VOLUME - 11, ISSUE - 08, AUGUST - 2022 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper | Clinical Microbiology

A STUDY OF BACTERIAL PATHOGENS CAUSING OPHTHALMIC INFECTIONS AND THE ANTIBIOTIC RESISTANCE PATTERN OF THE BACTERIAL ISOLATES AT A TERTIARY EYE CARE HOSPITAL IN HYDERABAD -INDIA

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ABSTRACT

Background & Aim: Ocular infections are common worldwide and vary from self-limiting to sight threatening diseases. The present study was undertaken to determine the prevalence of bacterial infections of the eye and to assess the aetiology and in vitro antibiotic susceptibility pattern of bacterial isolates to the generally used antibiotics at a Tertiary Eye Care Hospital. **Material And Methods:** A total of 530 samples were collected from patients with various infections of the eye like keratitis, conjunctivitis, dacryocystitis, blepharitis, hordeolum, and endophthalmitis and processed. All the clinical samples were subjected to Gram's staining, potassium hydroxide (KOH) mount examination, and later inoculated on to culture media. Antibiotic susceptibility testing was performed for bacterial growth. **Results:** Out of total 530 samples that were processed, 273 samples were positive for bacterial culture. Out of this, culture positivity was most seen in keratitis cases. The predominant bacterial species isolated was Coagulase Negative Staphylococcus. Among Gram negative bacteria Pseudomonas aeruginosa was most commonly isolated species. All the isolates were highly susceptible to fluoroquinolones i.e., Gatifloxacin (80%), Ofloxacin (60%) and protein synthesis inhibitor like Chloramphenicol (65%.), and among all antimicrobials least susceptibility was seen with Amikacin. **Conclusion:** To alleviate the burden of bacterial ocular infections, monitoring and studying the challenges of the antibiotic susceptibility testing and the patterns of antimicrobial resistance could help in the management, selection and evolving empiric treatment guidelines

KEYWORDS : Ophthalmic Infections, gram positive cocci, Pseudomonas aeruginosa, fluoroquinolones

INTRODUCTION

Eye is a distinctive organ with strong defence mechanisms that is impermeable to almost all external organisms, and invasions occur only when these barriers are broken by either infection or injury.

In the eye tear film production will keep the cornea and conjunctiva moist and washes away debris and noxious irritants, and prevents infection due to presence of antibacterial substances, these mechanisms can specifically reduce bacterial colonization of the ocular surface [1]. At birth bacteria form the normal microbial flora of the external ocular surface while the inner parts of the eye remain sterile.[2] Several protective mechanisms operate on the eye surface and prevent eye infections; however, a breach in surface epithelium due to trauma or lowering of local or systemic immune response may predispose the eye to bacterial infections [3], trauma, surgery and systemic diseases also contribute to infections of the eye. Besides external invasion of eye by microbes, micro-organisms that are carried by the blood stream may also invade the eye and cause infection.

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 [4]. Bacteria are generally associated with many types of ocular infections such as conjunctivitis, keratitis, blepharitis, hordeolum, dacryocystitis and endophthalmitis. Precise diagnosis with pertinent expeditious therapy will trivialize ocular morbidity and curtail permanent loss of vision.

Incidental to the geographical area of the patient, occupation immune condition it has been observed that the bacterial profile and antibiotic susceptibility of ocular infections will differ.

The purpose of this study is to identify the bacterial pathogens responsible for the development of ocular infections and to determine their in-vitro susceptibilities to commonly used antibacterial agents in our hospital.

MATERIALS AND METHODS

This is a retrospective institutional ethics committee approved study, conducted in a tertiary eye care hospital in Hyderabad, India during the period from August 2020 to July 2021.

A total of 530 patients with clinically suspected eye infections were included in this study and all the patients were examined on the slit-lamp bio-microscope and diagnosed as having ocular infection by ophthalmologist using standard procedures.

Following various standardised protocols, specimens for culture and smear were obtained from these clinically diagnosed eye infections cases and sent immediately to Microbiology laboratory in the hospital for processing.

