitis cases. For cases of dacryocystitis purulent material was collected from everted punta by pressure applied over the lacrimal sac area.

The obtained ocular specimens were subjected to culture onto the sheep blood agar, chocolate agar, Mac conkey agar, Sabouraud's dextrose agar, thioglycollate medium and brain heart infusion broth. In corneal scraping the specimen was cultured on Blood agar in the form of 'c' shape streak. Direct microscopic examinations such as 10% Potassium Hydroxide (KOH) wet mounting, Gram-stain, Kinyoun's acid-fast stain were also done. For fungus identification Slide culture method and LPCB staining were used.

Microbial cultures were considered significant if growth of the same organism was demonstrated on more than one solid phase medium, and/or if there was a confluent growth at the site of inoculation on one solid medium, and/or if growth of one medium to be consistent with direct microscopy findings (that is, appropriate staining and morphology with Gram-stain) and/or if the same organism was grown from repeated specimens [9]. The isolated bacterial strains were identified up to species level by using standard biochemical tests [10].

The antibiotic susceptibility testing was done by the Kirby Bauer disc diffusion method, as per the CLSI guidelines, 2011 [11]. The antimicrobial discs which were used were those of Ampicillin (20µg), Gentamicin (10µg), Amikacin (30µg), Cefazolin (30 µg), Cefuroxime (30µg) Ceftazidime (30µg), Cefotaxime (30µg), Piperacillin/tazobactam(100/10µg), Imipenem (10µg) and Meropenem (10 µg), for the Gram negative bacilli. Penicillin, Ampicillin, Cefoxitin (30µg), Cefotaxime (30µg), Chloramphenicol (30µg), Clindamycin (2µg), Erythromycin (15µg), Oxacillin (1µg), Vancomycin (30µg), Teicoplanin (30µg)), Ciprofloxacin (5µg), Linezolid (30µg) and Tetracycline (30µg) were used to study the susceptibility patterns of the Gram positive cocci . Antibacterial discs were obtained from Hi-Media, (Chennai, India).

RESULTS

Out of 125 patients with external ocular infection culture positivity were found in 80(64%) patients and rest of 45(36%) patients were culture negative. Off the 125 patients, 55(44%) were female patients and 70(56%) were male patients. Male patients were affected more when compared to the female patients. Age and Sex distribution of patients suffering from external ocular infection was shown in [Table/Fig-1]. Both male and Female patients of age group >60, 55(44%) were highly affected with External ocular infections. Among the 100 patients 25 of them were diabetic.

Among the 80 culture positive patients, 45(56%) patients had conjunctival infections such as Conjunctivitis, Blepharitis, Dacryocystitis and 35(44%) patients had Corneal infections, most commonly Keratitis. 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 was due to infection with paddy husk.

Out of 45 culture positive conjunctival infections patients, 41(91%) patients had infection with single species of bacteria and the remaining 4(9%) patients had infection with two type of bacterial species and thus a total of 49 bacterial isolates were recovered. The predominant bacterial isolate was Coagulase negative *Staphylococci* 21(43%) followed by *Staphylococcus aureus* 12(24%).

Among 35 culture positive keratitis patients, 10(29%) patients had bacterial infection, 23(66%) patients had fungal infections and 2(5%) patients had mixed infections with bacteria and fungi. The predominant fungus was Fusarium species 12(48%) followed by *Aspergillus flavus* 6(24%). The Fusarium species colony morphology and microscopic picture- LPCB mount

Sl. No.	Age	Male	Female	Total
1.	0-15	9	11	20 (16%)
2.	15-30	9	4	13 (10%)
3.	30-45	11	9	20 (16%)
4.	45-60	12	5	17 (14%)
5.	>60	29	26	55 (44%)
Total	-	70	55	125

[Table/Fig-1]: Age and Sex distribution of patients suffering from external ocular infections

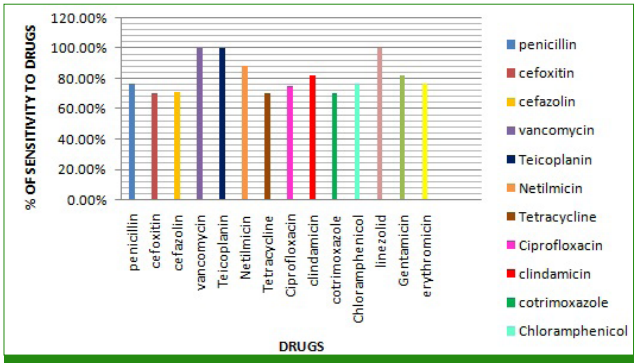
[Table/Fig-2]: Fusarium species colony morphology and Microscopic LPCB mount

