

ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF ALLIED HEALTH SCIENCES
DEPARTMENT OF MEDICAL LABORATORY SCIENCES



Bacterial and Fungal Pathogens from External Ocular Infection at St. Paul Hospital
Millennium Medical College, Addis Ababa, Ethiopia

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Acronyms

AAU.....	Addis Ababa University
ATCC.....	American Type Culture Collection
BAP	Blood Agar Plate
BHIB	Brain Heart Infusion Broth
CA	Chocolate Agar
CLSI	Clinical and Laboratory Standard Institute
CoNS	Coagulase negative Staphylococci
DRERC	Department of Research and Ethical Review Committee
DST	Drug Susceptibility Testing
FMOH	Federal Ministry of Health
H ₂ S	Hydrogen Sulfide
KOH	Potassium Hydroxide
LPCB	Lactophenol Cotton Blue
MDR	Multidrug Resistance
MRCoNS.....	Methicillin Resistant coagulase negative Staphylococci
MRSA	Methicillin Resistant Staphylococcus Aureus
SPHMMC.....	St. Paul Hospital Millennium Medical College
SPSS	Statistical Package for Social Science
WHO	World Health Organization

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Operational definitions/Definition of terms

Conjunctivitis: - is an inflammation of conjunctiva, the membrane that lines the eye lids and covers the exposed surface of the eyeball/sclera.

Dacryocystitis: - is an inflammation of the lacrimal sac (tear sac), which often occurs due to an obstruction of the nasolacrimal duct (tear duct).

Blepharitis: - is a chronic inflammation of the eyelids, a common problem in both children and adults.

Blepharo-conjunctivitis: - is a condition that causes swelling of the outer eyelids and the conjunctiva, the thin mucous layer that acts as a protective layer for the inner eyelids and front of the eyeball.

Keratitis: - is inflammation of cornea which is clear and transparent front part of the eye and is caused by an infection.

Previous surgery- means when patients who had the eye surgery from one week up to three months before sample collection.

Hospitalization for long period- means patient who admitted inpatient department and stays for 15 and more days before data collection.

Frequency of face washing-

Less frequent- means when patient not washing at all and wash once per day.

Frequent- means when patient wash two up to three times per day.

More frequent- means when patient wash more than three times per day.

Multidrug resistance- means bacterial isolate resistant for two or more classes of antibiotics tested.

Abstract

Background:-Ocular infection is a major public health problem in developing countries. Bacteria and fungi associated with external ocular infection are responsible for increased incidence of morbidity and blindness worldwide.

Objective:-The aim of this study was to determine the profile of bacterial and fungal pathogens from external ocular infection at St. Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia.

Methodology:-A cross sectional study conducted using convenient sampling technique. A total of 215 patients recruited from April to August, 2016. Conjunctival and eyelid margin swabs and corneal scraping were collected. Demographic data were collected using structured questionnaire. All Specimens were processed for microbiological analysis as per standard procedures and antibiotic susceptibility test was done using disc diffusion technique. The data was analyzed by using SPSS version 20 and appropriate statistical tools were used.

Result: - A total of 215 patients were enrolled in this study. Of which 133(61.4 %) were culture positive. Of this 118(54.9%) and 24(11.2%) were bacterial and fungal pathogens respectively. There were 9 (6.8%) bacterial and fungal co-infections. Majority were males 109 (50.7%). The mean age of the study participants was 42.34 (Sd. \pm 20.55) and majority of participants age were within the age range of 25-44 years 72(33.5%). Majority had conjunctivitis 87(40.5%) followed by blepharitis 48(22.3%). The predominant bacterial pathogen isolated were gram positives 88(74.6%). Of this *S. aureus* 32(27.1%) was the commonest isolate and *K. pneumoniae* 9(7.6%) was the predominant from gram negative bacterial pathogens. *Aspergillus* species 14(58.3%) were the most predominant fungal isolates. Gram positive cocci and gram negative rods were highly resistant for Penicillin 66/88(75%) and Ampicilin 20/27(81.5%) respectively. 71.2% of MDR bacteria were isolated. There was statistically significant association between prevalence of bacterial isolates and age variation ($P=0.023$) and trauma with fungi prevalence ($P=0.002$).

Conclusion:-The prevalence of bacterial and fungal pathogens was higher among external ocular infection. The drug resistance among ocular bacterial infection was also higher.

Keywords: - External ocular infections, conjunctivitis, blepharitis, keratitis, dacryocystitis, susceptibility

1. Introduction

1.1. Background

Eye is the most important sensory organ of vision function. It is exposed to the external environment, but is relatively impermeable to microorganisms. Any part of the eye can be infected by microbes from the environment and they can form transient flora or invade the tissue and they become potential pathogenic and cause infection when conditions allowed [1, 2, 3].

Ocular infection is a major public health problem in developing countries including Ethiopia. It can affect different eye structures and their manifestations and treatment vary accordingly. The conjunctiva, eye lid and cornea are most frequently infected parts of the eye. The most common manifestations are blepharitis, conjunctivitis, canaliculitis, dacrocystitis, keratitis, scleritis, orbital cellulitis, endophthalmitis, panophthalmitis and other infections. Infections associated with the surface of the eye like blepharitis, conjunctivitis, canaliculitis, dacrocystitis, keratitis are said to be external ocular infections. Bacteria, viruses, fungi and even parasites can cause these ocular infections [1, 4].

Bacteria are the most common microorganisms that cause external ocular infections. This is because the bacterial pathogens inhabit the ocular surface (i.e. mucous membrane of the conjunctiva), though the lysozymes, lactoferrin, defensins and antibodies in tear and blinking mechanism reduce bacterial colonization of ocular surface [3, 5].

The three most common causes of conjunctivitis are infection (infective conjunctivitis), allergic reactions and irritation (loose eyelash). Infective conjunctivitis is most commonly caused by bacteria and viruses. Viral conjunctivitis causes a watery discharge where as bacterial conjunctivitis is a common infectious disease, characterized by Conjunctival hyperemia, eyelid edema, and purulent discharge and it is the cause of red eye [6]. Furthermore, general features associated with fungal conjunctivitis are; Redness, itching, mucopurulent discharge and irritation and often fungal infections are chronic [7].

Pathogenic microorganisms cause ocular disease due to their virulence and host's reduced resistance because of the factors like personal hygiene, living conditions, socio-economic status, decreased immune status, trauma, surgery, and systemic diseases. Especially eye trauma is

believed to be the cause of fungal keratitis in most cases. Fungal infections of the cornea (mycotic or fungal keratitis, keratomycosis) present as Suppurative, usually ulcerative, lesions. Such a corneal infection possess a challenge to the ophthalmologist because of its tendency to mimic other types of stromal inflammation, and its management is restricted by the availability of effective antifungal agents and the extent to which they can penetrate into corneal tissue [4, 5, 8].

According to studies the most common pathogens responsible for bacterial conjunctivitis, blepharitis, keratitis, dacryocystitis are *Haemophilus influenza* and *Streptococcus pneumoniae* in children and *Staphylococcus aureus* in adults. Methicillin-resistant *S. aureus* (MRSA) is emerging as a more important pathogen, even in non-hospitalized populations. *Chlamydia trachomatis*, *Staphylococcus epidermidis*, *Streptococcus viridians*, *Moraxella catarrhalis* and Gram negative intestinal bacteria are also common. But generally gram positive pathogens are responsible for 60% to 80% of acute infections [3, 9, 10]. The common fungal genera in Mycotic eye infections are *Fusarium*, *Alternaria*, *Aspergillus* species and *Candida* species can affect cornea, orbit and other ocular structures [5, 7, 11].

The diagnosis of external ocular infections should be supported by laboratory investigation. However, they are usually treated on empirical basis with topical broad spectrum antibacterial drugs without laboratory confirmation due to several reasons such as lack of microbiology laboratory in most health institutions, cost and time consuming of culturing and sensitivity testing [4, 6]. Bacterial resistance has been emerging worldwide; this may be due to widespread and inappropriate dosing of broad-spectrum antibiotics for systemic infections and even for eye infections [5].

1.2. Statement of the problem

The external ocular infections are responsible for increased incidence of morbidity and blindness world widely [1]. In this case, keratitis is a major cause of vision loss and blindness second to cataract and is the most common in developing countries. But the etiological cause for keratitis may vary at different geographical locations, time to time and population to population [11, 12]. Blepharitis can also result in patient discomfort and decrease in vision. Moreover, untreated lacrimal abscess can progress to orbital cellulitis, superior ophthalmic vein thrombosis, and cavernous sinus thrombosis and these may lead to life threatening condition. Infections of the conjunctiva can also spread to the cornea and can cause a perforation [6, 13].

Mycotic keratitis has been reported from many different parts of the world, particularly tropical areas, where it may account for more than 50% of all ocular mycoses. On the other hand bacterial infection is a common cause of conjunctivitis and accounts for up to 50% of all cases of conjunctivitis in adults and 70% to 80% of all cases in children [3, 7, 14].

As world health organization (WHO) estimates that 285 million individuals were visually impaired world widely and 90% of these individuals live in low-resource countries [15]. Infectious corneal disease is one of an important cause of visual impairment and blindness, with an annual incidence of between 1.5 to 8 million cases world widely, being more prevalent in developing countries [16]. It was also estimated that 6 million corneal ulcers occur annually in the 10 countries of south-east Asia region encompassing a total population of 1.6 billion and globally it is anticipated that ocular trauma & corneal ulceration result in 15-20 million new cases of corneal blindness [17].

In Ethiopia the prevalence of blindness was about 1.6 % and it was estimated that 87.4 % of the cases were due to avoidable causes like bacterial, viral and fungal infections when untreated. Studies in some areas of the country showed that the higher prevalence of bacterial infections like in Jimma (74.4%), Borumeda (59.4%) and Hawassa (48.8%) and the higher incidence of drug resistance reported 69.4% of multidrug resistance. These data are somehow old though it can indicate that there is the higher burden of ocular infections in our country [4, 6, 19].