Sample collection: In case of Blepharitis specimens were obtained using sterile moistened cotton swabs, corneal scrapings for keratitis cases were taken using no .15 parker blade under topical anaesthesia, for Conjunctivitis cases samples were obtained by wiping a sterile moistened swab across the lower conjunctival cul-de-sac and in cases of Hordeolum the abscesses were incised and the drained pus was obtained.

For cases of Dacryocystitis purulent material was collected from everted Punta by pressure applied over the lacrimal sac area, and surgically excised lacrimal sac was also collected. Similarly for Patients suspected of infectious Endophthalmitis, specimens from anterior chamber and vitreous fluids were obtained.

Microbiological processing:

All the clinical samples were subjected to direct Gram's staining, potassium hydroxide (KOH) mount examination, and later inoculated into thioglycolate medium and brain heart infusion broth as well as on to Seaboard's dextrose agar (SDA) for the fungal growth. Following in house protocol all the specimens showing turbidity, were sub cultured on to bacterial culture media like the Sheep Blood agar, Chocolate agar and Macon key agar. The isolated bacterial strains were identified up to species level using standard biochemical tests. In-vitro susceptibility testing was performed by Kirby-Bauer disc diffusion method and results were interpreted as per the CLSI guidelines

RESULTS:

A total of 530 samples were collected from the patients diagnosed as having ocular infections and attending Sarojini Devi Eye Hospital a tertiary eye care hospital in Hyderabad. Out of 530 samples processed, 273(51.51%) yielded pure bacterial growth and out of the rest 128 (24.16%) showed fungal growth and 89 (16.79%) showed mixed growth and in 40(7.54%) cases there was no growth. (Table 1)

Among the 273 bacterial culture positive samples,153 (56.05%) processed samples were from Keratitis cases, 64 (23.44%) were from Conjunctivitis cases, 20 (7.33%) were from cases of Dacrocystitis, 18 (6.59%) were cases of Blepharitis,15 (5.49%) was from Hordeolum cases and 03 (1.1%). from Endophthalmitis cases. (Table 2)

The most common ocular infection was keratitis and among the153 samples taken from Keratitis cases which yielded bacterial growth 87 were Coagulase Negative Staphylococcus ,23 was staphylococcus aureus ,18 were Streptococus pneumoniae,16 was Pseudomonas aeruginosa, in 04 cases Klebsiella pneumoniae was isolated, from 03 samples Escherichia coli was grown and from 02 samples Citrobacter species were isolated.

Out of 64 conjunctivitis samples which were culture positive, the bacteria that was isolated were 33 Coagulase Negative Staphylococcus, 17 were Staphylococcus aureus, 09 were Streptococus pneumoniae from 03 samples Escherichia coli was isolated and 02 were Klebsiella pneumoniae.

Among the 20 Dacryocystitis cases which yielded bacterial growth, 12 were Coagulase Negative Staphylococci, 04 were Staphylococcus aureus, 02 were Streptococus pneumoniae, Escherichia coli 01 and Klebsiella pneumoniae 01.

Out of 18 Blepharitis cases that showed culture positivity, Coagulase Negative Staphylococcus were 02, 14 were Staphylococcus aureus, and 01each were species of Escherichia coli and Klebsiella pneumoniae.

Among the 15 Hordeolum case which showed bacterial growth 09 cases were of Coagulase Negative Staphylococci, 05 Staphylococcus aureus cases and one of Streptococus pneumoniae.

In case of 03 endophthalmitis case which showed bacterial growth, one each of Staphylococci aureus, Pseudomonas aeruginosa and Escherichia coli were isolated (Table3 &4)

The in-vitro antimicrobial sensitivity pattern of all the bacterial isolates was performed by Kirby-Bauer disc diffusion method and results were interpreted as per the CLSI guidelines (Table 5).

