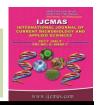


International Journal of Current Microbiology and Applied Sciences ISSN: 2319-7706 Volume 6 Number 7 (2017) pp. 4343-4352 Journal homepage: http://www.ijcmas.com



Original Research Article

https://doi.org/10.20546/ijcmas.2017.607.452

Microbiological Profile of External Ocular Infections in a Tertiary Care Hospital in South India

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ABSTRACT

The eye may be infected from external sources or through intra-ocular invasion of microorganisms carried by the blood stream [1]. Bacteria are the major cause of ocular infections [2]. This study was undertaken to isolate and identify the microorganisms causing external ocular infections and to determine the in-vitro susceptibilities of bacteria to commonly used antibacterial agents. A cross sectional study was conducted among 110 patients with external ocular infections at Govt. Mohan kumaramangalam medical college hospital, Salem over a period of 6 months from July to December 2016. Socio-demographic and clinical data were collected using structured questionnaire. External ocular specimens were collected using sterile swabs and inoculated on mac conkey agar, chocolate agar, blood agar, mannitol salt agar and sabouraud's dextrose agar culture Medias. Presumptive isolates were further identified by a series of biochemical tests. The antimicrobial susceptibility patterns of the isolates were determined by disc diffusion method. Out of 110 samples processed only 54(49%) showed growth. The overall prevalence of bacterial pathogens among external ocular samples was 46.3 % (n = 51/110) and of fungal pathogens was 2.7% (n = 3/110). The majority of the isolates (67 %; 36/54) were Gram positive bacteria and 28 % (15/54) Gram negative bacteria. The percentage of fungal isolates i.e., Candida spp. was 5 % (n = 3/54). The proportion of Staphylococcus aureus was 47% (n = 24/51) and Coagulase negative Staphylococci was 23.5 % (n = 12/51) among the Gram positive bacterial isolates. The proportion of Klebsiella spp. is 23.5 % (n = 12/51) and Pseudomonas aeruginosa 5.8% (n = 3/51) among the Gram negative bacterial isolates. All Gram positive isolates were susceptible to vancomycin. Majority of the Gram negative bacterial isolates showed susceptibility to ofloxacin and ciprofloxacin. The prevalence of bacterial pathogens among external ocular samples was high and the predominant isolate was Staphylococcus aureus. Conjunctivitis was found to be the dominant infection.

Keywords

Bacterial isolates, External ocular infections, Drug susceptibility pattern.

Article Info

Accepted: 30 June 2017 Available Online: 10 July 2017

Introduction

The eye is a unique organ that is impermeable to almost all external organisms. Continuous tear flow, aided by the blink reflex, mechanically washes substances from the ocular surface and prevents the accumulation of microorganisms. In addition, lysozyme, lactoferrin, secretory immunoglobulin's, and

defensins, which are present at high levels in tears, can specifically reduce bacterial colonisation of the ocular surface [3,4]. The eye may be infected from external sources or through intra ocular invasion of microorganism caused by blood stream [1]. Pathogenic micro-organisms cause ocular

disease and the most frequently affected parts of the eye are the conjunctiva, lid and cornea [6]. Bacteria are major causative agents that frequently cause infections in eye and possible loss of vision [2]. Frequently reported clinical manifestations include conjunctivitis, scleritis, keratitis, blepharitis, canaliculitis and dacryocystitis [6]. Conjunctivitis is the most common cause of "red eve" [14] and corneal ulceration is a major cause of mono-ocular blindness in developing countries [14]. The three most common causes of conjunctivitis infections (infective conjunctivitis), irritation (loose reactions allergic and eyelash). Infective conjunctivitis is most commonly caused by bacteria and viruses. Viral conjunctivitis causes a watery discharge the discharge from bacterial while conjunctivitis contains pus [16] Infective keratitis is a major cause of vision loss and blindness second to cataract [3, 4]. Ocular trauma is a far more common predisposing factor of infectious keratitis in developing countries, whereas pre-existing ocular disease and wearing contact lens are common risk factors in developed countries [25] Blepharitis is an inflammation of the eyelid margins which can result in patient discomfort and decline in visual function [19]. Dacryocystitis is an inflammation of the lacrimal sac and duct [20, 21]