Visually impaired individuals, as well as their families, may face serious social and economic challenges. According to a study in 2013, the cost of treating bacterial conjunctivitis alone was estimated to be \$377 million to \$857 million per year in United States [20]. On the other hand mobility was constrained among 83% of people with blindness compared to 49% for visually impaired and only 13% for sighted individuals in sub-Saharan Africa [18].

Drug resistance or multidrug resistance is becoming the very serious problem. The emergence of bacterial resistance towards antimicrobial agents may increases the risk of treatment failure with potentially serious consequences. In our context the blindly use of antibiotic eye drops without physicians prescription can contribute the increasing of drug resistance and also due to lack of access to microbiology laboratory in most health institutions, clinicians uses broad spectrum or empirical therapy and this leads to the risk of multidrug resistance development [4, 6, 19, 21]. Thus, to make a rational choice of initial antibiotic therapy, the current trends in the etiology of bacterial ocular infections and their susceptibility must be evaluated periodically. There is also scarcity of published data about fungal external ocular infections that can show the prevalence of fungal pathogens associated with ocular surface infections. Therefore, the aim of this study was to isolate and identify the etiology of external ocular bacterial and fungal pathogens, and to assess the in-vitro susceptibility pattern of those ocular bacterial isolates to commonly used antibiotics at St. Paul hospital millennium medical college, Addis Ababa, Ethiopia.

1.3. Significance of the study

The result of this study will

- ✓ Provide up to date information about the profile bacterial and fungal pathogens associated with external ocular infection.
- ✓ Provide information for clinicians to select the best antibacterial drug for bacterial external eye infection.
- ✓ Assist in the development of treatment guidelines in which the concerned bodies used as a reference.
- ✓ It can also serve as base line information for further studies of fungal infection.

2. Literature review

A cross sectional study done by Khauv P, et al., in Cambodia from March to October 2012 revealed that the wide range of pathogens were identified in all ophthalmia neonatorum patients and 85.7% of patients with an external eye infection. Pathogens were not detected in either of the intra-ocular infection patients. Most commonly isolated bacteria were *S. aureus* (23 isolates), coagulase-negative staphylococci (13), coli-forms (7), Haemophilus species (6), *S. pneumoniae* (4), and *N. gonorrhoeae* (2). 95.7% of *S. aureus* isolates were Methicillin sensitive. Isolates of *N. gonorrhoeae* were resistant to penicillin and fluoroquinolone but susceptible to ceftriaxone [22].

A recent bacteriological investigation done by Namitha B N, et al., in Navodaya in 2014 showed that the most common bacterial pathogens associated with ocular infection were *S. aureus* (32.8%) followed by *S. epidermidis* (25%), *S. pneumonia* (14.1%), *K. pneumonia* (6.3%), and *E. coli* (4.7%). Bacterial isolates were highly susceptible to Vancomycin (100%), Gentamycin (94.3%) among Gram positive organisms and among Gram negative organisms it is highly susceptible to Tobramycin (100%) and Imipenem (90.9%) [1].

A study conducted by Moreno N P, et al., in which was aimed to determine the aerobic bacterial microbiota of the conjunctiva in diabetic patients with normal and altered glycosylated hemoglobin levels in two regions in Brazil. The result revealed that the percentage of cultures in which bacterial growth observed was greater in diabetic patients. The microorganism most frequently detected in this study was *S. epidermidis*, followed by *S. aureus*, *P. mirabilis*, and *E. coli* [23].

A study conducted by Abid A J, et al., in Babylon in 2012 to investigate the causes of bacterial eye infections and the culture result showed that 176 positive bacterial growth out of 191. 119 isolate represent gram positive and 57 isolate gram negative, in Gram positive bacteria *S. aureus* showed high percentage(38.06%), While in gram negative *E. coli* showed high percentage (22.7%) from different types of eye infections studied including Conjunctivitis 60(34.09%), eye lid infection (Blepharitis) 58 (32.95%), Dacryocystitis 58 (32.95%) [9].

Another cross sectional study done by Umamageswari S S M, et al., in 2013 in India showed that out of 125 patients with external ocular infection, 80(64%) were culture positive. Among the 80(64%) culture positive patients, 45(56%) patients had Conjunctival infections and 35(44%)

had keratitis. From Conjunctival infections 49 bacterial isolates were recovered. The predominant bacterial isolate was found to be Coagulase negative *Staphylococci* 21(43%) followed by *S. aureus* 12(24%). Among keratitis patients, 10(29%) patients had bacterial infection, 23(66%) patients had fungal infections. The predominant fungus was *Fusarium* species 12(48%) followed by *A. flavus* 6(24%). The gram positive isolates were susceptible to Vancomycin 100% followed by Ciprofloxacin 75%. Gram negative isolates were susceptible to Imipenem 100%, Amikacin 100% and Ciprofloxacin 96% [5]. The same type of study at the same place done by Hemavathi, et al., in 2014 revealed that 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. 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. One post-trauma endophthalmitis sample yielded both *Candida* and *Pseudomonas* from vitreous fluid. Bacterial strains were susceptible to gatifloxacin (86.4%), tetracycline (65.4%), and chloramphenicol (69.1%) [2].

A cross sectional study conducted by Kunimoto D Y, et al., in south India revealed that the main predisposing factors includes ocular disease (38.2%), previous ocular surgery in the same eye (29.4%), trauma (17.6%), and severe systemic disease (16.7%). Contact lens wear was associated with only two cases (2.0%). 99 organisms were isolated in cultures of corneal scrapings from 74 (72.5%) of the 102 cases. *S. epidermidis* (31.1%), filamentous fungi (25.7%), and *S. pneumoniae* (13.5%) were the most common isolates [24]. Similarly, according to reviewed and personal observational study done by Thomas P A revealed that the fungal infections of the cornea were frequently caused by species of *Fusarium*, *Aspergillus*, *Curvularia*, and *Candida*. The author suggested as trauma was the most important predisposing cause; ocular and systemic defects and prior application of corticosteroids were also important risk factors identified [8].

A retrospective study conducted by Bharathi M J, et al., in south India revealed that a total of 4417 ocular samples were submitted for microbiological evaluation from 2002 to 2007, of which 2599 (58.8%) had bacterial growth, 456 (10.3%) had fungal growth, 15 (0.34%) had Acanthamoeba growth, 14 (0.32%) had mixed microbial growth. The rate of culture-positivity was 88% in eyelids' infection, 70% in Conjunctival, 69% in lacrimal apparatus, 67.4% in

corneal, 51.6% in intraocular tissues, 42.9% in orbital and 39.2% in scleral infections. The most common bacterial species isolated were *S. aureus* (26.69%) followed by *S. pneumoniae* (22.14%). *S. aureus* was more prevalent in eyelid infections (51.22%) coagulase-negative staphylococci in endophthalmitis (53.1%), *S. Pneumoniae* in lacrimal apparatus and corneal infections (64.19%) *P. aeruginosa* in keratitis and Dacryocystitis (66.5%), *Haemophilus* species in Dacryocystitis and conjunctivitis (66.7%) [25].

Another cross sectional study carried out by Ansari M R, et al., in Iran, which was aimed to evaluate the Conjunctival bacterial flora and its antibiotic susceptibility pattern in eyes of patients undergoing cataract surgery in 2008. Out of the 170 patients 89 cases (52.4%) had positive cultures in the eyes. In 79 eyes (88.8%) found coagulase-negative *Staphylococcus* (CoNS). Eighty two cases (95.3%) of isolated *Staphylococcus* were susceptible to Amikacin, 86 (100%) sensitive to Ciprofloxacin and 42 (48.8%) sensitive to Ceftazidime. Average susceptibility and resistance to antibiotics was 2.6 (\pm 1.8) antibiotics in women and 1.6(\pm 1.4) in men and this was statistically significant [26].

A cross sectional study conducted by Abdel-Sater M A, et al., in Yemen showed that a total of 200 specimens from infected eyes were collected from 2005 to 2006. Of which 139 cases (69.5%) were infected (126 by bacteria, 7 by fungi and 6 by both). Eye infections in relation to sex- female, age between 21-30 years, the farmers and those who had no definite work were highly susceptible. 7 fungal species related to 4 genera were identified of which *Aspergillus* species were the most prevalent and ten species and 4 unidentified species of bacteria belongs to 10 genera were recovered. *Staphylococci*, *P. aeruginosa* and *E. coli* were the most common agents of eye infections. Five species of fungi and 4 species and 1 unidentified species of bacteria were isolated from the mixed cases [27].

Another cross sectional study done by Musa A A, et al., in 2014 in Libya which was aimed to investigate bacterial pathogens and the result revealed that a total of 56 anterior blepharitis cases including 22 ulcerative blepharitis and 34 seborrheic blepharitis cases. The isolated bacteria from anterior blepharitis were *S. aureus* 14 (25%), *S. epidermidis* 14 (25%), similar *Klebsiella* species 10 (18%), *S. viridians* 5 (9%), *Proteus* species four (7%) and other gram negatives. The common

isolates in both samples were *S. aureus*, *S. epidermidis*, and *Proteus* species. The gram positive cocci *S. aureus* were resistant to four antibiotics and *S. viridians* were resistant to three antibiotics, whereas the gram negative bacteria were resistant to two antibiotics [28].

According to a study done by Hefni H M et al., in Egypt in 2011 87.8% of ocular samples was positive for bacterial infection out of 296 patients. *S. aureus* was the most common causative agent, being responsible for (31.2%) of the all cases, followed by *S. epidermidis* (24.2%) and *K. pneumonia* (20%). The frequency of bacterial ocular infection in children aged 2-<14 years was higher than other age groups. The fluoroquinolones and third generation cephalosporin's were highly effective against all bacterial isolates. But most of them were resistant to penicillin, ampicillin, tetracycline and chloramphenicol [29].

