AEROBIC BACTERIOLOGICAL AND MYCOLOGICAL STUDY OF OPHTHALMIC INFECTIONS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSPITAL AT RAJAHMUNDRY, ANDHRA PRADESH

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ABSTRACT

BACKGROUND
Eye is one of the most vulnerable area of infection and is constantly exposed to variety of pathogens, but infections occur when the normal defence of the eye is compromised. The bacterial and fungal aetiologies and the antimicrobial susceptibility pattern vary according to geographical and regional location. In this region of East Godavari District, no recent data is available regarding this, which highly justifies the study.

AIMS AND OBJECTIVES
To identify the prevalent bacteria and fungus causing ocular infection that prevail in the community. To characterise the predisposing factors leading to ocular infection. To establish effective empiric therapy for control of common ocular bacteriological infection by the antibiotic sensitivity study.

MATERIALS AND METHODS
The present study was conducted in Ophthalmology OPD/IPD of GSL Medical College and General Hospital during the period from October 2011-February 2013. Samples collected under aseptic precautions. A total number of 170 culture-positive ocular infections out of 210 clinically diagnosed cases served as study group and 30 culture-positive cases out of 40 normal persons served as control group.

RESULTS
Among the 170 culture-positive cases, 72.95% yielded pure bacterial isolates, 22.94% yielded pure fungal isolates and 4.11% yielded a mixed culture. The overall male: female ratio is 1.54:1. The Gram-positive cocci were resistant to clindamycin and penicillin and Gram-negative bacilli were highly resistant to gentamicin, aztreonam and 3rd generation cephalosporins.

CONCLUSION
More importance should be given to microbiological evaluation of each and every ocular infection along with their antimicrobial susceptibility study in order to treat them with appropriate antibiotics thereby preventing ocular morbidity to formulate empiric therapy guidelines to prevent further emergence of resistance of the existing sensitive drugs.

KEYWORDS
Ocular Infections, Gram-positive Cocci, Gram-negative Bacilli, Fungal Isolates, Antimicrobial Susceptibility Pattern, Empirical Therapy.

HOW TO CITE THIS ARTICLE: Rao KS, Basu R, Rao SGV. Aerobic bacteriological and mycological study of ophthalmic infections and their antimicrobial susceptibility pattern in a tertiary care hospital at Rajahmundry, Andhra Pradesh. J. Evid. Based Med. Healthc. 2016; 3(48), 2409-2414. DOI: 10.18410/jebmh/2016/530

INTRODUCTION: Eye is constantly exposed to variety of pathogens, but infections occur when the normal defence of the eye are compromised. Ocular infections may be caused by bacterial, viral, chlamydial, fungal and acanthamoebic agents. Most prevalent infections of the eye that are commonly encountered include conjunctivitis, keratitis, stye, blepharitis and dacryocystitis.

Financial or Other, Competing Interest: None.
Submission 21-05-2016, Peer Review 04-06-2016,
Acceptance 13-06-2016, Published 15-06-2016.
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DOI: 10.18410/jebmh/2016/530

Indiscriminate usage of broad spectrum antibiotics and corticosteroids has led to the emergence of drug-resistant strains of Gram-negative bacteria and fungus among the leading causes of ocular infections. The bacterial and fungal aetiologies and the antimicrobial susceptibility pattern may vary according to geographical locations. Therefore, up-to-date information is essential to treat the cases appropriately and to start the empirical therapy for the clinically similar cases before culture report is obtained.

The related studies done in other areas revealed high incidence of multidrug resistance in ocular pathogens leaving the ophthalmologists with very few choice of drugs for treating ocular infections. In this region of East Godavari district, no recent data is available on the spectrum of bacterial and fungal aetiologic agents responsible for ocular infection and the antimicrobial susceptibility pattern of the bacterial isolates to commonly used antibiotics, which highly justifies the purpose of this study.

MATERIALS AND METHODS: A study was conducted on 170 culture-positive cases out of 210 different ophthalmic infections who attended the Ophthalmology Outpatient Department (OPD) or Inpatient Department (IPD) of GSL Medical College and General Hospital during October 2011-February 2013 as the study group whereas 30 culture-positive cases out of 40 normal individuals without any obvious ocular symptoms were included as control group.
In the study group, sample collection was done under aseptic precautions, which include ophthalmic swabs, aspirations by aqueous tap in cases of hypopyon, corneal scrapings using Bard Parker blade no-15 in keratitis and in dacryocystitis cases, if any discharge expressed on pressing the medial canthus of eye from the punctum was collected by sterile swab as specimen; in cases of dacryocystectomy, the dissected tissues or sacs were collected from operation theatre, and conjunctival swabs under aseptic precautions were taken from the control group.

