Bacteriological Profile and Antimicrobial Susceptibility Pattern of Isolates from Ocular Infections among the Patients Attending Ophthalmology Department of a Tertiary Care Hospital

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INTRODUCTION

Bacteria are the major cause of ocular infections around the world. Infection may either be due to single microbe or polymicrobial and is mostly associated with factors like age, contact lens usage, trauma, surgery, poor ocular hygiene or any prior ocular pathology [1]. Ocular infections manifest commonly in the form of blepharitis, conjunctivitis, keratitis, endophthalmitis, orbital cellulitis or dacryocystitis [2]. The source of eye infection may be exogenous which is the most common mode or endogenous invasion of the micro-organisms which can be carried in the bloodstream. External or exogenous bacterial infection of the eye are usually localised to some extent or may spread to the adjacent tissues [3].

The eye is normally protected by the continuous flow of tears and the blink reflex which mechanically prevents foreign substances invading it. Tear film also possess few enzymes like lysozyme, secretory IgA, lactate dehydrogenase, beta-lysin, defensins, etc which are present at high levels in tears that is capable of reducing microbial colonisation on the ocular surface thereby preventing bacterial infections [4]. The virulence factors of pathogenic microorganisms and impaired host resistance favours the ocular infections. The common organisms that cause eye infections are Propriobacterium acne, Staphylococcus aureus, Streptococcus pneumoniae, Moraxella spp [2]. Conjunctivitis, inflammation of the mucosa of conjunctiva, is the most frequent ocular case with economic and social burdens [5]. Blepharitis which is an inflammation of the eyelid can cause loss of eye lash [6]. Keratitis, the most serious eye infection is the leading cause of corneal blindness. Moreover, the disease can also progress to endophthalmitis if not diagnosed early [7]. Dacryocystitis is an inflammation of the nasolacrimal duct [8]. Exogenous endophthalmitis is an infective complication of primary cataract, intraocular surgery and ocular trauma due to the introduction of infectious pathogens like bacteria whereas the endogenous one is commonly due to systemic dissemination of the pathogens [9].

The eye infections if left untreated can damage the structures of the eye, causing visual impairment and blindness [1]. Knowledge on the specific aetiology is essential for the effective management of ocular infections. However these infections are mainly managed empirically and very less is known about the specific aetiology [10, 11]. The purpose of this study is to identify the specific bacterial pathogen responsible for the development of particular ocular infection and to determine their in-vitro susceptibility to commonly used antibacterial agents in clinical practice.

METHODOLOGY

A hospital based cross sectional study was conducted among the patients attending the ophthalmology department in a tertiary care hospital, Tirupati from October 2019 to September 2020 with the approval from the Institutional Ethical Committee. All the patients attending the Ophthalmology Department with ocular infections were included in the study. Patients who were already on antibacterial therapy were excluded from the study.

OPERATIONAL DEFINITIONS

Conjunctivitis: Inflammation or infection of the conjunctiva [12]

Blepharitis: Inflammation or infection of the eyelid margin [13]

Dacryocystitis: Inflammation of the lacrimal sac [13]

Infective uveitis: Inflammation or infection of the uveal tissue of the eye [13]

Endophthalmitis: Endopthalmitis is a purulent inflammation of the intraocular fluids [vitreous and aqueous] usually due to infection [13]

DATA COLLECTION

The study included 290 patients with ocular infections who attended the Ophthalmology Department of tertiary care hospital, Tirupati. The socio-demographic data of each study participants were collected. The ocular examination was performed using slit lamp bio-microscopy to identify any focus of infection and inflammation, to come to a provisional diagnosis. The provisional diagnosis was recorded and the specimen was collected by the attending ophthalmologist using a standard protocol from the study participants.

SPECIMEN COLLECTION

Conjunctival swab: Swabs are collected prior to the start of anti-microbial therapy. After the clinical examination with the help of a sterile cotton swab the discharge collected in the conjunctival cul-de-sac and lacus lacrimalis is collected by a gentle stroke from lateral canthus to medial canthus. This procedure is done without the use of any anaesthetic drops. Two such swabs are collected from each eye and sent for microbiological examination [13]

Corneal scrapping: Two drops of 0.5 % proparacaine is instilled in the lower fornix of the affected eye. A lid speculum is applied to separate the eyelids. Under slit lamp or operating microscope the affected eye is examined using direct illumination and any debris or mucus is cleaned using a sterile swab. Kimura spatula or a number 15 Bard Parker knife is used to scrape the leading edge and base of the ulcer. The specimen was directly inoculated into the blood agar plate. Multiple specimens were obtained to enhance the yield [14]. Smears are prepared by transferring the specimen on to the glass slide over an area of approximately 1 cm in diameter fro microscopic examination.