A study conducted by Mshangila B, et al., in Uganda in 2013 showed that the eyelid margin and Conjunctival samples were culture positive in 59.5% and 45.8% out of 131 preoperative cataract patients respectively. The predominant organisms identified were Coagulase-negative *Staphylococci* (CoNS) (65.9%) and *S. aureus* (21.0%). CoNS showed the highest resistance to tetracycline (58.2%) and erythromycin (38.5%), whereas in *S. aureus* the resistance to tetracycline and erythromycin were 55.2% and 31.0% respectively. Methicillin resistant CoNS (MRS) and Methicillin resistance *S. aureus* (MRSA) were 31.9% and 27.6% respectively. Ciprofloxacin, gentamycin, Tobramycin and vancomycin showed the lowest resistance rates [30].

A retrospective study conducted by Muluye D, et al., in Gondar university hospital from 2009 to 2012 showed that a total of 102 eye discharges were submitted for microbiological evaluation, of which 60.8% had bacterial growth. The most frequent bacterial isolates were gram positive bacteria (74.2%). The predominant isolate was Coagulase-negative *staphylococci* (27.4%) followed by *S. aureus* (21%). Most of the bacterial isolates were resistance to ampicillin (71%), amoxicillin (62.9%), erythromycin (43.5%), gentamycin (45.2%), penicillin (71%), trimethoprim-sulphamethoxazole (58.1%), and tetracycline (64.6%) while Ceftriaxone and Ciprofloxacin showed (75.8%) and (80%) susceptibility respectively. From the total bacterial isolates, (87.1%) were showed multi drug resistance (MDR) [31]. Another cross sectional study which was conducted in this hospital by Assefa Y, et al showed that 60.8% different bacterial

species were isolated from 51 dacryocystitis cases. The dominant isolates were Coagulase negative *Staphylococci* (CoNS) 9(29.0%), *S. aureus* (*S. aureus*) 6(19.4%), and *Pseudomonas* species 3(9.7%). Amoxicillin 38.7%, ciprofloxacin 25.8%, chloramphenicol 25.8%, cotrimoxazole 25.8%, and ampicillin 19.4% were resistant to the overall bacterial isolates identified. Only *Citrobacter* species were sensitive to all antibiotics tested. 9(29.0%) of the bacterial isolates were resistant to only one antibiotics and resistance to two, three and four antibiotics each accounted 5(16.1%) rate [32].

A cross sectional study conducted by Shiferaw B, et al., revealed that the overall prevalence of bacterial pathogens among external ocular samples was 59.4 %. The majority of the isolates 93.7 % (89/95) were gram positive and the other 6.3 % (6/95) gram negative bacteria. The proportion of coagulase negative *Staphylococci* among the gram positive bacterial isolates was 53.7 % (51/95). All gram positive isolates were susceptible for vancomycin but 67.4 % (60/95) of them were resistant against amoxicillin. Resistance to tetracycline, norfloxacin, ceftriaxone and ciprofloxacin were observed among gram negative bacteria [6]. Similarly, study done by Tesfaye T, et al., in Jimma in 2013, a total of 198 ocular samples were collected for microbiological evaluation, of which 148 (74.7%) had bacterial growth. The gram-positive cocci comprised 52.0% and the most frequently isolated was *S. aureus* (28.4%) and gram-negative bacteria accounted for 48.0%, from which the predominant isolate was *P. aeruginosa* (20.9%). Majority of gram positive cocci were susceptible to ciprofloxacin 71 (92.2%) and vancomycin 70 (90.9%) and gram negative isolates to amikacin 67 (94.4%) and ciprofloxacin 57 (91.5%) [4].

Another cross sectional study done by Amsalu A, et al., revealed that a total of 281 consecutive, non-repetitive ocular specimens were collected among 140 conjunctivitis, 55 blepharitis, 31 keratitis, 19 Dacryocystitis cases, and 36 other cases. Out of 281 specimens submitted to culture, 137 (48.8%) were culture positive. The predominant bacterial isolates were gram positive cocci 88 (61.5%). The commonest species isolated was *S. aureus* 30 (21.0%) followed by coagulase negative *Staphylococci* (CoNS) 26 (18.2%) and *S. pneumoniae* 20 (14.0%). Ciprofloxacin was effective against 86% of isolated pathogen. Multi-drug resistance was observed in 69.9% of the bacterial isolates [19].

3. Objectives

3.1. General Objective

- To assess the bacterial and fungal pathogens from external ocular infection at St. Paul Hospital Millennium Medical College, Addis Ababa, Ethiopia

3.2. Specific Objectives

- To determine the profile of bacterial and fungal pathogens from external ocular infections
- To determine the drug susceptibility patterns of the bacterial isolates

4. Method and materials

4.1. Study design and period

A cross sectional study was conducted at St. Paul's Hospital Millennium Medical College from April to August, 2016.

4.2. Study area

This study was conducted at St. Paul's Hospital Millennium Medical College, which is a referral hospital in Addis Ababa under the Ethiopian Federal Ministry of Health (FMOH). It is the second largest public hospital in the nation, built by the Emperor Haile Selassie in 1961 with the help of the German Evangelical Church. The hospital was established to serve the economically under privileged population, providing services free of charge to about 75% of its patients. In 2007 it became a medical college and its core services include the provision of medical care, teaching and research. It has 800 clinical and non-clinical staff members that provide medical specialty services to an estimated 110,000 people annually who are referred from all over the country. Ophthalmology department is one of the largest ward serving for about 60-70 patients per day and around 12-15 ophthalmological surgeries performed weekly [from the hospital website and magazine].

4.3. Population

4.3.1. Source population

All patients who were attended St. Paul hospital millennium medical college ophthalmology department

4.3.2. Study population

All patients attended St. Paul hospital ophthalmology department and suspected with external ocular infections during the study period.

4.4. Inclusion and exclusion criteria

4.4.1. Inclusion criteria

- Clinically diagnosed patients suspected with external ocular infections attending Department of Ophthalmology, St. Paul hospital millennium Medical College were included.
- Patients who were willing to give their consent were enrolled in this study.

4.5.2. Exclusion criteria

- Patients with clinical ocular diseases.
- Patients on topical antibiotics treatment

4.6. Variables

4.6.1. Dependent variables

- Bacterial and fungal profile
- Antimicrobial susceptibility pattern

4.6.2. Independent variables

- Sex
- Age
- Educational status
- Residence
- Occupational status
- Trauma
- Previous surgery
- Wearing of contact eye lenses
- Systemic diseases
- Face washing frequency
- Clinical presentations/features

4.7. Sampling technique and sample size determination

4.7.1. Sampling technique

Convenient sampling technique was used.

4.7.2. Sample size determination

215 convenient non repeatable samples were collected from April to August 2016. Therefore all willing patients who had external ocular infection were included in this study within the time allocated.

4.8. Data collection and laboratory analysis

4.8.1. Specimen collection and transportation

Upon admission to the study, patients were examined physically and with the help of slit lamp microscope for external ocular infections by the ophthalmologist. During examination 2 to 4 clinical specimens were collected aseptically from study participants. Conjunctival and eyelid swabs were collected using sterile cotton tipped swab pre-moistened with sterile physiological saline by asking the patient to look up, the lower lid was pulled down using thumb with an absorbing tissue paper and the swab was rubbed over the lower Conjunctival sac from medial to

lateral side and back again. Pus from lachrymal sac was collected using dry sterile cotton tipped swab either by applying pressure over the lachrymal sac and allowing the purulent material to reflux through the lachrymal punctum. In cases of acute lachrymal abscess or chronic Dacryocystitis pus was drain and taken on a dry sterile cotton tipped swab. Corneal scraping was collected after instilling 2 to 3 drops of local anesthetic (Tetracaine hydrochloride 0.5%) into the conjunctiva and patient was asked to wait for 2 to 3 minutes and corneal surface was cleaned for debris and discharge using dry sterile cotton tipped swab and with the help of slit lamp the edge of the ulcer was scraped using 21 gauge needle. All swabs and the scraped material obtained on the needle directly were transferred into brain heart infusion broth 2ml (BHIB) (Oxoid, Basingstoke, UK) [33, 34]. All samples were labeled and transported to clinical bacteriology and mycology laboratory of Ethiopian Public Health Institute with the minimum delay possible (30 minutes). All ocular samples were collected by the ophthalmologist.

Demographic data, clinical data and some associated factors of study participant were collected by using structured questionnaire by principal investigator.

4.8.2. Laboratory processes

4.8.2.1. Inoculation

One of the brain heart infusion broths was inoculated onto Blood agar base (Oxoid, Basingstoke, UK) to which 10% sheep blood is incorporated, chocolate agar/heated blood agar (Oxoid, Basingstoke, UK) and MacConkey agar (Oxoid, Basingstoke, UK). The second brain heart infusion broth then inoculated onto Sabouraud's Dextrose agar (SDA) supplemented with chloramphenicol (Oxoid, Basingstoke, UK).

4.8.2.2. Incubation

The inoculated cultures were incubated at 35-37 °C for 24 hours with in candle jar (5-10% CO₂) except MacConkey agar and if no growth re-incubated for further 24 hours. SDA was incubated at 27 °C for at least four weeks aerobically and examining every two days for fungal growth.

4.8.2.3. Bacterial Identification

Pure isolates of bacterial pathogen were preliminary characterized by colony morphology, gram stain, and catalase and haemolytic reactions on blood agar plates. Identification of bacteria down to genus and/or species level was done by employing an array of routine biochemical tests such as catalase, coagulase, Optochin test and Bacitracin test for gram positive identification and

oxidase test, motility test, indole production test, Urease test, citrate utilization test, lysine decarboxylation test, carbohydrate fermentation, gas production and H₂S production for gram negative bacterial identification and using X and V factors and Satellitism test for *Haemophilus* species identification [35, 36].