Samples were Processed by the Following Procedures:

- Gram-staining and KOH mounting.
- Inoculation on to blood agar, chocolate agar and Sabouraud dextrose agar (SDA).

Bacterial Species Isolated were Subjected to Various Biochemical Reactions like:

- Catalase test, coagulase test, bile solubility test, optochin sensitivity test, etc, for Gram-positive cocci.
- Oxidase test, indole production test, methyl red reaction, Voges-Proskauer test, urease test etc., for Gram-negative bacilli.
- Fungal isolates on SDA were subjected to LPCB (Lactophenol Cotton Blue) mount, germ tube test, slide culture test etc.

RESULTS: Out of total number of 210 ophthalmic samples from different clinically diagnosed ophthalmic infections, 170(80.95%) culture-positive cases were obtained which constituted the study group whereas 30(75%) culture-positive cases were obtained out of 40 normal individuals without any obvious ocular symptoms who served as control group.

	Culture-positive N=200	Culture-negative N=50
Clinically Infective Population n=210(100%)	170(80.95%) [Study Group]	40(19.05%)
Normal Population n=40(100%)	30(75%) [Control Group]	10(25%)

Table 1: Culture-Positivity among Infective and Normal Population

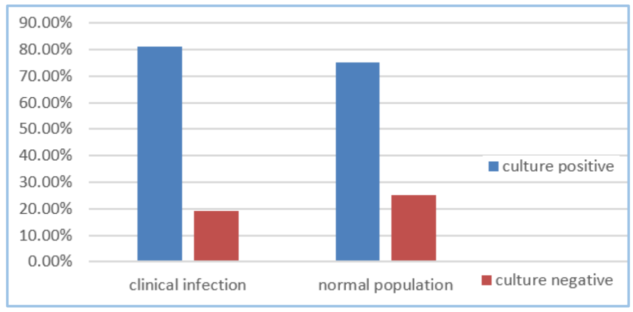


Fig. 1: Culture-Positivity among Clinically Infective and Normal Population

Table 2 shows the number of ocular samples collected from various infective conditions

Sl. No.	Ocular Samples	Number (%)
1.	Corneal Scrapings	70(41.18%)
2.	Conjunctival Swabs	32(18.82%)
3.	Swabs from Non-Conjunctival areas (Include Swabs from Dacryocystitis Cases) and Aspirations by Aqueous Taps	48(28.23%)
4.	Tissues or Dacryocystectomy Sacs	20(11.77%)
	Total	**170(100%)**

Table 2: Specimen-wise Distribution of Study group (n=170)

Table 3 Represents Area-Wise Distribution of Culture-positive Individuals

Culture-positive Cases	Rural No. (%)	Urban No. (%)
Study Group n = 170 (100%)	140(82.35%)	30(17.65%)
Control group n = 30 (100%)	19(63.33%)	11(36.67%)
P value = 0.0260* (<0.03) *Statistically significant association		

Table 3: Area-wise Distribution of Culture-Positive Individuals

The age-wise distribution of culture-positive cases in study group and control group is presented in Figure 2.

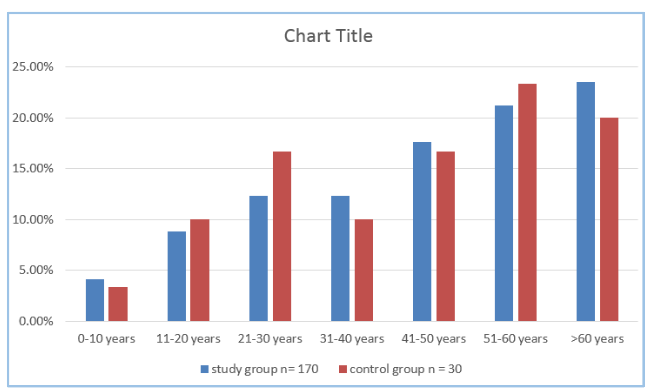
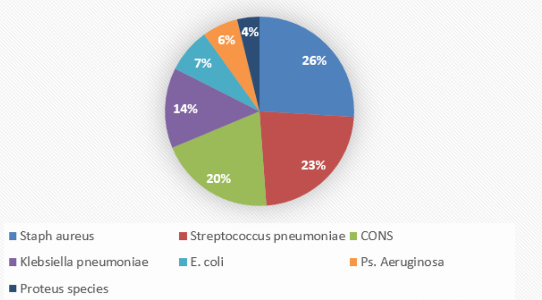