All Gram-positive bacteria showed maximum sensitivity to Gatifloxacin; and in the descending order to the following antibiotics, Chloramphenicol; Ofloxacin; Ceftazidime; Ciprofloxacin and the least sensitivity to Amikacin (Table 6)

Table 1: Distribution Of Total And Culture Positive Samples

Total Samples	530
Pure Bacterial growth	273 (51.51%)
Pure fungal growth	128 (24.15%)
Mixed growth	89 (16.79%)
No growth	40 (7.55%)

Table 2: Ocular Infections With Bacterial Culture Positivity

Ocular Disease	No. of Cases	Percentage
Keratitis	153	56.05%
Conjunctivitis	64	23.44%
Dacrocystitis	20	7.33%
Blepharitis	18	6.59%
Hordeolum	15	5.49%
Endophthalmitis	03	1.1%

Total Culture Positive Cases: 273

Table 3: Pattern Of Ocular Infections And The Distribution Of Isolates With Percent

Organism	KERATITIS (153)	CONJUNCTIVITIS (64)	DACRO CYSTITIS (20)	BLEPHARITI (18)	HORDEOLUM (15)	END (03)
Coagulase Negative Staphylococcus (143)	87 (60.84%)	33 (23.08%)	12 (8.39%)	02 (1.40%)	09 (6.29%)	0
Staphylococcus aureus (64)	23 (35.93%)	17 (26.56%)	04 (6.25%)	14 (21.88%)	05 (7.81%)	01
Streptococus pneumoniae (30)	18 (60%)	09 (30%)	02 (6.67%)	-	01 (3.33%)	-
Pseudomonas aeruginosa (17)	16 (94.12%)	-	-	-	-	01 (5.88)
Escherichia coli (09)	03 (33.33%)	03 (33.33%)	01 (11.11%)	01 (11.11%)	-	01 (11.11%)
Klebsiella pneumoniae (08)	04 (50%)	02 (28.57%)	01 (14.29%)	01 (14.29%)	-	-
Citrobacter species (02)	02 (100%)	-	-	-	-	-

Table 4: Bacteria Isolated And Their Percent

ORGANISM ISOLATED	NUMBER	PERCENT
Coagulase Negative Staphylococcus	143	52.38%
Staphylococcus aureus	64	23.44%
Streptococus pneumoniae	30	10.99%
Pseudomonas aeruginosa	17	6.23%
Escherichia coli	09	3.30%
Klebsiella pneumoniae	08	2.93%
Citrobacter species	02	0.73%

Table 5: Sensitivity Pattern Of Bacterial Isolates

Bacterial Isolates	NO.	Gat	CHL	OF	CFZ	CIP	AK
Coagulase Negative Staphylococcus (CONS)	143	118	83	72	21	78	08
Staphylococcus aureus	64	56	58	38	17	17	04
Streptococus pneumoniae	30	21	20	30	22	30	01
Pseudomonas aeruginosa	17	12	08	14	15	07	02
Escherichia coli	09	06	05	05	05	06	03

Klebsiella pneumoniae	08	03	02	03	05	04	02
Citrobacter species	02	02	01	01	02	0	02
TOTAL	273	218 (80.15%)	177 (65.07%)	163 (59.92%)	87 (31.98%)	142 (52.21%)	22 (8.08%)

GA: Gatifloxacin; CHL: Chloramphenicol; OF: Ofloxacin; CFZ: Ceftazidime; CIP: Ciprofloxacin; AK: Amikacin

Table 6 Antimicrobial Susceptibility Pattern Of All Bacterial Isolates

Antimicrobial	%
Gatifloxacin	80.15%
Chloramphenicol	65.07%
Ofloxacin	59.92%
Ceftazidime	31.98%
Ciprofloxacin	*52.21%*
Amikacin	8.08%

DISCUSSION:

A total of 530 samples from clinically suspected ocular infections were collected and processed in the Department of Microbiology, SD Eye hospital and in 273 (51.51%) cases bacteria was the causative pathogen.

In our study bacterial Keratitis (153) was the predominant infection accounting up to 56.05% of the total cases.