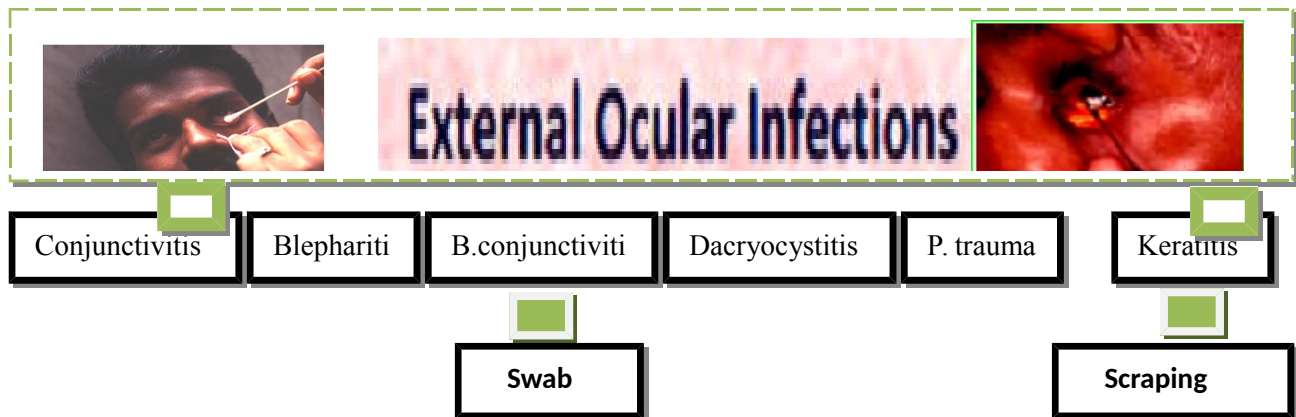
4.8.2.3. Fungal Identification

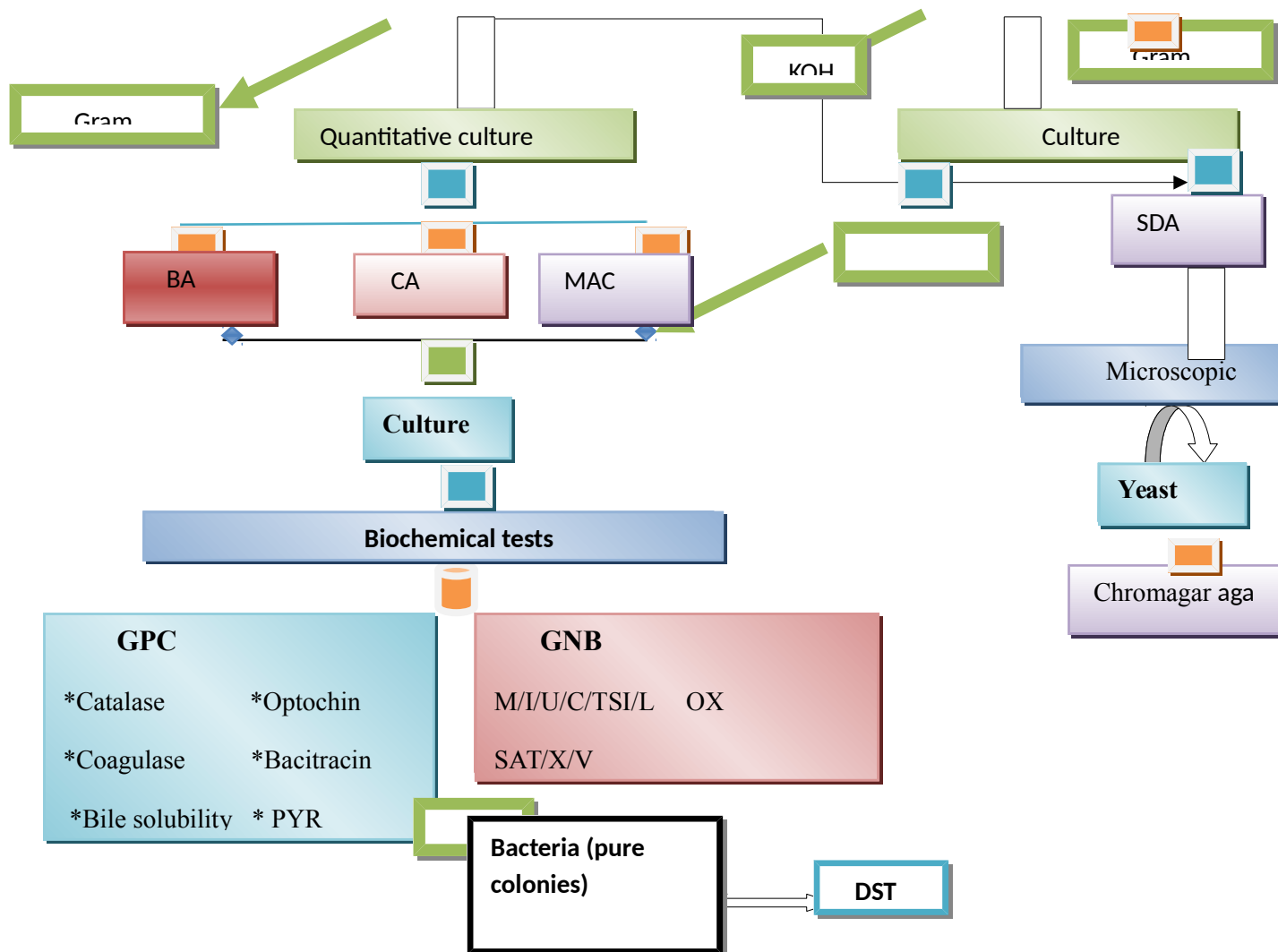
Fungi were identified by studying their macroscopic, microscopic characteristics and by using an array of biochemical and assimilation tests according to Kern and Blevins [37]. Briefly, cultures of mycelia fungi (molds) were identified by examining macroscopic and microscopic characteristics of their colony, texture, rate of growth, topography and pigmentation of the front and the reverse side of the culture were employed macroscopic identification. Microscopic identification of mold isolates was performed by placing piece of a colony from SDA to clean microscopic slide and staining with lactophenol cotton blue after placing a cover slip, the characteristics of conidia and mycelia of each isolate were studied microscopically. Yeasts were identified by employing an array of biochemical and assimilation test procedures and using Chromagar *Candida* medium (Becton Dickinson) as per the instruction of the manufacturer.

4.8.2.2. Drug susceptibility testing

A modified Kirby-Bauer disc diffusion technique for drug susceptibility test (DST) was performed among all identified bacterial isolates as recommended by Clinical and Laboratory Standard Institute (CLSI), 2015 on Mueller-Hinton agar and Mueller-Hinton agar supplemented with 5% sheep blood for fastidious bacterial isolates (Oxoid Ltd Basingstoke, Hampshire, UK). The bacterial suspension prepared equivalent to the McFarland standard (0.5 CFU) was seeded on Muller-Hinton agar and after few minutes put the paper impregnated antibiotic disks (Oxoid Ltd Basingstoke, Hampshire, UK) then incubate for 18-24hrs at 37°C based on the organisms tested. Diameters of the zone of inhibition around the discs were measured to the nearest millimeter using a caliper and classified as sensitive, intermediate, and resistant. The following antibiotics which are currently recommended by CLSI version 2015 were tested such as: Amoxicillin-clavulanic acid (10µg), Ampicilin(10µg), Amikacin (30µg), Gentamycin (10µg), Erythromycin (15µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Norfloxacin (10µg), Tetracycline (30µg), Trimethoprim-sulphamethoxazole (1.25/23.75µg), Penicillin (10µg), Vancomycin (30µg), Clindamycin (2µg), Oxacilin (30µg), Chloramphenicol (30µg), Piperacilin (100 µg), Tobramycin (10 µg), Ceftazidime (10 µg) and Meropenim (10 µg). Bacterial isolates

which were resistant for two or more classes of antibiotics were considered as multidrug resistant (MDR) [30, 36]. All antibiotics disks were generously provided by Ethiopian Public Health Institute.





GPC=Gram positive cocci, GNB=gram negative bacilli, MIUCTL=motility/Indole/Urea/Citrate utilization test/ Kligler iron agar/lysine decarboxylase test, OX=Oxidase test, X/V/SAT=X/V Factors/Satellitism, BA=Blood agar, CA=chocolate agar, MAC=MacConkey agar, SDA=Sabouraud's dextrose agar, DST=Drug susceptibility test

Figure4.1. Flow chart for bacterial and fungal identification of external ocular infection

4.9. Quality control

To maintain the quality of the work from sample collection up to final laboratory identification and data management the standard operating procedure of sample collection and laboratory analysis were followed strictly. All the equipment were checked for their functionality. The prepared culture media were checked for sterility by incubating the five percent of prepared media for overnight and observe for the presence of any growth. Abilities of the prepared media supporting the growth of organisms were checked by inoculating control strains. We also used

these control strains before we performed culture and sensitivity tests in the whole process of this study. The known control organisms used were *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853). Questionnaires used to collect demographic data and associated factors were pretested prior to data collection and supervision of the data collection were done regularly on daily basis and in which incompletely filled questionnaires were discarded.

4.10. Statistical analysis

Data were collected, entered, cleaned and analyzed using SPSS version 20 software according to the study objectives. The descriptive summaries were presented with tables and graphs. Chi-square and binary logistic regression were used to determine the association between the prevalence of bacterial and fungal pathogens and selected demographic characteristics and associated risk factors. P-value less than 0.05 were considered as statistically significant.

4.11. Ethical Consideration

Ethical clearance was obtained from Departmental Research and Ethical Review Committee (DRERC) of Medical laboratory Science, School of Allied Health Science, College of Health Science, Addis Ababa University and St. Paul's Hospital Millennium Medical College. The permission from the hospital management office was obtained. Written informed consent was also obtained from each study participants. Study participant's confidentiality were strictly maintained during the interview process as well as anonymity was kept during data processing and report writing. Laboratory confirmed cases were reported to and managed by the clinicians and patients treated accordingly. So that patients were benefited from this study.

5. Result

5.1. Demographic characteristics

A total of 215 patients with external ocular infection were enrolled in this study. Majority of the participants were males 109 (50.7%). The mean age of the study participants was 42.34 (Sd. \pm 20.55) and majority of participants age were within the age range of 25-44 years 72(33.5%). More than half of the participants lives in urban 152 (70.7%). Majority of participants were literate 109 (50.7%). 51 (23.7%) of study participants were house wives in occupation.