Anterior chamber paracentesis: After a local anaesthetic drops of 0.5% proparacaine instilled in lower fornix of the affected eye, a 26 or 30 gauge needle with insulin or 2 ml syringe was used for sample collection. Lid speculum was used to separate the eyelids. Under operating microscope or slit lamp needle was entered in to the anterior chamber by a valvular self-sealing paracentesis obliquely through the stroma via the lower limbus. 0.1

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to 0.3 ml of aqueous was collected avoiding damage to endothelium or the lens. The needle was withdrawn and an external pressure was applied to the entrance with sterile cotton tip applicator. A drop of antibiotic was instilled in the conjunctival sac and eye was patched [15].

Specimen/swabs collected were labelled and transported immediately to microbiology laboratory

CULTURE AND IDENTIFICATION

The first swab was subjected to Gram stain where the presence of bacteria its Gram's reaction and presence of pus cells were assessed.

The second swab was inoculated on to 5% sheep blood agar, MacConkey agar, and Mannitol salt agar and inoculated aerobically at 37°c for 24 hours.

All the plates were examined for pure growth. The pure colonies were obtained for further identification methods using standard microbiological techniques, i.e Gram stain, colony morphology and biochemical tests.

The identification of Gram positive bacteria was based on the type of haemolysis on sheep blood agar followed by catalase test, coagulase test, bile solubility and Optochin disk test [16, 17].

The identification of Gram negative bacteria was based on catalase test, oxidase test, hanging drop motility test, indole test, Methyl red test, Voges Proskauer test, Citrate utilisation test, triple sugar iron agar test, urease test and Arginine ornithine decarboxylase test[18].

Antimicrobial susceptibility testing [AST]

AST was carried out for each bacterial isolate using Kirby Bauer's disc diffusion technique based on CRSI [Clinical and Laboratory Standard Institute] guidelines [19]

Briefly, 3 to 5 pure colonies of bacteria were transferred into a test tube containing 1 ml of sterile normal saline mixed homogenously and adjusted to 0.5 Mc Farland standards.

The suspension was inoculated on to Muellar Hilton agar [MHA] for non-fastidious organism and in 5% sheep blood agar for fastidious like Streptococcus pneumoniae.

Each antibiotic disc was placed manually on agar plate and incubated at 37 °c for 24 hours and the zone of inhibition around the disc was measured to the nearest milli-meter using graduated scale. The isolate were classified as susceptible, intermediate and resistance according to CLSI guidelines.

As there are no antibiotic susceptibility break points for topical antibiotic therapy higher antibiotic concentration comparatively are achieved in ocular tissue while using topical therapy.

RESULTS

In the present study a total of 290 patients had attended ophthalmology department for ocular infections. Among the study participants 164 [56.52%] were males, and 174 [60%] belongs to rural areas. The most affected age group belongs to > 60 [40.41%] years.

TABLE 1: GENDER WISE DISTRIBUTION OF THE STUDY PARTICIPANTS

Gender	Males	Females	Total
Total No. Of Study Participants	164 [56.55%]	126 [43.45%]	290

CHART 1: RESIDENCE WISE DISTRIBUTION OF THE STUDY PARTICIPANTS

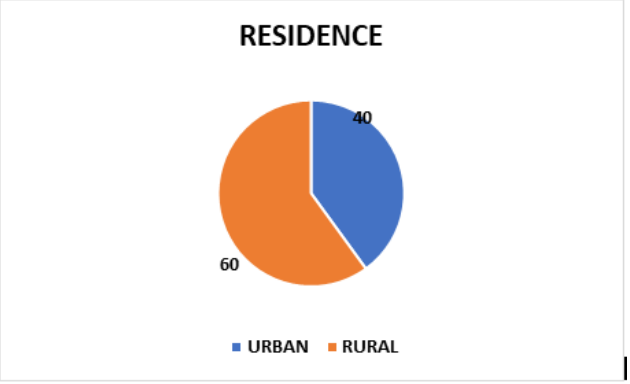

TABLE 2: AGE WISE DISTRIBUTION OF THE STUDY PARTICIPANTS

Age Group	Total Participants [%]	Positive For Bacterial Culture [%]
20-Jan	37 [12.76]	14 [9.54]
21-40	56 [19.31]	28 [19.18]
41-60	83 [28.12]	45 [30.28]
>60	114 [39.31]	59 [40.41]
TOTAL	290	146

CLINICAL DATA

The various clinical conditions seen among 290 patients were conjunctivitis 108 [37.24%], keratitis 37 [12.76%] dacryocytitis 53 [18.28%], blepharitis 52 [17.93%], trauma 24 [8.28%], infective uveitis 10 [3.45%] and endophthalmitis 6 [2.07%].