Fig. 2: Age-wise Distribution of Study and Control Groups

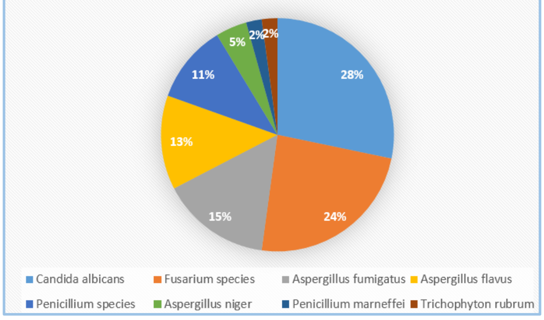
Among the 170 culture-positive specimens, 124(72.95%) were pure bacterial isolates, 39(22.94%) were pure fungal isolates and 7(4.11%) yielded a mixed culture. The overall male: female ratio is 1.54:1. The most prevalent bacteria causing ophthalmic infections in this study is Staphylococcus aureus (25.95%) followed by Streptococcus pneumoniae (22.9%), Coagulase-Negative Staphylococcus (CONS) (19.85%), Klebsiella pneumoniae (13.74%), Escherichia coli (7.63%), Pseudomonas aeruginosa (6.11%) and Proteus species (3.82%). (Figure: 3).

Figure 3. Aerobic bacterial etiology of ocular infections



The most common fungal aetiology revealed in this study was Candida albicans (28.26%) followed by Fusarium species (23.91%), Aspergillus fumigatus (15.21%), Aspergillus flavus (13.05%), Penicillium species (10.87%), Aspergillus niger (4.34%), Penicillium marneffei and Trichophyton rubrum of 2.17% each. (Figure 4).

Figure 4. Fungal etiology of ocular infections



Sl. No.	Name of Organisms	No. (%)
1.	Coagulase-negative staphylococcus (CONS)	12(40%)
2.	Staphylococcus aureus	9(30%)
3.	Streptococcus pneumoniae	6 (20%)
4.	Aspergillus fumigatus	2(6.67%)
5.	Aspergillus flavus	1(3.33%)
Total		**30(100%)**

Table 4: Isolates Obtained from Control Group (n=30)

The dacryocystitis cases in total are 50, out of which 13(26%) were acute cases with male and female distribution of 4(8%) and 9(18%) respectively; 34(68%) were chronic cases with male and female distribution of 12(24%) and 22 (44%) respectively and 3(6%) cases were congenital among which 1(2%) was male and 2(4%) were females. The overall male: female ratio observed was 0.51:1

Among the bacterial aetiologies, Staphylococcus aureus was the commonest bacterial pathogen causing keratitis, dacryocystitis and blepharitis; the commonest fungal agent causing keratitis was Aspergillus species; Candida albicans was the commonest fungal agent causing dacryocystitis and conjunctivitis. Among all ocular infections, trauma was the most common predisposing factor. The antimicrobial susceptibility patterns of the bacterial isolates are presented in Tables 5 and 6.

	Staph aureus n=34		**CONS n=30**		**Str. Pneumoniae n=26**	
	Sensitive No. (%)	**Resistant No. (%)**	**Sensitive No. (%)**	**Resistant No. (%)**	**Sensitive No. (%)**	**Resistant No. (%)**
Penicillin G	5(14.71%)	29(85.29%)	8(26.67%)	22(73.33%)	11(43.31%)	15(57.69%)
Piperacillin+Tazobactam	25(76.08%)	9(23.92%)	25(83.33%)	50(16.67%)	18(69.23%)	8(30.77%)
Oxacillin	19(55.88%)	15(44.12%)	21(70%)	9(30%)	18(69.23%)	8(30.77%)
Vancomycin	34(100%)	0(0%)	30(100%)	0(0%)	26(100%)	0(0%)
Ciprofloxacin	18(52.94%)	16(47.06%)	20(66.67%)	10(33.33%)	18(69.23%)	8(30.77%)
Levofloxacin	31(91.18%)	3(8.82%)	23(76.67%)	7(23.33%)	22(84.62%)	4(15.38%)
Gatifloxacin	30(88.23%)	4(11.77%)	26(86.67%)	4(13.33%)	21(80.77%)	5(19.23%)
Erythromycin	13(38.24%)	21(61.76%)	7(23.33%)	23(76.67%)	18(69.23%)	8(30.77%)
Clindamycin	20(58.82%)	14(41.18%)	18(60%)	12(40%)	9(34.61%)	17(65.38%)
Gentamycin	14(41.18%)	20(58.82%)	13(43.33%)	17(56.67%)	20(76.92%)	6(23.08%)
Amikacin	18(52.94%)	16(47.06%)	22(73.33%)	8(26.67%)	22(84.62%)	4(15.28%)
Tobramycin	30(88.23%)	4(11.77%)	26(86.67%)	4(13.33%)	23(88.46%)	3(11.54%)
Chloramphenicol	20(58.82%)	14(41.18%)	18(60%)	12(40%)	20(76.92%)	6(23.08%)
Co-trimoxazole	20(58.82%)	14(41.18%)	18(60%)	12(40%)	16(61.54%)	10(38.46%)