34(15.8%) and 32(14.9%) of study participants had trauma history and previous eye surgery respectively. Most of study participants had less frequent face washing habit 109(50.7%) and 21(9.8%) had systemic diseases. However, there was no contact lens wearer in this study (table5.1).

Table5.1. Demographic and Clinical characteristics of study participants at St. Paul’s Hospital Millennium Medical College Eye Clinic, 2016

Variables		Frequency	Percentage
Sex	Male	109	50.7
	Female	106	49.3
Residence	Rural	63	29.3
	Urban	152	70.7
Age in years*	1-14	19	8.0

	15-24	24	11.2
	25-44	72	33.5
	45-64	56	26.0
	≥65	44	20.5
Educational background	Illiterate	94	43.7
	Preschool	12	5.6
	Literate	109	50.7
Occupation	Farmer	32	14.9
	Student	26	12.1
	Merchant	19	8.8
	Office worker	49	22.8
	House wife	51	23.7
	Labor worker	13	6.0
	Preschool	11	5.1
	No job	14	6.5
Trauma	Yes	34	15.8
	No	181	84.2
Previous Surgery	Yes	32	14.9
	No	183	85.1
Systemic disease	Yes	21	9.8
	No	194	90.2
Contact lens wearing	Yes	0	0
	No	215	100
Frequency of face washing	Less frequently	109	50.7
	Frequently	87	40.5
	More frequently	19	8.8
Hospitalization of long period	Yes	6	2.8
	No	209	97.2

* WHO age classification standard [37].

5.2. Clinical features of study participants

Out of 215 patients attended 87(40.5%) had conjunctivitis followed by blepharitis 48(22.3%), blepharo-conjunctivitis 27(12.6%), Dacryocystitis 22(10.2%) and keratitis 9(4.2%), post-traumatic infection 9(4.2%) and other cases accounted 6%. The Majority of conjunctivitis cases were seen in female 48 (55.2%) but blepharitis was equal in both sexes 24(50.0%). The overall

distribution of clinical feature among sex is presented below (Fig.5.1).

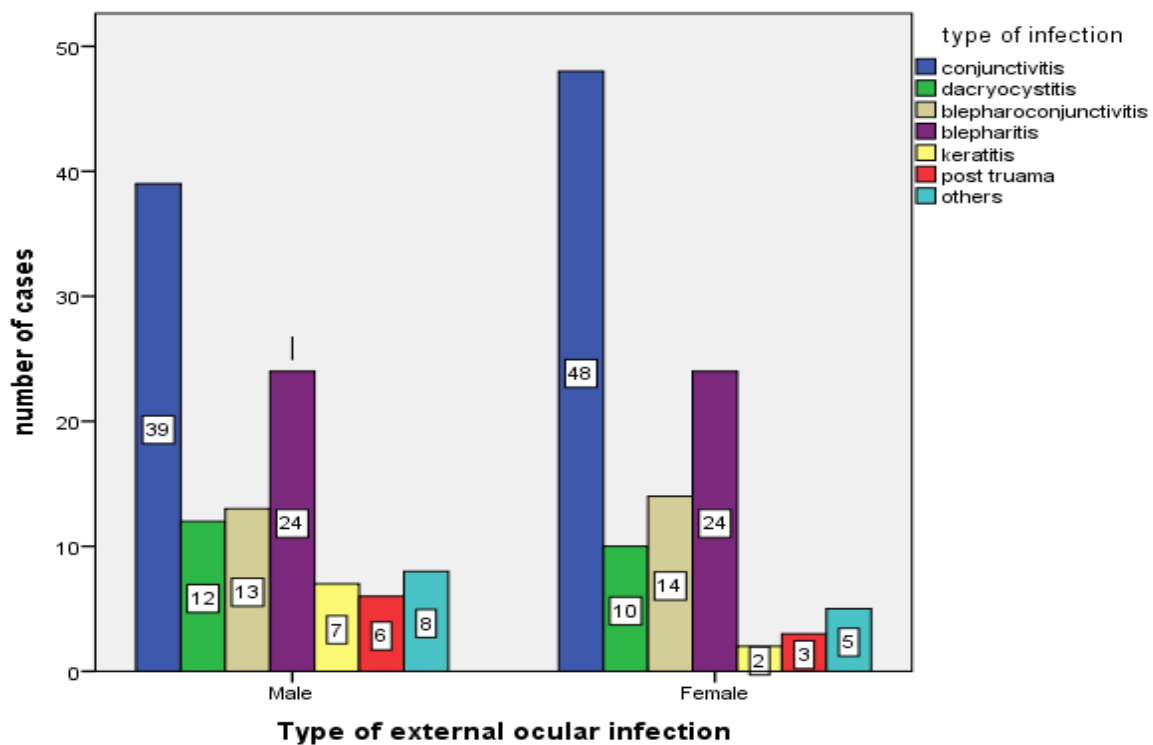


Figure5.1. Prevalence of external ocular infection among sex at St. Paul Hospital Millennium Medical College, 2016

5.3. Spectrum of bacterial and fungal isolates

Among 215 samples collected 133(61.4%) were found to be culture positive. The bacterial and fungal culture positivity were 118(54.9%) and 24 (11.2%) respectively. There were 9(6.8%) bacterial and fungal co-infection. The predominant bacterial pathogen isolated were gram positives 88(74.6%). *S. aureus* 32(27.1%) and *K. pneumoniae* 9(7.6%) were the dominant gram

positive and gram negative bacterial pathogens respectively. *A. fumigatus* 6(25%) was also the commonest fungal pathogen followed by *A. niger* 5(20.8%) (table 5.2).

Table5.2. Spectrum and prevalence of bacterial and fungal isolates at Paul’s Hospital Millennium Medical College Eye Clinic, 2016

Bacterial isolates	Frequency	Percentage	Fungal isolates	Frequency	Percentage
<i>S. aureus</i>	32	27.1	<i>A. fumigatus</i>	6	25.0
CoNS	25	21.2	<i>A. niger</i>	5	20.8
<i>S. pneumoniae</i>	18	15.3	<i>Aspergillus</i> spp.	3	12.5
<i>S. pyogenes</i>	4	3.4	<i>Penicillium</i> spp.	5	20.8
<i>S. viridians</i>	9	7.6	<i>Acremonium</i> spp.	1	4.2
<i>Moraxella</i> spp	3	2.5	<i>C. albicans</i>	3	12.5
<i>H. influenzae</i>	5	4.2	<i>C. krusei</i>	1	4.2
<i>E. coli</i>	3	2.5			
<i>K. pneumoniae</i>	9	7.6			
<i>P. aeruginosa</i>	3	2.5			
<i>E. aerogenes</i>	2	1.7			
<i>P. mirabilis</i>	2	1.7			
<i>Citrobacter</i> spp.	2	1.7			
<i>Alkaligenes</i> spp.	1	0.8			
Total	118	100		24	100

5.4. Bacterial and fungal isolates and clinical features

In this study, 53(60.9%) of conjunctivitis, 24(50%) of blepharitis cases were bacterial culture positive. The prevalence of both bacterial and fungal were higher accounting 15(68.2%) among Dacryocystitis and 5(55.5%) among keratitis respectively. There were 9 (6.8%) bacterial and fungal co-infections with the highest percentage in blepharo-conjunctivitis cases 2(15.4%) (table5.3).

Table5.3. Frequency of bacterial and fungal isolates among different clinical features at St. Paul’s Hospital Millennium Medical College Eye Clinic, 2016

Clinical feature	Total cases	Bacterial isolates	Fungal isolates	Co-infection	Total
Conjunctivitis	87(40.5)	53(60.9)	10(11.5)	5(8.6) ^a	58(43.6)
Blepharitis	48(22.3)	24 (50)	2(4.1)	1(4.0) ^b	25(18.8)
Blepharoconjunt.	27(12.6)	13(48.1)	2(7.4)	2(15.4) ^c	13(9.8)
Dacryocystitis	22(10.2)	15(68.2)	0	0	15(11.3)
Keratitis	9(4.2)	2(22.2)	5(55.5)	0	7(5.3)
Post-truamatic	9(4.2)	5(55.5)	2(22.2)	0	7(5.3)

Others*	13(6.0)	6(46.2)	3(23.1)	1(12.5) ^d	8(6.0)
Total	215	118(54.9)	24(11.2)	9(6.8)	133(100)

*Eye lid abscess, Malignancy super infection, External hordeolum, Pre-septal cellulitis, a-(1 CoNS + *C. albicans*, 1 *S. pneumoniae* + *Acremonium* spp., 1 *H. influenzae* + *Penicillium* spp., 1 *S. pneumoniae* + *A. fumigatus* and 1 *H. influenzae* + *Aspergillus* spp.), b-(1 CoNS + *C. krusei*), c- (1 *S. aureus* + *A. fumigatus* and 1 *S. aureus* + *Penicillium* spp.), d-(1 *H. influenzae* + *A. niger*)

S. aureus was the commonest organism in conjunctivitis, blepharitis, and blepharo-conjunctivitis. However, in Dacryocystitis CoNS were the dominant isolate. *S. aureus* and *S. pneumoniae* were also isolated from keratitis cases (table5.4).