	Organism	Conjuctival Infection			Total Conjunctival infection	Keratitis	Total
		Conjuctivitis	Blepharitis	Dacryocystitis			
GPC	CONS	20	1	-	21(43%)	2	23(38%)
	Staphylococcus aureus	11	-	1	12(24%)	4	16(26%)
	Streptococcus pneumonia	3	1	-	4(8%)	3	7(11%)
GNB	*Pseudomonas aeruginosa*	7	-	1	8(16%)	2	10(16%)
	Acinetobacter	1	-	-	1(2%)	-	1(2%)
	Klebsiella	1	-	-	1(2%)	1	2(3%)
	Citrobacter	1	-	-	1(2%)	-	1(2%)
	Enterobacter	1	-	-	1(2%)	-	1(2%)
	TOTAL	45	2	2	49	12	61

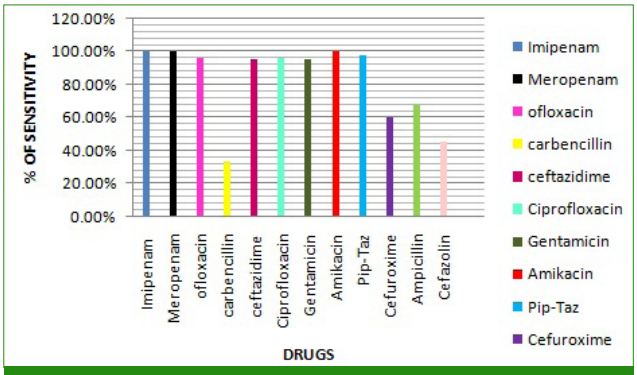
[Table/Fig-3]: Bacterial isolates from External eye infections

Sl. No.	Fungus in Keratitis	Number	%
1.	Fusarium sps	12	48
2.	*Aspergillus flavus*	6	24
3.	Aspergillus niger	4	16
4.	Aspergillus fumigates	2	8
5.	*Candida albicans*	1	4
	TOTAL	25	

[Table/Fig-4]: Fungus isolated in Keratitis



[Table/Fig-5]: Antibiotic sensitive pattern of Gram positive cocci



[Table/Fig-6]: Antibiotic sensitive pattern of Gram negative bacilli

are shown in [Table/Fig-2]. The bacterial isolates recovered from external ocular infections are presented in [Table/Fig-3]. The fugal isolates recovered from Keratitis are shown in [Table/Fig-4]. So total number of organisms isolated from external eye infections was 86, out of which bacterial isolate were 61(71%) and fungal isolates were 25(29%).

The Gram positive isolates were susceptible to Vancomycin 100% and Ciprofloxacin 75.25% shown in [Table/Fig-5]. The Gram negative organisms were mostly sensitive to Amikacin 100%, Imipenam 100% and fluroquinolones like ciprofloxacin 96% shown in [Table/Fig-6].

DISCUSSION

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 [12]. 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 [13], the site from which it is taken, the media used for culture and also on the empirical treatment received before collection of the samples [14]. Hence, the culture-positivity varies from center to center. In this study the culture positivity is 64% which is similar to the study conducted in Arvind Eye Hospital Tamilnadu [15].

In the present study external ocular infections were predominantly seen in male sex due to their outdoor activities, patients of low socio-economic group [16] like farmers and patients above 60 years of age 55(44%). The study conducted by Srinivasan M et al at Madurai observed patients of low socioeconomic group [16] like farmers were more affected by external ocular infections. The study conducted by Rahman et al., [17] showed that 44.4% of patients belonged to the age group of > 60 years.

As seen in Idu F et al., [3] studies Bacterial conjunctivitis was the most commonly seen external ocular infection which was similar in the present study also. The predominant bacterial isolate isolated was Coagulase negative *Staphylococci* 21(43%) which was the commensal of the normal conjunctival flora [18]. The causes of bacterial conjunctivitis was due to the alteration in the normal flora, which can occur by external contamination, by infection spread from adjacent sites or via blood-born path way and disruption of epithelial layer covering the conjunctiva [19].

In this study the Gram positive isolates were susceptible to Vancomycin 100% followed by Ciprofloxacin 75% and Gram negative isolates susceptible to Ciprofloxacin 96% similar to the study conducted in Tirunelveli – South India [20] where Vancomycin 100% susceptible but Ciprofloxacin is 90% susceptible, the other study conducted in Hyderabad showed Ciprofloxacin 70% sensitive [21]. 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 [20]. 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 [20].

Corneal injury was the major cause of corneal ulcer as seen in the study conducted by Chander J Sharma A [22] and Fungi were identified as the predominant aetiological agent for corneal ulceration as in study of Sundaram BM et al., [23]. Both these conditions correlate with the present study. As in the study *Fusarium* species and *Aspergillus flavus* were the commonest organism in corneal ulcers which was similar to the study conducted by Venugopal PL-North India [24].

This article documents the prevalence of bacterial species and fungus causing external ocular infection in a tertiary care hospital along with the drugs susceptible pattern to the bacterial isolates that will help the clinician in prompt treatment of external ocular infections.

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