Ocular Infections caused by the bacteria is most common which is followed by fungal and then viral infections. The bacterial aetiology and their susceptibility as well as resistant patterns may vary with geographical location according to the local population [8], [10], [11], [12], [13]. Bacteria cultured from the conjuctival sac are usually similar to those found in the upper respiratory tract and the skin, with majority being Gram positive bacteria and commonly *Staphylococcus spp. and Corynebacterium spp.* In addition the eyelid margins and conjuctival sacs of healthy subjects can also contain Gram negative

[24] Aspergillus pathogens Staphylococcus aureus are the most common fungus and bacteria isolated frequently. These infections if not treated promptly may lead to complications. The morbidity due to ocular infections can vary from self-limiting, trivial infection to sight threatening condition and blindness [22]. A variety of factors determine clinical outcome in microbes causing eye infection and the epidemiological patterns vary from one country to the other and in different geographical areas in the same country [23]. The ability to isolate the causative organism depends on a variety of factors including the amount of inoculum, the site from which it is taken, the media used for culture (whether enriched media are used or not) and also on the empirical treatment received before collection of the samples. If they are not treated properly it may lead to sight threatening condition.

The management of bacterial eye infections may involve treatment with broad spectrum indiscriminate use antibiotics. The antibiotics led to the development of resistance to many commonly antimicrobial medications. The emergence of resistance towards topical bacterial antimicrobial agents may increases the risk of treatment failure with potentially serious consequences [7]. Therefore, up to date information is essential for appropriate antimicrobial therapy and management of ocular infections ^[23]. Thus, the aim of this study is to identify the dominant bacterial common pathogen to external infections, and to assess the in vitro drug susceptibility patterns of these isolates to commonly prescribed antibiotics among patients with external ocular infections.

Materials and Methods

A cross-sectional study was conducted from July to December 2016 at Govt. Mohan

Kumaramangalam Medical College Hospital, Salem. All patients who had signs and symptoms of external ocular infections were included as study population. One hundred and ten consecutive patients attending the ophthalmology outpatient department of the hospital from July to December 2016 were included in this study.

Patients who had treatment with antibiotics within the last 5 days or who had undergone previous ocular surgery within the last 7 days of recruitment of study subjects were excluded from the study.

Sample collection and laboratory investigation

Sample collection

After detailed eye examination, using standard techniques external ocular specimens were collected for culture and smears were obtained by scraping and swabbing the eyelid margin using sterile blade or Bard Parker handle and sterile broth moistened cotton swabs in case of conjunctivitis and blepharitis. Corneal/Conjuctival scraping and swabbing were performed and subjected to direct microscopy and culture.

Briefly, patient was requested to look up, lower eye lid was pulled down and then swabs were collected. The sample collector holds the palpebra apart and gently collects discharge from the surface of the eye using sterile cotton swab that has been premoistened with sterile saline. The sterile normal saline moistened swab was rubbed over the lower conjunctival sac from medial to lateral side and back again. Purulent material in cases of dacryocystitis was collected by everted puncta then applying pressure over the lacrimal sac area from the infected eye [26, 27]. The swab was immersed in 3 ml of brain heart infusion (BHI), placed

in a cold box and transported to the laboratory for investigation.

Isolation and identification of bacterial pathogens

Specimens were subjected to (10 % potassium hydroxide) wet mounting & Gram staining as first step and microbial culture identification was done by inoculating the specimens on to mac conkey agar, mannitol salt agar, blood agar, chocolate agar and sabouraud's dextrose agar plates and incubated at 37 °C for 48 hours. All plates were initially examined for growth after 24 hours and cultures with no growth were incubated for further 48 hours. After getting pure colonies. further identification was done using standard microbiological techniques, which includes Gram stain, colony morphology and biochemical tests.

Presumptive Gram negative bacteria were identified using triple sugar iron agar, citrate utilization test, urease test and indole test and Gram positive bacteria were identified using catalase, coagulase, bacitracin and optochin tests ^[28]. Antimicrobial susceptibility test was carried out on each identified bacterium using disc diffusion method on Muller Hinton agar based on clinical and laboratory standard institute (CLSI) guidelines.