Table5.4. Bacterial pathogens distributions in different clinical features at St. Paul's Hospital Millennium Medical College Eye Clinic, 2016

Bacteria Isolate	Conjunctivitis N= 87	Blepharitis N= 48	Blepharo conjunct. N= 27	Dacryocystitis N=22	Keratitis N=9	Post-trauma N= 9	Others N=13	Total N=215
<i>S. aureus</i>	14(26.4)	4(16.7)	3(23.1)	7(46.7)	1(50.0)	0	3(50)	32(27.1)
CoNS	12(22.6)	0	2(15.4)	8(53.3)	0	2(40)	1(16.7)	25(21.2)
<i>S. pneumoniae</i>	10(18.8)	2(8.3)	1(7.7)	3(20)	1(50)	1(20)	0	18(15.3)
<i>S. pyogenes</i>	1(1.9)	1(4.16)	1(7.7)	0	0	0	1(16.7)	4(3.4)
<i>S. viridians</i>	3(5.7)	2(8.3)	2(15.4)	1(6.7)	0	1(20)	0	9(7.6)
<i>Moraxella</i> spp.	2(3.8)	1(4.2)	0	0	0	0	0	3(2.5)
<i>H. influenzae</i>	2(3.8)	2(8.3)	0	0	0	0	1(16.7)	5(4.2)
<i>E. coli</i>	3(5.7)	0	0	0	0	0	0	3(2.5)
<i>K. pneumoniae</i>	4(7.5)	1(4.2)	1(7.7)	2(13.3)	0	1(20)	0	9(7.6)
<i>P. aeruginosa</i>	0	1(4.2)	1(7.7)	1(6.7)	0	0	0	3(2.5)
<i>E. aerogenes</i>	1(1.9)	0	1(7.7)	0	0	0	0	2(1.7)
<i>P. mirabilis</i>	0	1(4.2)	0	1(6.7)	0	0	0	2(1.7)
<i>Citrobacter</i> spp.	0	0	1(7.7)	1(6.7)	0	0	0	2(1.7)
<i>Alkaligenes</i> spp.	1(1.9)	0	0	0	0	0	0	1(0.8)
Total	53(44.9)	24(20.3)	13(11.0)	15(12.7)	2(1.7)	5(4.2)	6(5.1)	118(100)

Out of 215 samples, 24(11.2%) fungal pathogens of four genus and seven species were isolated. *A. fumigatus* 6 (25%) was the commonest followed by *A. niger* 5 (20.8%), *Penicillium* species (5 20.8%), *C. albicans* 3 (12.5%), *C. krusei* and *Acremonium* species 1 (4.2% each) were also isolated from different clinical features (table5.5).

Table5.5. Fungal pathogens distribution in different clinical features at St. Paul's Hospital Millennium Medical College Eye Clinic, 2016

Fungal Isolate	Conjunctivitis N= 87	Blepharitis N= 48	Blepharo conjunct. N= 27	Dacryocystitis N=22	Keratitis N=9	Post-trauma N= 9	Others N=13	Total N=215
<i>A. fumigatus</i>	2(20.0)	0	2(100)	0	1(20.0)	1(50.0)	0	6(25.0)
<i>A. niger</i>	0	0	0	0	3(60.0)	0	2(66.7)	5(20.8)
<i>Aspergillus</i> spp.	2(20.0)	0	0	0	0	1(50.0)	0	3(12.5)
<i>Penicillium</i> spp.	2(20.0)	1(50.0)	0	0	1(20.0)	0	1(33.3)	5(20.8)
<i>Acremonium</i> spp.	1(10.0)	0	0	0	0	0	0	1(4.2)
<i>C. albicans</i>	3(30.0)	0	0	0	0	0	0	3(12.5)
<i>C. krusei</i>	0	1(50.0)	0	0	0	0	0	1(4.2)
Total	10(41.6)	2(8.3)	2(8.3)	0	5(20.8)	2(8.3)	3(12.5)	24(100)

Table5.6. Age wise distribution of bacterial isolates at St. Paul's Hospital Millennium Medical College Eye Clinic, 2016

Bacterial isolates	Age in years					Total (N=215)
	1-14 (N=19)	15-24 (N=23)	25-44 (N=73)	45-64 (N=56)	≥65 (N=44)	
Staphylococci						
<i>S. aureus</i>	5(41.3)	1(10.0)	12(32.4)	12(37.5)	2(8.7)	32(27.1)
CoNS	3(18.8)	2(20.0)	10(27.0)	3(9.4)	7(30.4)	25(21.2)
Streptococci						
<i>S. pneumoniae</i>	1(6.3)	1(10.0)	2(5.4)	8(25.0)	6(26.1)	18(15.3)
	2(12.5)	0	2(5.4)	0	0	4(3.4)
<i>S. pyogenes</i>	2(12.5)	1(10.0)	3(8.1)	1(3.2)	2(8.7)	9(7.6)
<i>S. viridians</i>						
<i>Moraxella</i> species	0	0	1(2.7)	0	2(8.7)	3(2.5)
<i>H. influenza</i>	3(18.8)	0	0	2(6.3)	0	5(4.2)
Gram negative bacilli						
<i>E. coli</i>	0	2(20.0)	1(2.7)	0	0	3(2.5)
<i>K. pneumoniae</i>	0	2(20.0)	3(8.1)	4(12.5)	0	9(7.6)
<i>P. aeruginosa</i>	0	0	1(2.7)	1(3.2)	1(4.3)	3(2.5)
<i>E. aerogenes</i>	0	1(10.0)	0	0	1(4.3)	2(1.7)
<i>P. mirabilis</i>	0	0	2(5.4)	0	0	2(1.7)
<i>Citrobacter</i>	0	0	0	1(3.2)	1(4.3)	2(1.7)
species	0	0	0	0	1(4.3)	1(0.8)
<i>Alkaligenes</i>						
species						

Total (N; %)	16(13.6)	10(8.5)	37(31.3)	32(27.1)	23(19.5)	118(100)
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S. aureus was the commonest bacteria in the age group of 1-14 years (5; 41.5%) followed by CoNS and *H. influenza* (3; 18.8%) each. In age groups of 25-44 and 45-64 *S. aureus* (12; 37.5%) was the dominant pathogen isolated followed by CoNS (10; 27%) and *S. pneumoniae* (8; 25%) table 5.6. On the other hand a total 6 fungal pathogens were isolated from age group of 1-14 years of which *A. fumigatus* (3) was dominant followed by *Penicillium* species (2). And in age group of 45-64 years around 8 fungal pathogens were isolated, *A. niger* (3) was the commonest.

5.5. Antibiotic susceptibility patterns of bacterial isolates

In this study a total of 118(54.9%) bacterial pathogens were recovered. For all those isolates the drug sensitivity test was done by disc diffusion method. The most effective antibiotics for both gram positive and gram negative bacteria were Ceftriaxone 38/39(97.4%), Gentamycin 76/79(96.2%), Tobramycin 70/79(88.6%), and Ciprofloxacin 75/87(86.2%). Gram positive cocci were sensitive for Erythromycin 67/88(76.1%), Clindamycin 75/88(85.2%). *Streptococci* were sensitive for Vancomycin 24/31(77.4%) and penicillin 20/31(64.5%) but 55/57(94.7%) of *staphylococci* isolates were penicillin resistant. 7/57(12.3%) of Oxacilin resistant *staphylococci* were isolated. Of which 4/32(12.5%) were MRSA (Methicillin Resistant *S. aureus*) and 3/25(12%) MRCoNS(Methicillin Resistant Coagulase Negative *Staphylococci*). Gram negative rods were 100% sensitive for Amikacin, Ceftazidime, and Meropenim, however, resistant for Ampicilin 20/27(81.5%), Amoxicillin 16/27(59.3) (table 5.7 and 5.8). All *Moraxella* species were sensitive for all antibiotics tested. *S. aureus* was highly sensitive for Tobramycin 32(100%), Gentamycin 31(96.9%), Clindamycin 30(93.8%), and Ciprofloxacin 29 (90.6%) whereas Penicillin 31(96.9%) and Tetracycline 18(56.2%) were showed less susceptibility. Gentamycin 24(96.0%) and Oxacilin 22(88.0) were the most effective antibiotics against CoNS whereas Penicillin 2(8.0%), Tetracycline 9(36.0%) and Erythromycin 16(64.0) were showed lower susceptibility. Oxacilin 17(94.4%), Clindamycin 16(88.9%), Erythromycin 15(83.3%) and Vancomycin 15(83.3%) were showed better susceptibility against *S. pneumoniae*. *K. pneumoniae* was highly sensitive for Ciprofloxacin 9(100%), Gentamycin 9(100%), Ceftriaxone 9(100%), Amikacin 9(100%), Ceftazidime 9(100%), Meropenim 9(100%), and Tobramycin 8(88.8%) but

resistant for Ampicilin 8(88.9%), Amoxicillin 6(66.7%), and Tetracycline 5(55.6%) (table 5.7 and 5.8).

Table5.7. Antibiotics susceptibility pattern of gram positive bacterial isolates at St. Paul's Hospital Millennium Medical College Eye Clinic, 2016

Bacteria	Antibiotics												
	P	PE	OX	TE	E	DA	CIP	CN	TOB	SXT	C	VA	CRO
<i>S. aureus</i> (N=32)	S	3.1	87.5	43.8	81.3	93.8	90.6	96.9	100	81.2	78.1		
	I	0	0	0	3.1	0	3.1	0	0	0	0	ND	ND
	R	96.9	12.5	56.2	15.6	6.2	6.3	3.1	0	18.8	21.9		
CoNS (N=25)	S	8.0	88.0	36.0	64.0	76.0	72.0	96.0	76.0	76.0	72.0		
	I	0	0	0	0	0	8.0	0	4.0	4.0	4.0	ND	ND
	R	92.0	12.0	64.0	36.0	24.0	20.0	4.0	20.0	20.0	24.0		
<i>S. pneumoniae</i> (N=18)	S	66.7	94.4	72.2	83.3	88.9				72.2	77.8	83.3	
	I	0	0	0	5.6	0	ND	ND	ND	0	5.5	0	ND
	R	33.3	5.6	27.8	11.1	11.1				27.8	16.7	16.7	
<i>S. pyogenes</i> (N=4)	S	100		50.0	75.0	100				25.0	100	100	
	I	0	ND	0	25.0	0	ND	ND	ND	25.0	0	0	ND
	R	0		50.0	0	0				50.0	0	0	
<i>S. viridians</i> (N=9)	S	44.4		88.9	77.8	66.7				77.8	88.9	55.6	100
	I	0	ND	0	0	0	ND	ND	ND	0	0	0	0
	R	55.6		11.1	22.2	33.3				22.2	11.1	44.4	0
Total isolates (N=88)	S	26.1	76.1	52.3	76.1	85.2	82.5	96.5	89.5	75.0	78.4	77.4	100
	R	73.9	23.9	47.7	23.9	14.8	17.5	3.5	10.5	25.0	21.6	22.6	0

