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Original Article

Microbiology Section

Profile of Microbial Isolates in Ophthalmic Infections and Antibiotic Susceptibility of the Bacterial Isolates: A Study in an Eye Care Hospital, Bangalore

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ABSTRACT

Ocular infections are common and vary from self-limiting to sight-threatening. All the structures of the eye can be infected by various microbes.The present study was undertaken to determine the prevalence of bacterial and fungal infections of the eye and also to assess the antibiotic susceptibility pattern of bacterial isolates at an eye care hospital in Bangalore, India.

Material and Methods: Two hundred thirty five samples were received from infections of the eye-conjunctivitis including dacrocystis, corneal ulcers, endophthalmitis and post-traumatic infections. Culture, Gram's stain and potassium hydroxide (KOH) mount was done for allclinical specimens. Giemsa staining was done occasionally as required. Antibiotic susceptibility testing was performed for bacterial growth.

Results: Out of 235 samples processed, 81(34.5%) yielded bacterial growth and 32 (13.6%) yielded fungal growth. One corneal scraping showed the presence of *Acanthamoeba* by microscopy. Predominant bacterial isolates were-*Staphylococcus* species 36 (39.9%), *Pseudomonas* species 20 (22.2%) and *Escherichia coli* 12 (13.3%). Among 25 fungal isolates, *Aspergillus flavus* 11(44%) and *Fusarium* 8 (32%) predominated. Bacterial strains were susceptible to gatifloxacin (86.4%), tetracycline (65.4%), chlorampenicol (69.1%) and least sensitive to the beta- lactam group like amoxicillin (23.5%).

Conclusion: Conjunctival specimens yielded mainly bacterial growth with *Staphylococcus* species being the predominant isolate followed by *Pseudomonas* species. Maximum fungal isolates were from corneal scrapings with *Aspergillus flavus* and *Fusarium* being predominant. Majority of the bacteria were susceptible to gatifloxacin.

Keywords: Brain Heart Infusion (BHI) broth, Eye infection, Antibiotics

INTRODUCTION

The eye is exposed to the external environment, but is relatively impermeable to microorganisms. Trauma,surgery and systemic diseases can contribute to infections of the eye. Any part of the eye can be infected by microbes from the environment. They can form transient flora or invade the tissue and cause infection [1]. We have done a retrospective analysis of samples collected between June 2010 to May 2011 to know the pattern of microbial infections of the eye and their antibiotic susceptibility.

MATERIAL AND METHODS

A total of 235 specimens from clinically suspected eye infections were processed in the Microbiology laboratory at an eye hospital in Bangalore, India. Conjunctival specimens including pus from dacrocystis were collected with swabs presoaked in Brain Heart Infusion (BHI) broth. Other specimens included corneal scrapings, anterior chamber tap(AC tap), vitreous fluid, post-trauma infections. All the specimens were inoculated immediately on Blood agar, MacConkey's agar and Sabouraud's Dextrose agar are examined by Gram's stain and KOH mount. Giemsa staining was done occasionally as required. The growth was identified by standard laboratory procedures and susceptibilitytesting of the bacterial isolates were performedand interpreted by the Kirby-Bauer method.

RESULTS

Out of 235 specimens processed, 113(48%) showed growth. 81(34.5%) were bacterial and 32(13.6%) were fungal isolates. Conjunctival swabs yielded 39(52%) bacterial isolates. Corneal scrapings grew 20(22.2%) bacterial and 30(33.3) fungal isolates. In addition one corneal sample showed the presence of *Acanthamoeba* by microscopy, which could not be cultured.Vitreous fluid yielded 12(42.9%) bacterial and 1(3.6%) fungal isolates. Post-trauma swabs yielded 2(12.5) bacterial and 1(6.3) fungal isolates [Table/Fig-1]. One post-trauma endophthalmitis sample yielded both *Candida* and *Pseudomonas* from vitreous fluid.

Specimen	Total no. of samples	Bacterial isolates (%)	Fungal isolates (%)	Total growth (%)
Conjunctival swabs	75	39 (52.0)	0	39 (52.0)
Corneal scrapings	90	20 (22.2)	30 (33.3)	50 (55.5)
Vitreous fluid	28	12 (42.9)	1 (3.6)	13 (46.4)
AC tap	26	8 (30.8)	0	8 (30.8)
Post-trauma swabs	16	2 (12.5)	1 (6.3)	3 (18.8)
Total	235	81 (34.5)	32 (13.6)	113 (48.0)

[Table/Fig-1]: Total number of bacterial and fungal isolates from different clinical specimens