When the corneal epithelial barrier is challenged due to injury or trauma, causing ulceration and infiltration of inflammatory cells it leads to a potentially dangerous ocular infection - the Bacterial keratitis.[5]

In the present study, out of the total of 153 bacterial isolates of keratitis the causative organisms were Gram-positive cocci in 128 cases (83.66%). A similar incidence of 69.1% and 65.65% [6] was reported by other Indian researchers.

The next frequent bacteria involved in the causation of keratitis was the invasive Pseudomonas aeruginosa (16) accounting for 10.46% out of the total153 keratins cases.

This is in line with a study made by Jayaraman Kalia Murthy et al in south India. [7]

The second most common ocular infection in our study is conjunctivitis (64) accounting for 23.44 % of the total cases and the majority of infections were due to CONS followed by Staphylococcus aureus and streptococci pneumoniae.

The conjunctiva is the outermost layer of the white part of the eye and the inner surface of the eyelids which is vulnerable part of the eye with increased risk of infection by various routes like, hand-to-eye contact, and spread from the ocular adnexa, including the lacrimal system, nose, and paranasal sinuses [8]. Gram positive cocci have been reported as causative agents of conjunctivitis in many parts of the world [9,10,11] infection of the lacrimal apparatus which is caused by the blockage of the lacrimal duct system resulting in accumulation of tears and creation of a fertile environment for secondary bacterial infection, and dacryolith formation. [12,13]

In our study Gram positive bacteria especially, CONS followed by Staphylococcus aureus and S. pneumoniae were the predominant bacteria that were isolated from Dacryocystitis infection. Though Gram positive bacteria have been isolated in other similar Indian studies the causative Gram-positive bacteria were different like Bareja et al.[14] from North India reported higher rate of S. pneumoniae, whereas Chaudhry et al.[15] reported higher prevalence of Staphylococcus spp.

In our study next in order of the ocular infection's prevalence was Blepharitis, an inflammatory condition of the eyelid margin, a common cause of ocular discomfort and irritation in all ages and a condition which can lead to permanent alterations in the eyelid margin or vision loss from superficial keratopathy, corneal neovascularization, and ulceration.

The predominant organism in its causation was found to be Staphylococcus aureus 14 out of the total 18 cases of Blepharitis.

The microbial pathogens recovered from eyes with endophthalmitis (3) in our study was staphylococcus aureus, pseudomonas aeruginosa, and Escherichia coli one each.

The causative pathogen of endophthalmitis can come from the outside environment (exogenous) or endogenous from systemic infections transported through the bloodstream.

Similar pathogens were isolated in study by. Krissoff MS et al, and Boldt HC [16,17]

Over all in our study the major pathogens were the gram-positive cocci especially Coagulase-negative Staphylococci and Staphylococcus aureus which is in comparison with the studies done by S Ramesh1 et al.[4]

As per our study it has been assayed that in ardent and injudicious use of antibiotics for bacterial ocular infections has resulted in development of resistance to the most of the antibiotics that are routinely used.

Quinolones like Gatifloxacin and Ofloxacin along with Chloramphenicol have shown good efficacy and can be a righteous option for treating ocular infections.

CONCLUSION

The ophthalmologists must avoid empirical treatment and to access the changing patterns in the causation of ocular infections microbiological study through culture along with drug susceptibility testing must be made mandatory before start of treatment. To improve the patient outcome and to prevent ocular morbidity excessive and inappropriate use of antibiotic must be discouraged along with prescribing safe and effective topical antibiotic which will go a long way in improving patients' outcomes and quality of life.

Conflict Of Interest: The authors declare that there are no conflicts of interest.

Source Of Funding: None.

Acknowledgement

Authors would like to acknowledge with deep gratitude, all residents and staff members of the Department of Ophthalmology and Microbiology, Sarojini Devi Eye Hospital for their care to the patients involved in the study and their help and input on the research project.

Author Contributions: BMSV designed and planned the study, analysed the data; wrote the manuscript and supervised the whole study. PGB extracted data, contributed in the processing, under the guidance of BMSV .UT and GAN performed data collection and processing, and also contributed in preparing the tables under the guidance of BMSV.

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