Table 5: Antibiogram of Gram-positive Cocci

	Klebsiella Pneumoniae n=18		Escherichia coli n=10		Proteus Species n=5		Pseudomonas Aeruginosa n=8	
	Sensitive No. (%)	Resistant No. (%)	Sensitive No. (%)	Resistant No. (%)	Sensitive No. (%)	Resistant No. (%)	Sensitive No. (%)	Resistant No. (%)
Ampicillin	7 38.89%	11 61.11%	3 30%	7 70%	1 20%	4 80%	2 25%	6 75%
Piperacillin	10 55.56%	8 44.44%	6 60%	4 40%	3 60%	2 40%	5 62.5%	3 37.5%
Piperacillin/tazobactam	14 77.78%	4 22.22%	8 80%	2 20%	4 80%	1 20%	7 87.5%	1 12.5%
Ceftriaxone	4 22.22%	14 77.78%	2 20%	8 80%	1 20%	4 80%	2 25%	6 75%
Cefotaxime	4 22.22%	14 77.78%	3 30%	7 70%	1 20%	4 80%	3 37.5%	5 62.5%
Ceftazidime	5 27.78%	13 72.22%	4 40%	6 60%	2 40%	3 60%	2 25%	6 75%
Cefepime	11 61.11%	7 38.89%	6 60%	4 40%	3 60%	2 40%	5 62.5%	3 37.5%
Imipenem	14 77.78%	4 22.22%	8 80%	2 20%	5 100%	0 0%	7 87.5%	1 12.5%
Aztreonam	8 44.44%	10 55.56%	6 60%	4 40%	3 60%	2 40%	5 62.5%	3 37.5%
Ciprofloxacin	11 61.11%	7 38.89%	7 70%	3 30%	3 60%	2 40%	4 50%	4 50%
Levofloxacin	14 77.78%	4 22.22%	9 90%	1 10%	4 80%	1 20%	6 75%	2 25%
Gatifloxacin	15 83.33%	3 16.67%	8 80%	2 20%	5 100%	0 0%	7 87.5%	1 12.5%
Gentamycin	9 50%	9 50%	6 60%	4 40%	3 60%	2 40%	4 50%	4 50%
Amikacin	12 66.67%	6 33.3%	7 70%	3 30%	3 60%	2 40%	5 62.5%	3 37.5%
Tobramycin	16 88.89%	2 11.11%	9 90%	1 10%	5 100%	0 0%	6 75%	2 25%

Table 6: Antibiogram of Gram-negative Bacilli

The Gram-positive cocci were resistant to Clindamycin and penicillin and the Gram-negative bacilli being resistant to gentamicin, aztreonam and third generation cephalosporins.

DISCUSSION: The present study was undertaken to study and evaluate aerobic bacteria and fungus causing ocular infections along with antibiotic susceptibility pattern of the bacterial isolates. Among different ocular infections obtained, the dacryocystitis cases were taken as special reference in this study as it is an important cause of ocular morbidity. The culture positivity rate (80.95%) of the study group seen in our study was very close with the studies by Tewelde Tesfaye et al and M. Jayahar Bharathi et al of 74.7% and 69.8% respectively.(1,2)

Among the 170 cases of the study group, 140(82.35%) were from rural population and 30(17.65%) were from urban population whereas among 30 control group 19(63.33%) were from rural and 11(36.67%) were from urban area. The association was proved to be statistically significant (p value=0.026). the findings were closely corroborated with the study of Reema Nath et al which revealed 87.9% rural and 11.5% urban population involved in their study.(3)

As represented in figure 2, it establishes that culture positivity was more with advancement of age as immunity tend to decrease with old age, thereby facilitating growth of pathogens yielding more culture positivity and the observations closely corroborated with the studies by Gulnaz Bashir et al, M Jayahar Bharathi et al and Khokar N. et al(1,4,5)