C-Chloramphenicol (30 µg), CIP- Ciprofloxacin (5 µg), CN- Gentamycin (10 µg), CRO- Ceftriaxone (30 µg), DA- Clindamycin (2 µg), E- Erythromycin (15 µg), OX- Oxacilin (30 µg), PE- Penicillin (10 unit), SXT- Trimethoprim-Sulphamethoxazole (1.25/23.75µg), TE- Tetracycline (30 µg), TOB-Tobramycin (10 µg), VA- Vancomycin (30 µg), CoNS- Coagulase Negative Staphylococci, P- Pattern, N- Number and ND- Not done

Table 5.8. Antibiotic susceptibility pattern of gram negative bacterial isolates at St. Paul's Hospital Millennium Medical College Eye Clinic, 2016

Bacteria	Antibiotics														
	P	AMP	AMC	TE	AK	NOR	CIP	CN	TOB	SXT	C	CRO	CAZ	MEM	PEP
<i>E. coli</i> (N=3)	S	0	66.7	66.7	100	66.7	33.3	66.7	33.3	33.3	0	100	100	100	
	I	0	0	0	0	0	0	0	33.3	0	0	0	0	0	ND
	R	100	33.3	33.3	0	33.3	66.7	33.3	33.3	66.7	100	0	0	0	
<i>K. pneumoniae</i> (N=9)	S	0	33.3	44.4	100	100	100	100	88.9	88.9	66.7	100	100	100	
	I	11.1	0	0	0	0	0	0	11.1	0	0	0	0	0	ND
	R	88.9	66.7	55.6	0	0	0	0	0	11.1	33.3	0	0	0	
<i>E. aerogenes</i> (N=2)	S	0	0	100	100	100	100	100	100	100	100	100	100	100	
	I	50.0	50.0	0	0	0	0	0	0	0	0	0	0	0	ND
	R	50.0	50.0	0	0	0	0	0	0	0	0	0	0	0	
<i>P. aeruginosa</i> (N=3)	S	0	0	66.7	100	100	100	100	100	33.3	66.7	100	100	100	100
	I	0	0	0	0	0	0	0	0	0	33.3	0	0	0	0
	R	100	100	33.3	0	0	0	0	0	66.7	0	0	0	0	0
<i>P. mirabilis</i> (N=2)	S	0	0	100	100	100	100	100	100	100	100	100	100	100	
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	ND
	R	100	100	0	0	0	0	0	0	0	0	0	0	0	
<i>Citrobacter</i> Species (N=2)	S	0	0	50.0	100	100	100	100	100	100	50.0	100	100	100	
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	ND
	R	100	100	50.0	0	0	0	0	0	0	50.0	0	0	0	
<i>Alkaligenes</i> species (N=1)	S	0	0	100	100	100	100	100	100	100	100	0	100	100	
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	ND
	R	100	100	0	0	0	0	0	0	0	0	100	0	0	
<i>Moraxella</i> species (N=3)	S			100			100			100	100	100	100	100	
	I	ND	ND	0	ND	ND	0	ND	ND	0	0	0	0	0	ND
	R			0			0			0	0	0	0	0	
<i>H. influenzae</i> (N=5)	S	100	100	60.0			100			40.0	100	100			
	I	0	0	0	ND	ND	0	ND	ND	0	0	0	ND	ND	ND
	R	0	0	40.0			0			60.0	0	0			
Total isolates	S	18.5	35.5	66.7	100	95.5	93.3	95.5	86.4	73.3					

(N=30)	R	81.5	64.5	33.3	0	4.5	6.7	4.5	13.6	26.7				
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AMP- Ampicilin (10 µg), AMC- Amoxicillin-Clavulanic acid (10 µg), AK- Amikacin (30 µg), CAZ- Ceftazidime (10 µg), MEM- Meropenim (10 µg), NOR- Norfloxacin (10 µg), PEP- Piperacilin (100 µg), P- Pattern, N- Number and ND- Not Done

5.6. Multidrug resistance of bacterial isolates

In this study, the overall prevalence of multidrug resistance (bacteria resistant for two or more antibiotics tested) was 84 (71.2%). Twelve (10.2%) bacterial isolates were sensitive for all antibiotics tested. Most of the gram negative enteric bacteria isolated were resistant for at least one antibiotic (table 5.9).

Table5.9. Multiple antibiotics resistance pattern of bacterial isolates at St. Paul’s Hospital Millennium Medical College Eye Clinic, 2016

Bacteria	Multidrug resistance						Total
	R0	R1	R2	R3	R4	R5 and above	
<i>S. aureus</i>	1(8.3)	8(36.4)	13(32.5)	4(22.2)	3(17.6)	3(33.3)	32(27.1)
CONS	0	3(13.6)	8(20.0)	2(11.1)	9(52.9)	3(33.3)	25(21.2)
<i>S. pneumoniae</i>	5(41.7)	5(22.7)	4(10.0)	3(16.7)	0	1(11.1)	18(15.3)
<i>S. pyogenes</i>	1(8.3)	1(4.5)	1(2.5)	1(5.6)	0	0	4(3.4)
<i>S. viridians</i>	0	3(13.6)	5(12.5)	0	1(5.9)	0	9(7.6)
<i>Moraxella</i> spp.	3(25.0)	0	0	0	0	0	3(2.5)
<i>H. influenzae</i>	2(16.7)	1(4.5)	2(5.0)	0	0	0	5(4.2)
<i>E. coli</i>	0	0	0	0	1(5.9)	2(22.2)	3(2.5)
<i>K. pneumoniae</i>	0	0	4(10.0)	3(16.7)	2(11.8)	0	9(7.6)
<i>P. aeruginosa</i>	0	0	0	2(11.1)	1(5.9)	0	3(2.5)
<i>E. aerogenes</i>	0	1(4.5)	1(2.5)	0	0	0	2(1.7)
<i>P. mirabilis</i>	0	0	2(5.0)	0	0	0	2(1.7)
<i>Citrobacter</i> spp.	0	0	0	2(11.1)	0	0	2(1.7)
<i>Alkaligenes</i> spp.	0	0	0	1(5.6)	0	0	1(0.8)
Total	12(10.2)	22(18.6)	40(33.9)	18(15.3)	17(14.4)	9(7.6)	118(100)

R0-sensitive for all antibiotics tested, **R1**-resistant for 1 antibiotic, **R2**- resistant for 2 antibiotics, **R3**- resistant for 3 antibiotics, **R4**- resistant for 4 antibiotics, **R5**- resistant for 5 and above antibiotics

5.7. Bacterial and fungal prevalence and associated risk factor

Out of the total 215 patients enrolled in this study. Of 109 male participants 54.1% and 12.8% were positive for bacterial and fungal culture respectively. On the other hand, from 106 females 55.7% and 9.2% were bacterial and fungal culture positives respectively. The predisposing factors such as sex, residence, educational background, occupational status, trauma, presence of systemic diseases, previous surgery, hospitalization for long time and age were assessed. The binary logistic regression was done to determine the association. However, there was no significant statistical association between bacterial and fungal prevalence and those associated factors except bacterial prevalence with age variations P-value=0.023; AOR=4.87(95% CI; 1.24-19.12) and fungal prevalence with trauma P-value=0.002; AOR=0.245(95% CI; 0.096-0.623) (table 5.10 and 5.11).

Table5.10. Association between Selected socio-demographic and clinical characteristics and bacterial positivity at St. Paul’s Hospital Millennium Medical College eye clinic, 2016

Variables		Bacteria				
		Positive N (%)	COR (CI; 95%)	P-value	AOR (CI; 95%)	P-value
Sex	Male	59(54.1)	1.064(0.622-1.821)	0.821		
	Female	59(55.6)	1			
Age group	1-14	16(84.2)	0.205(0.052-0.806)	0.023	4.870(1.240-19.12)	0.023*
	15-24	10(43.5)	1.424(0.516-3.928)	0.495	0.702(0,255-1.937)	0.495
	25-44	37(50.7)	1.066(0.504-2.253)	0.868	0.938(0.444-1.984)	0.868
	45-64	32(57.1)	0.821(0.371-1.817)	0.627	1.217(0.550-2.692)	0.627
	≥65	23(52.3)	1			
Residence	Rural	29(46.0)	1.656(0.917-2.992)	0.094		
	Urban	89(58.5)	1			
Education level						
	Illiterate	50(53.2)	1.100(0.465-2.601)	0.828		
	Preschool	10(83.3)	0.250(0.046-1.365)	0.109		
	Literate	58(53.2)	1			
Occupation						
	Labor worker	31(48.4)	1.31(0.689-2.490)	0.41		
	Employee	38(63.3)	0.689(0.354-1.342)	0.27		
	Unemployed	49(53.8)	1			
Trauma	Yes	17(50.0)	1.262(0.606-2.629)	0.533		
	No	101(55.8)	1			
Systemic disease	Yes	9(42.8)	1.710(0.689-4.246)	0.248		
	No	109(56.1)	1			
Face washing frequency						
	Less frequent	54(49.5)	3.819(1.191-12.24)	0.024	3.064(0.895-10.499)	0.07
	Frequent	49(56.3)	2.908(0.892-9.478)	0.077	2.099(0.611-7.206)	0.24
	More frequent	15(78.9)	1			

Previous surgery	Yes	18(56.3)	0.937(0.440-1.997)	0.866
	No	100(54.6)	1	
Hospitalization	Yes	2(33.3)	2.495(0.447-13.92)	0.297
	No	116(55.5)	1	

1-reference category, *- statistically significant association (P-value < 0.05), COR- Crude Odds Ratio, AOR- Adjusted Odds Ratio, CI-Confidence interval

Table5.11. Association between Selected socio-demographic and clinical characteristics and fungal positivity at St. Paul’s Hospital Millennium Medical College eye clinic, 2016