Briefly, 3–5 colonies of the test organism were emulsified in 5 ml of nutrient broth and mixed gently. The suspension was incubated at 37 °C until the turbidity of the suspension becomes adjusted to 0.5 McFarland standards. The suspension was uniformly rapped on to Mueller-Hinton agar for non-fastidious organisms and Mueller-Hinton agar with defibrinated sterile sheep blood (10 % V/V) for fastidious organisms.

The antimicrobial impregnated disks were placed using sterile forceps on the agar surface and the plates were incubated at 37 °C for 24 hours and the zone of inhibition was determined.

All specimens were collected following standard operating procedure for ophthalmic specimen collection. The sterility of culture media was ensured by incubating 5 % of each batch of the prepared media at 37 °C for 24 hours.

Performances of all prepared media were also checked by inoculating standard-strains such as *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853). The qualities of biochemical testing procedures were checked by these reference strains.

Microbial cultures were considered significant if growth of the same organisms were demonstrated on more than one solid phase medium and/or if there was confluent growth at the site of inoculation on one solid medium, and/or if growth of one medium to be consistent by direct microscopy findings and/or if the same organism was grown from repeated specimens. The isolated strains were identified upto species level by standard biochemical steps.

Results and Discussion

Socio-demographic characteristics of the study participants

A total of 110 patients were enrolled in this study. The majority of the study subjects were males (74/110; 67.2 %).

The mean age of the study participants was 55.11 (SD \pm 17.85) years. Most of the study participants were illiterate (82/110, 74.5 %), majority (85/110, 77.2%) were rural dwellers and (72/110, 65.4%) were farmer by occupation.

Clinical findings

In this study, 42.7 % (47/110) patients were suffering from conjunctivitis followed by keratitis 28.1 %, (31/110) as shown in chart 4. The dominant type of ocular infection among male patients was conjunctivitis (44/74; 59.4%) where as in female patients a higher number of cases of dacryocystitis (41.6 %; 15/36) was observed.

When the different types of eye infection were stratified by sex, higher prevalence of conjunctivitis cases were observed among male patients than females but the prevalence of dacryocystitis was higher among female patients.

Prevalence of bacterial pathogens and associated risk factors

Out of 110 samples processed only 54(49%) showed growth. The overall prevalence of bacterial pathogens among external ocular samples was 46.3% (n = 51/110). The majority of the isolates (67 %; 36/54) were Gram positive and 28 % (15/54) Gram negative bacteria. The percentage of fungal isolates i.e., *Candida spp.* was 5 % (n = 3/54). The proportion of *Staphylococcus aureus* was 47% (n = 24/51) and *Coagulase negative Staphylococci* was 23.5 % (n = 12/51) among the Gram positive bacterial isolates.

The proportion of *Klebsiella spp*. is 23.5 % (n = 12/51) and *Pseudomonas aeruginosa* 5.8% (n = 3/51) among the Gram negative bacterial isolates. The details are shown in chart 1, 2 &3. In this study, occupation, residence, education, frequency of face washing, the occurrence of systemic disease like diabetes mellitus, usage of contact lens, steroid abuse, history of trauma prior to infection and cigarette smoking were used as possible risk and predisposing factors for ocular infections.

Antimicrobial susceptibility patterns of bacterial isolates

The antimicrobial susceptibility patterns of bacterial isolates from ophthalmic patients showed that a significant number of bacterial isolates were resistant to one or more than one antimicrobials. All Gram positive isolates were susceptible to vancomycin (100%) with cefoxitin (92 %). The number of isolates of Staphylococcus aureus showing susceptibility to amikacin 18(75%), gentamicin 23(96%), cotrimoxazole 20(83.3%), ciprofloxacin 10(42%). The number of isolates Coagulase negative Staphylococci showing susceptibility to amikacin 4(33%), gentamicin 3(25%), cotrimoxazole 5(42%), ciprofloxacin 9(75%). 2/24 isolates of Staphylococcus aureus were MRSA and 1/12 isolates of Coagulase negative Staphylococci was MR -CoNS. In case of Gram negative bacterial isolates most of them showed susceptibility to ofloxacin and ciprofloxacin. All the 3 (100%) isolates of *Pseudomonas aeruginosa* showed susceptibility to ofloxacin and ciprofloxacin with susceptibility to gentamicin 2(67%), tetracycline 1(33.3%), amikacin 2(67%) and cefotaxime 2(67%). The number of isolates of *Klebsiella spp.* showing susceptibility to ofloxacin 11(92%), ciprofloxacin 10(83.3%), gentamycin 2(67%), amikacin 0(0%), cefotaxime 0(0%), tetracycline 0(0%). The details are shown in tables 1 and 2.