Among the 39 conjunctival swabs which yielded growth 14(35%) were Coagulase Negative *Staphylococci* (CONS), 10(25%) were *S. aureus*; 6(15%) were *E.coli*; 3(7.7%) were *Pseudomonas*; with 2(5%) each of *Pneumococcus, Klebsiella* and *Citrobacter.*

The 20 corneal scrapings yielded 6(30%) CONS, 4(20%) *Pseudomonas*; and 2(10%) each of *S.aureus, Pneumococcus, E.coli, Klebsiella* and *Nocardia.*

The 12 vitreous fluid samples yielded 2(16.7%) each of *S.aureus,E. coli, Citrobacter, H.influenzae* and 4(33.3%) *Pseudomonas.* AC tap yielded 2(25%) each of *S.aureus, S.viridans, H.influenzae* and *Acinetobacter*. Post-trauma swabs yielded 2(100%) isolates of *Pseudomonas* [Table/Fig-2].

Specimen	Conjunctival swab%	Corneal scraping%	Vitreous fluid*%	AC tap%	Post-trauma swab%	Total %
CONS	14(35.9)	6 (30.0)	-	-	-	20 (24.6)
S. aureus	10(25.6)	2(10.0)	2(16.6)	2(25.0)	-	16 (19.8)
Pneumococci	2 (5.1)	2(10.0)	-	-	-	4 (4.9)
S. viridans	-	-	-	2(25.0)	-	2 (2.5)
E.coli	6(15.4)	2(10.0)	2(16.6)	-	-	10(12.3)
Klebsiella	2(5.1)	2(10.0)	-	-	-	4(4.9)
Citrobacter	2(5.1)	-	2(16.6)	-	-	4(4.9)
H.influenza	-	-	2(16.6)	2(25.0)	-	4(4.9)
Pseudomonas	3(7.7)	4(20.0)	4(33.3)	-	2(100)	13(16.0)
Acinetobacter	-	-	-	2(25.0)	-	2(2.5)
Nocardia	-	2(10.0)	-	-	-	2(2.5)
Total Isolates	39	20	12	8	2	81

[Table/Fig-2]: Distribution of different bacterial isolates from eye.

Specimen	Corneal scraping (%)	Vitreous fluid* (%)	Post- trauma swab (%)	Total (%)
A. flavus	10(33.3)	-	1(100)	11(34.3)
A. niger	2(6.7)	-	-	2(6.2)
Fusarium	8(26.7)	-	-	8(25.0)
Curvularia	1(3.3)	-	-	1(3.1)
Cladosporium	1(3.3)	-	-	1(3.1)
Acremonium	1(3.3)	-	-	1(3.1)
Candida	-	1(100)	-	1(3.1)
Sterile Mycelia	7(23.3)	-	-	7(21.9)
Total (%)	30(92.8)	1(3.1)	1(3.1)	32

[Table/Fig-3]: Distribution of fungal isolates from eye

Out of 32 fungal isolates, 30(92.8%) were from the cornea which yielded *Aspergillus flavus* 10(33.3), *A.niger* 2(6.7), *Fusarium* 8(26.7) and 1(3.3) each of *Curvularia, Cladosporium* and *Acremonium*. *Acremonium* occurs uncommonly, but unlike infections due to other filamentous fungi, usually affects immunocompetent individuals [2]. One sample each of vitreous fluid and post-trauma swabs yielded *Candida and A. flavus* respectively. The vitreous fluid which yielded *Candida* showed mixed growth along with *Pseudomonas*. There was no fungal isolate from conjunctival swab and AC tap fluid [Table/Fig-3].

Antibiotic susceptibility was done for 81 isolates. The pattern was – gatifloxacin 70(86.4%), chloramphenicol 56(69.1%), ofloxacin 54(66.7%), tetracyclin 53(65.4%), ciprofloxacin 51(62.9%) followed by amikacin 48(59.3%) [Table/Fig-4]. In addition the 13 *Pseudomonas* strains were tested for moxifloxacin and tobramycin to which they were uniformly susceptible (not shown in [Table/Fig-4].

DISCUSSION

Conjunctivitis can be caused by bacteria,fungi,viruses and parasites. Most common bacteria causing acute bacterial conjunctivitis are *Staphylococci, S.pneumoniae, H.influenzae, group A streptococci* and *Neisseria;* however fungal infections of conjunctiva are rare [1]. In our study, among the 39 conjunctival isolates, predominant bacteria were *Staphylococcus species*, out of which 35% were CONS (higher as compared to a study conducted by Tesfaye et al.,) [3] and 25.6% were *S. aureus* (similar to the above study). Others isolates were *E.coli, Pseudomonas, Pneumococcus, Klebsiella* and *Citrobacter;* the pattern of isolation being similar to other studies [4,5]. Neisseria and fungi were not found in our study.