TABLE 3: DISTRIBUTION OF CLINICAL CONDITIONS AMONG STUDY PARTICIPANTS.

Types Of Clinical Presentations	Clinical Presentation	Frequency Of Bacteria Among Clinical Presentation
	n [%]	n [%]
Conjuctivitis	108 [37.24%]	79 [73.14%]
Corneal Ulcer	37 [12.76%]	11 [29.73%]
Dacryocystitis	53 [18.28%]	21 [39.62%]
Periocular Burns +Trauma	24 [8.28%]	6 [25%]
Infective Uveitis	10 [3.45]	-
Endophthalmitis	6 [2.07%]	1 [16%]
Blepharitis	52 [17.93%]	28 [53.85%]
TOTAL	290	146

BACTERIAL AETIOLOGY OF OCULAR INFECTION

Among the 290 participants with ocular infections, 146 [50.34%] were positive for culture. Among the total isolated, 110 [75.34%] were Gram positive and 36 [24. 66%] were Gram negative. The predominant bacteria among the isolate was Staphylococcus aureus [Table 4].

TABLE 4: DISTRIBUTION OF BACTERIA ISOLATED FROM STUDY SPECIMENS

Bacteria	Number [n=146]	Percentage

Staphylococcus aureus	62	42.47
Coagulase negative Staphylococcus	35	23.97
Streptococcus pneumoniae	13	8.9
Escherichia coli	11	7.53
Klebsiella pneumoniae	13	8.9
Pseudomonas aeruginosa	4	2.74
Moroxella spp	5	3.42
Acinetobacter spp	3	2.05

The most common organism isolated among blepharitis specimens was coagulase negative Staphylococcus and in other clinical conditions Staphylococcus aureus was the most common organism to be isolated [Table 5].

TABLE 5: FREQUENCY OF BACTERIA ISOLATED AMONG THE VARIOUS CLINICAL CONDITIONS

	BACTERIA ISOLATED							
Clinical Condition	**S.aureus**	**CONS**	**S.pneumoniae**	**E.coli**	**K.pneumoniae**	**P.aeruginosa**	**Moroxellasp**	**Acinetobacter spp**
Conjuctivitis	34	23	6	5	7	1	3	-
Corneal Ulcer	5	3	-	-	-	2	-	1
Dacryocystitis	17	-	-	2	1	-	1	-
Trauma	-	-	2	1	1	1	-	1
Endophthalmitis	-	-	-	-	-	-	-	1
Blepharitis	6	9	5	3	4	-	1	-
TOTAL	62	35	13	11	13	4	5	3

ANTIMICROBIAL SUSCEPTIBILITY PROFILE

Among the 110 Gram positive bacteria isolated, the most sensitive antibiotic was ciprofloxacin [86.36%] followed by gentamycin [82.72%] and clindamycin [80%] [Table 6]

TABLE 6: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF THE GRAM POSITIVE BACTERIA ISOLATED

BACTERIA [n=110]	**AMX**	**COT**	**E**	**CL**	**NFX**	**CIP**	**TE**	**GE**	**VA**
S.aureus [n=62]	8	43	13	57	5	53	11	47	62
CONS [n=35]	3	21	17	31	12	29	4	31	35
S.pneumoniae [n=13]	13	1	13		13	13	13	13	-
Total [n=110]	24	65	43	88	30	95	28	91	97
	-21.82	-59.09	-39.09	-80%	-27.27	-86.35	-25.45	-82.72	-100

AMX: Amoxcillin, CONS: Coagulase Negative Staphyloccus, CIP: Ciprofloxacin, CL: Clindamycin, COT: Cotrimoxazole, E: Erythromycin, GE: Gentamycin, NFX: Norfloxacin, TE: Tetracycline, VA: Vancomycin

Among the 62 Staphylococcus aureus isolated the highest resistance was seen with amoxicillin 54 [87.09%] followed by tetracycline 51 [82.26%] and erthyomycin 49 [79.03%].

The total number of Streptococcus pneumoniae isolated were 100% sensitive to amoxicillin, erythromycin and gentamycin.

Among the 36 Gram negative bacteria isolated, 35 [97.22%], 33 [91.67%], and 31 [86.11%] were sensitive to gentamycin, ciprofloxacin, ceftriaxone respectively. [Table 7]

The Gram negative bacteria showed high resistance to tetracycline 7[19.44%], followed by amoxicillin clavulanic acid [30.56%].