The organisms that cause ocular infection are generally exogenous. However, in certain circumstances they gain accesses to enter the eye and cause infection. In this study, the prevalence of bacteria causing eye infection was 46.3% and it is slightly lower when compared with other previous study reports from Gondar, India and Ethiopia (60.8 %, and 58.8 %, 59.4 % respectively) [29,30,31]. Majority of the patients were rural dwellers and farmer by occupation as reported in study from Ethiopia [31].

Table.1 In vitro antimicrobial susceptibility pattern of Gram positive bacterial isolates

	VAN	FOX	AK	GEN	COT	CIP
S.aureus	24(100%)	22(92%)	18(75%)	23(96%)	20(83.3%)	10(42%)
CoNS	24(100%)	11(92%)	4(33%)	3(25%)	5(42%)	9(75%)

VAN-Vancomycin, FOX-Cefoxitin, AK-Amikacin, Gen-Gentamycin, COT-Cotrimoxazole, CIP-Ciprofloxacin

Table.2 *In vitro* antimicrobial susceptibility pattern of Gram negative bacterial isolates

	AK	CTX	CIP	OF	TE	GEN
Pseudomonas aeruginosa	2(67%)	2(67%)	3(100%)	3(100%)	1(33.3%)	2(67%)
Klebsiella spp.	0(0%)	0(0%)	1(33.3%)	3(25%)	0(0%)	2(67%)

AK-Amikacin, CTX- Cefotaxime, CIP – Ciprofloxacin, OF- Ofloxacin, TE – Tetracycline, GEN - Gentamycin

Chart.1 Percentage of culture positivity in ocular specimens

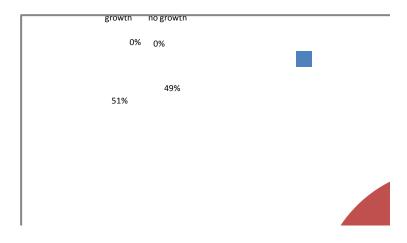


Chart.2 Percentage of different microbial flora grown in culture

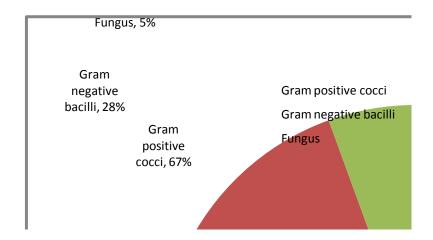


Chart.3 Distribution of different pathogens grown in culture

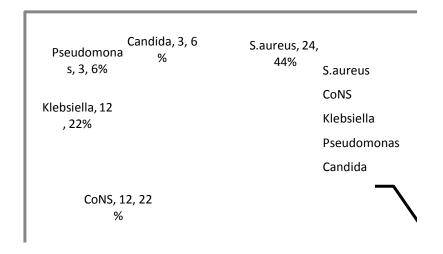
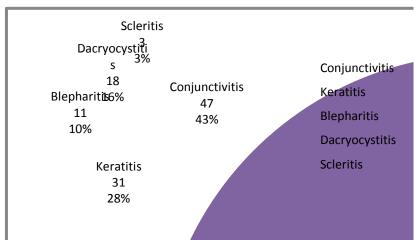


Chart.4 Prevalence of different types of external ocular infections



The conjunctiva is predisposed to infection by diverse micro-organisms. The principal routes of inoculation are airborne droplets, hand-to-eye contact, and spread from the ocular adnexa, including the lacrimal system, nose, and paranasal sinuses [36]. In the current study, conjunctivitis (43%) is the dominant type of eye infection followed by keratitis (31%), blepharitis (11%), dacryocystitis (18%) and scleritis 3(%) similar to the study conducted by Birtukan *et al.*, [31] where conjunctivitis was the dominant type of eye infection (43.1 %).