The overall male:female ratio is 1.54:1. The observations revealed that in both bacteriological and fungal aetiologies of ocular infections, the males were more affected than females; the male preponderance may be due to more exposure to external environment in comparison to females. The findings were consistent with various studies from India by Reema Nath et al, Chowdary A. et al, Chandar J. et al(3,6,7)

The prevalence of bacteriological (72.95%) and fungal (22.94%) aetiologies in our study were corroborated closely with the study by M. Jayahar Bharathi et al which observed 58.8% of bacterial and 10.3% of fungal prevalence among ocular infections. The Staphylococcus aureus was noted in general to be the most common isolate among all the ocular infections observed in this study which is corroborated by various other studies in India and abroad by M. J. Bharathi et al, Prakash et al, Tewelde Tesfaye et al, Ubani U.A. et al, Ogbolu, D.O. et al etc.(1,2,8,9,10)

The most common fungal aetiology among all the ocular infections revealed in this study was Aspergillus species. The fungal agents responsible for individual ocular infective conditions in this study revealed that Aspergillus (32.6%) were the most common fungal isolates from keratitis cases, in dacryocystitis Candida albicans (4.34%) was the most common isolate followed by Aspergillus fumigatus (2.18%); in conjunctivitis, only Candida albicans was isolated; no fungal pathogen was isolated from other ocular infections.

The normal flora observed among the control group mostly corroborated with the pathogens isolated from the study groups proving that in many cases endogenous sources are the major cause of ocular infections. The probable predisposing factors as per presentation and patients' history favouring ocular infections in this study include trauma (35.29%), nasolacrimal duct obstruction (29.41%), Natural infections (22.94%), Contact lens use (5.88%), post-operative infection (4.71%), Congenital and Foetomaternal transmission (1.18%) and Cranial Nerve palsy (0.59%).

The chronic dacryocystitis was most common (68%) compared to acute type (26%) and congenital type(6%) which were corroborated by Prakash R. et al who observed 63.75% of chronic type, 25% acute type and 11.25% congenital type of dacryocystitis in their study. Increased incidence of dacryocystitis cases in higher age groups may be due to reduced immunity with advancement of age. All these findings were closely corroborated with the study by Dr. Jyothi Bhuyan et al[11] Overall, Staphylococcus aureus 12% was the most common isolate in acute dacryocystitis whereas as CONS (14%) was the most common isolate in chronic dacryocystitis. All these findings were consistent with the studies by Prakash R. et al, Dr. Jyothi Bhuyan et al, Radhakrishna Mandal et al, Chaudhari M.et al.[8,11,12,13]

Overall result of antibiogram of Gram-positive cocci in the present study revealed that in addition to 100% sensitivity to Vancomycin all were highly sensitive to Tobramycin, Levofloxacin, Gatifloxacin and Piperacillin/Tazobactam with decreased susceptibility or resistance to Ciprofloxacin, Gentamycin, Amikacin and Clindamycin which closely corroborates with the studies by M.J. Bharathi et al, Kowalski RP et al[14] and differed with the study by Anand AR et al which observed high susceptibility pattern with Gentamycin and Ciprofloxacin.

Overall antibiogram of Gram-negative bacilli revealed that all were highly susceptible to Tobramycin, Gatifloxacin, Levofloxacin, Imipenem whereas high resistant patterns were observed with Gentamycin, Aztreonam and 3rd generation Cephalosporins. All these observations were closely corroborated with the studies by Xuguang Sun et al[15], Green M. et al[16]etc. and not in agreement with studies by Kim Usha et al. The relationship between antibiotic use and drug resistance is complex. Improper selection of antibiotics, inadequate dosage and poor compliance to therapy may play important role in increasing resistance.[1]

The bacterial sensitivity to various antimicrobial agents varies from place to place and time to time.[17,18] Therefore, the changing spectrum of microorganisms involved in ocular infection in the emergence of acquired microbial resistance dictate the need for continuous surveillance to guide empirical therapy.[17,18,19]

CONCLUSION: In the present scenario, it has been observed that different ocular infective conditions have varying aetiologies; in addition to commensal organisms, various other agents may also be the causative agents of ocular infections depending on age, sex or multiple predisposing factors.

The different bacterial agents have shown emergence of resistance to various commonly used antibiotics rendering them difficult to control if not diagnosed and treated properly in time. More importance should be given to microbiological evaluation of each and every ocular infection along with their antimicrobial susceptibility study in order to treat them with appropriate antibiotics thereby preventing ocular morbidity to formulate empiric therapy guidelines to prevent further emergence of resistance of the existing sensitive drugs.

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