Microbial keratitis is a common sight- threatening ocular infection, caused by bacteria, fungus, viruses and parasites. Fungal keratitis are more common than bacterial keratitis in some parts of the world [6]. In our study, the 90 corneal scrapings yielded 30 (33.3) fungal and 20 (22.2%) bacterial isolates.

Different bacterial agents like *Pneumococci, Staphylococci, Pseudomonas* and *Nocardia* species have been linked with keratitis. Many reports quote CONS as a leading cause of bacterial keratitis. [5,7,8]. In our study, the 20 corneal scrapings yielded 6 (30%) CONS, 4(20%) *Pseudomonas*; and 2 (10%) each of *S.aureus, Pneumococcus, E.coli, Klebsiella* and *Nocardia*.

Fungal isolates causing keratitis include the following: filamentous fungi-*Fusarium* and *Aspergillus* species, dematiaceous fungi-*Curvularia* and *Lasiodiplodia*,and Candida species [9]. Our 30 corneal fungal isolateswere *Aspergillus flavus* 10(33.3%), *A. niger* 2(6.7%), *Fusarium* 8(26.7) and 1(3.3%) each of *Curvularia, Cladosporium* and *Acremonium.* Seven (23.3%) isolates were nonsporulating. It has been mentioned that 9 -14% of fungal isolates may be nonsporulating, requiring PCR based DNA sequencing for identification [9].

Bacterial endophthalmitis occurs due to exogenous infection after trauma; following intraocular surgery or from endogenous systemic infections. The incidence of acute endophthalmitis following cataract surgery range from 0.04-0.5% and common organism is CONS [4,10]. The 12 vitreous fluid samples yielded, 2(16.7%) each of *S.aureus, E.coli, Citrobacter, H.influenzae* and 4(33.3%) *Pseudomonas*. Each of two samples of vitreous fluid from post trauma cases yielded *Candida* and *A. flavus*. One sample of vitreous fluid yielded a mixed growth of *Pseudomonas* and *Candida* [Table/Fig- 3].

Antibiotic susceptibility pattern of 81 bacterial isolates showed sensitivity to Gatifloxacin [11] 70(86.4%), Chloramphenicol 56

Organism	No.	P	Am	E	Cf	Of	Gf	Nx	T	C	Ak
CONS	20	0	2	4	13	12	18	9	16	16	8
S.aureus	16	0	6	0	4	6	15	2	14	12	8
S. pneumococci	4	3	4	4	4	4	4	4	4	4	2
S. viridans	2	2	2	2	0	2	2	0	2	2	0
E.coli	10	0	0	1	8	8	8	6	5	8	7
Klebsiella	4	0	0	0	4	2	1	2	2	2	4
Citrobacter	4	0	0	0	2	2	4	2	0	4	4
Pseudomonas	13	3	1	5	9	10	10	7	4	4	7
Acinetobacter	2	0	0	0	2	2	2	2	0	0	2
Nocardia	2	0	0	2	1	2	2	0	2	0	2
H. influenzae	4	4	4	4	4	4	4	4	4	4	4
Total %	81	12 14.8	19 23.5	22 27.2	51 62.9	54 66.7	70 86.4	38 46.9	53 65.4	56 69.1	48 59.3

[Table/Fig-4]: Antibiotic susceptibility pattern of bacterial isolates
(penicillin-P, Amoxicillin-Am, erythromycin-E, ciprofloxacin-Cf, ofloxacin-Of, Norfloxacin-Nx, gatifloxacin-Gf, tetracycline-T, chloramphenicol-C, amikacin-Ak)

(69.1%), Ofloxacin 54 (66.7%), Tetracyclin 53(65.4%), Ciprofloxacin 51(62.9%) followed by Amikacin 48(59.3%). Both Gram positive cocci and Gram negative bacilli were highly susceptible to gatifloxacin chloramphenicol and ofloxacin, but less susceptible in comparison with another study from South India which shows sensitivity of gatifloxacin (83.8%), chloramphenicol (83.7%) and ofloxacin (80.8%) [2]. This may be an indication of developing drug resistance. Penicillin showed resistance of 90% similar to another study which showed 90-100% resistance [3].

CONCLUSION

Due to the emergence of drug resistance it is imperative that all ophthalmological samples must be tested for antibiotic resistance as far as possible, and avoid indiscriminate use of over the counter antibiotic eye formulations and also it is important to know the changing pattern of pathogens.

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