In this study the dominant type of ocular infection among male patients conjunctivitis (44/74; 59.4%) where as in female patients a higher number of cases of dacryocystitis (41.6 %; 15/36) were observed. Higher rates of both acute and chronic dacryocystitis have been reported in previous studies among women [32, 33]. Data of the current study showed that external ocular infections were predominantly seen among male patients. This might be due to their outdoor activities and that was also supported by similar reports from Jimma and Gondar, Ethiopia [2, 34]

The occurrence of different bacteria as an etiological agent for external ocular infection

signifies differences in the environmental conditions, the standard of personal hygiene, age and the site of infection. Hence, the culture positivity varies from centre to centre. In this study, the overall culture-positivity was 49%. Bacteria (95%) were the most common pathogens and were involved in infections of all the tissues of the eye, whereas fungi (5%). The majority of the isolates (70.5 %; 36/51) were Gram positive and the other 29.4 % (15/51) were Gram negative bacteria. *Staphylococcus aureus* (44%) is the predominant bacterial pathogen isolated in this study similar to the study conducted in south India by Ramesh *et al.*, [35].

The prevalence of S. aureus in causing conjunctivitis has been reported to be higher in many parts of the world [37], [38], [39]. It is also inferred that 8.3% of the staphylococci isolated were MRSA and 8.3 % of CoNS were MR – CoNS. The prevalence of Gram negative bacteria as etiological agents of ophthalmic disease in the current study can be graded lower as only 28 % (n = 15/54) of the patients who were positive for these bacterial pathogens. This decrease prevalence could be due to the effective personal hygiene. Most of the ocular bacterial infections are due to Gram positive bacteria than Gram negative bacteria. The present

study mainly focused on Gram positive cocci causing eye infections. Several other studies in India, Nepal and other parts of world have shown similar results inferring Gram positive cocci as a primary cause of ocular infections.

The relationship between antibiotic use and resistance is complex. Improper selection of antibiotics, inadequate dosing and poor compliance to the therapy may play an important role in increasing resistance as their overuse.

The analysis on *in-vitro* susceptibility testing shows that vancomycin demonstrated greatest efficacy against Gram-positive isolates alone, ciprofloxacin while and ofloxacin demonstrated greatest efficacy against Gramnegative isolates. Frequent and routine use of antibiotics for all bacterial ocular infections in our population has decreased susceptibility to most of the tested antibiotics. Besides that, all fluoroquinolones tested in this ciprofloxacin and ofloxacin showed higher efficacy against Gram negative bacterial isolates and proving that in our community quinolones, especially ciprofloxacin ofloxacin are a good choice for treating ocular infections at present. The information provided in this article would aid the clinician in formulating rationale-based decisions in the antibiotic treatment of bacterial ocular infections that cause major public health problems.

In conclusion, the prevalence of bacterial infection among patients with external ocular infection in this study was high (46.3 %). The predominant isolates were *Staphylococcus aureus*, *Coagulase negative staphylococci and Klebsiella spp*. High antibiotic resistance to commonly prescribed antibiotics was observed. Methicillin resistance has been observed in both of the Gram positive isolates, *Staphylococcus aureus & Coagulase negative Staphylococci* for which vancomycin

showed 100 % susceptibility. Gram negative bacterial isolates showed resistance to amikacin, cefotaxime and tetracycline. Quinolones showed promising results in the treatment of Gram negative pathogens.

Therefore, to prevent the increasing rate of antimicrobial resistance the practice of starting empirical therapy to be avoided and the routine identification of bacteria through culture methods and conducting drug susceptibility testing should be practiced in addition to direct Gram staining and potassium hydroxide wet mounting, as a routine diagnostic procedure especially in the centres where ophthalmologists have access to microbiology lab facilities.

Improper selection of antibiotics, inadequate dosing and poor compliance to therapy may play an important a role in increasing resistance. Changes in bacterial resistance patterns have been a major problem in the effective management of ocular infections, better access to effective and safe topical antibiotics has been cited as the primary factor in improving patients outcomes and quality of life. Early access to diagnosis and appropriate treatment and better patient health education can prevent the ocular morbidity and mortality.

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How to cite this article:

Rajesh, B., B. Divya and Aruna, V. 2017. Microbiological Profile of External Ocular Infections in a Tertiary Care Hospital in South India. *Int.J.Curr.Microbiol.App.Sci.* 6(7): 4343-4352. doi: https://doi.org/10.20546/ijcmas.2017.607.452