TABLE 7: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF THE GRAM NEGAITIVE BACTERIA ISOLATED

BACTERIA	**AMC**	**COT**	**CIP**	**CTR**	**GE**	**TE**	**NFX**
Escherichia coli [n=11]	9	7	11	9	11	3	6
Klebsiella pneumoniae [n=13]	7	8	13	11	13	1	7
Pseudomonas aeruginosa [n=4]	1	-	2	4	4	-	1
Acinatobacter [n=3]	-	-	3	2	3	-	1
Moroxella [n=5]	1	2	4	5	4	3	1
Total [n=36]	11	17	33	31	35	7	16
	-30.56	-47.22	-91.67	-86.11	-97.22	-19.44	-44.44

AMC: Amoxcillin – Clavulanic acid, CIP: Ciprofloxacin, COT: Cotrimoxazole, CTR: Ceftriaxone, GE: Gentamycin, NFX: Norfloxacin, TE: Tetracycline

DISCUSSION

The present study includes 290 specimens, among which 146 [50.34%] specimens were culture positive with coincides with various study conducted by Mohammed et al [20] [59.6%], Bharathi et al [21] [58.8%] and Shiferaw et al [22] [59.4%].

In the present study the men were more susceptible for ocular infections [56.55%], which is similar to the study conducted by Mohammad et al [20] [53.3%] and Nuzhat et al [23] [54%].

The most common age group affected by ocular infections in the present study is > 60 years [40.41%] which correlates with the study by Shiferaw et al [22] [44.4%].

The ocular infections are more prevalent in rural areas [60%] in the present study which correlates with other studies namely Mohammed et al [20] [67.2%], Shiferaw et al [22] [75%].

In the present study the most common ocular infection reported was conjunctivitis [37.24%], which is similar to other studies by Mohammed et al [20] [32.8%], Shiferaw et al [23] [43.1%], Nuzhat et al [23] [42%] and Hemavathi et al [16] [52%].

The most common organism isolated from the specimens are Staphylococcus aureus [42.4%] followed by Coagulase negative Staphylococcus [23.97%].

These findings correlate with many other study conducted by Mohammed et al [20] [37.4%, 28.8%], Namitha et al [24] [32.8%, 25%], Nuzhat et al [23] [36%, 20%] by Staphylococcus aureus and Coagulase negative Staphylococcus respectively.

In the present study, the Gram negative bacteria most commonly reported is K.pneumoniae [8.90%] which correlates with the study performed by Namitha et al [24] [6.2%], Muluye et al [25] [14.5%].

Among the Staphylococcus aureus [62] isolated in the present study, methicillin sensitive Staphylococcus aureus strains were 12.90% while methicillin resistance Staphylococcus aureus were 87.10% and all MRSA strains were 100% sensitive to vancomycin.

Among the coagulase negative Staphylococcus [35], only 3 isolates showed sensitivity to methicillin, while rest 32 isolates were resistant to it. These isolates were sensitive to vancomycin [100%]. These correlate with the most of the study around the world i.e Shiferaw et al [22], Nuzhat et al [23], Bharathi et al [21].

The Streptococcus pneumoniae [13] isolates in the present study were 100% sensitive to amoxicillin, ciprofloxacin, erythromycin, gentamycin which is similar to Mohammad et al [20], Hemavathi et al [16].

Among the Gram negative bacteria [36%] isolated in the present study, the highest sensitivity was towards gentamycin [9.22%] followed by ciprofloxacin [91.67%] which coincides with the study by Mohammed et al [20]

Among the Gram Negative bacterial isolates, Klebsiella pneumoniae [13], Pseudomonas aeruginosa [4], Moraxella [5], showed 100% sensitive to ceftriaxone which is similar to studies by Mohammed et al [20]

In the present study the highest resistance among Gram positive and Gram negative bacteria was shown for amoxicillin [21.82%] and tetracycline [19.44%] respectively.

This is similar to the study performed by Shiferaw et al [22], Mohammed et al [20], and Muluye et al [25].

CONCLUSION

The most common ocular infection reported in our hospital was conjunctivitis followed by dacryocystitis. Staphylococcus aureus stands as the most common bacteria to cause ocular infection in the community. Most of the organisms are sensitive to ciprofloxacin and gentamycin and least sensitivity to amoxicillin and tetracycline. In order to prevent the increasing rate of Antimicrobial resistance, identification of bacteria followed by antimicrobial sensitivity testing should be made mandatory in routine practice.

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