Variables		Bacteria				
		Positive N (%)	COR (CI; 95%)	P-value	AOR (CI; 95%)	P-value
Sex	Male	14(12.8)	0.71(0.299-1.670)	0.43		
	Female	10(9.4)	1			
Age group	1-14	6(31.6)	0.278(0.073-1.064)	0.06		
	15-24	2(8.7)	1.346(0.240-7.544)			
	25-44	3(4.1)	2.991(0.678-13.19)			
	45-64	8(14.3)	0.769(0.233-2.54)			
	≥65	5(11.4)	1			
Residence	Rural	9(14.3)	0.657(0.271-1.591)	0.352		
	Urban	15(9.9)	1			
Education level						
	Illiterate	11(11.7)	0.762(0.308-1.883)	0.56		
	Preschool	3(25.0)	0.303(0.070-1.304)			
	Literate	10(9.2)	1			
Occupation						
	Labor worker	8(12.5)	0.838(0.311-2.257)	0.73		
	Employee	6(10.0)	1.098(0.377-3.196)			
	Unemployed	10(10.9)	1			
Trauma	Yes	9(26.5)	0.251(0.099-0.634)	0.003	0.245(0.096-0.623)	0.002*
	No	15(8.3)	1			
Systemic disease	Yes	4(19.0)	0.489(0.150-1.595)	0.24	0.450(0.126-1.611)	0.22
	No	20(10.3)	1			
Face washing Frequency						
	Less frequent	13(11.9)	1.969(0.567-6.844)	0.28		
	Frequent	7(8.0)	3.048(0.793-11.71)			
	More frequent	4(21.0)	1			
Previous surgery	Yes	4(12.5)	0.859(0.273-2.702)	0.79	1.034(0.298-3.585)	0.95
	No	20(10.9)	1			
Hospitalization	Yes	1(16.7)	0.618(0.069-5.53)	0.67		
	No	23(11.0)	1			

1-Reference category, *-statistically significant association (P-value <0.05), COR-Crude Odds Ratio, AOR-Adjusted Odds Ratio, CI- Confidence Interval

Presence of systemic diseases and previous surgery were significant in other studies though they were not statistically significant in our case, we used to determine adjusted odds ratio with trauma in this study.

Occupational status of study participants originally collected cannot clearly show the association with odds ratio and 95% CI, so that this variable was merged in to three categories for proper manipulation of the association between bacterial and fungal prevalence and occupational status.

6. Discussion

Ocular infection is a major public health problem especially in developing countries like Ethiopia. The external ocular infections are responsible for increased incidence of morbidity and blindness worldwide, their morbidity vary from self limiting light infection to sight threatening infection [1, 4].

In this study, out of 215 patients, 54.9% were found to be positive for bacterial pathogen. This finding is in agreement with the previous studies carried out in Gondar, 54.2% [38], in Iran, 52.4% [26], in Hawassa, 50.7% [19], and comparable with studies in Addis Ababa (47.4%) [39], in Borumeda (59.4%) [6] The same study in Jimma reported higher prevalence of bacterial pathogens 74.7% [4], and also in Babylon, 92.1% [9] and in India, 64% [5]. However, our finding showed higher prevalence than study conducted in Bangalore, 34.5% [2]. The possible reasons for varying rate of isolation of bacterial pathogens may be because of the variations in geographical location, study period, study design and socioeconomic status population studied.

Moreover, the prevalence of fungal pathogen from external ocular infection was 11.2%. This is prevalence was higher than studies conducted in Yemen 5.1% [27] and in South India, 10.3% [25]. But it is lower prevalence when compared to study in Bangalore, 13.6% [2], in South India, 25.7% [24] and another study in India, 18.4% [5]. This prevalence difference may be due to study design, climatic and geographical variations [12, 27].

In the current study, the most common type of external ocular infection was conjunctivitis (40.5%) followed by blepharitis (22.3%), Dacryocystitis (12.6%). This is the same with the previous works in our country like in Borumeda, Hawassa, Jimma, Addis Ababa [4, 6, 19, 39]. Keratitis cases were lower in number when compared to other studies in Ethiopia and abroad [4,

5, 19, 25, 39]. This may be due to the difference in geographical location, climate variation, the period study done. However, the coverage of fungal pathogens was higher this may be because 6 of 9 cases with keratitis came from rural region because in rural area they engaged in labor work. The bacterial culture positivity among different types of external ocular infections in this study was revealed that dacryocystitis 68.2%, followed by conjunctivitis 60.9%), blepharitis 50%. Whereas fungal prevalence was higher in keratitis 55.5% followed by conjunctivitis 10.5%. This is in line with other studies elsewhere, study in India showed that Conjunctival swabs yielded 52% bacterial isolates and 33.3% of corneal scrapings grew fungal isolates and a study in Addis Ababa also reported in agreement with our result [2, 39]. Fungal pathogens also isolated from blepharitis 2(4.2%) and post traumatic infections 2(22.2%) in this study. This is in agreement with studies in Addis Ababa isolated 1 fungal element from blepharitis [39] and in Bangalore reported 1(6.3%) fungal pathogen from post traumatic infections [2]. And comparable to our finding in India reported 13.7% of fungal conjunctivitis [8].

The most commonly isolated bacterial pathogens in this study were gram positive cocci 88(74.6%). In line with the current study, there are reports in other part of Ethiopia, like 93.7% in Borumeda [6], 52% in Jimma [4], 61.5% in Hawassa [19]. And other studies abroad also reported that gram positive cocci as the dominant bacteria in Navodaya, Cambodia, Babylon, Iran, China and Nigeria [1, 22, 23, 26, 40, 41]. In general most studies revealed that gram positive cocci were responsible for causing external ocular infections. This may be due to from abnormal proliferation of the native flora, contaminated fingers or skin [42].

In the present study, *S. aureus* 32(27.1%) was the predominant pathogen followed by CoNS 25(21.2%) *S. pneumoniae* 18(15.3%), *S. viridians* 9(7.6%), *K. pneumoniae* 9(7.6%). Our result is in agreement with other studies in Ethiopia like in Addis Ababa, Gondar, Hawassa, Jimma [4, 19, 38, 39] and in other parts of the world like in Bangalore, Yemen, Babylon and Cambodia also reported *S. aureus* as a predominant organism followed by coagulase negative *Staphylococci* [2, 9, 22, 27]. However, some other studies reported CoNS as the commonest isolate such as in South India [24], in India [5], in Uganda [30], in Borumeda [6] and two studies in Gondar [31, 32]. In this study *S. aureus* was the commonest bacterial isolate in conjunctivitis, blepharitis, blepharo-conjunctivitis. This finding is supported by studies conducted in Jimma [4], Nigeria [43], in India [25]. However, in Dacryocystitis, coagulase negative *Staphylococci* were the

predominant bacteria. This is consistent with a study in Gondar [32]. In contrast, studies in Hawassa [19] and Addis Ababa [39] reported that *S. pneumoniae* was the predominant bacteria in Dacryocystitis. The reason for high coverage of *S. aureus* and CoNS in blepharitis and blepharoconjunctivitis may be due to virulence factors such as exo-enzymes, surface slime may play for the pathogenesis [19, 28].

The result of this study showed that 22(18.8%) of enteric gram negative bacteria were isolated. Similar studies reported higher in locally (Gondar) 44.5% [38] and internationally (India) 29(35%) [2]. However, our study showed higher prevalence than studies in Borumeda and Jimma accounted 6.5% and 11.5% respectively [4, 6]. Among gram negative bacteria *K. pneumoniae* was the predominant. This is supported by studies conducted in Navodaya [1], and in Egypt [29]. But other studies in Jimma, Yemen and Gondar, reported *P. aeruginosa* as the dominant gram negative bacterial isolate [4, 27, 32]. *E. coli* also reported in other study [9].

In this study, *Aspergillus* species 14(58.3%) was frequently isolated from external ocular infection followed by *Penicillium* species 5(20.8%), *Candida* species 4(16.7%) and a single case of *Acremonium* species. Similarly, a study in Yemen reported *Aspergillus* species as the commonest fungal isolate followed by *C. albicans* [27] and also in Delhi India and Western India reported *Aspergillus* species as the dominant fungal pathogen [12, 44]. However, other studies like in India, South India, North Karnataka reported that *Fusarium* species as the predominant causative agent of mycotic eye infections followed by *Aspergillus* species, *Penicillium* species [5, 8, 17]. Furthermore, study in Ethiopia reported equal number of *Aspergillus* species and *Fusarium* species [39]. The difference in type of fungal pathogens implicated in causing external ocular infections may vary over geographical variation, climate change and time to time.

The current result showed that there was 9(6.8%) of bacterial and fungal co-infections. The same to ours, other studies like in India 2(5%), Yemen 6(4.3%) and South India 14(0.32%) of bacterial and fungal co-infections were reported [5, 25, 27].

In the current study, the demographic characteristics and associated predisposing factors were assessed. In the case of sex, 54.1% and 12.8% of males were positive for bacterial and fungal culture respectively whereas 55.7% and 9.2% of females were positive for bacterial and fungal pathogen respectively and this showed females were relatively more affected by bacterial

infection and the reverse is true for fungal infection. In agreement with our finding study in Yemen suggested that females were more affected and male were far more susceptible for fungal pathogens [27]. However, it is different from another study in Borumeda, males were more affected since most were rural and farmers engaged in outdoor activities [6]. Moreover, age group of 1-14 years were showed the higher frequency of bacterial isolates (84.2%) and it was statistically significant (P-value=0.023, AOR=4.87 (CI 95%; 1.24-19.12). In this case, the odds of having bacterial infection among age group of 1-14 is 4.8 times risky than age group of ≥ 65 . This is supported by a study conducted in Egypt [25] and also comparable with other works showed variation of bacterial isolates with age variation inferring that the age group of ≤ 2 was showed statistically significant association with bacterial infection like in Jimma, Gondar, and India [4, 29, 31]. The reason for increased susceptibility to infection in infants and children may be that they are at a greater risk after their maternal immunity has disappeared and before their own immunity system had matured and sometimes mothers ignorance of children hygiene [25, 31]. Traumatic history and fungal positivity were showed statistically significant association P-value=0.002, AOR=0.245 (95% CI; 0.096-0.623) in this study. So that, patients who had traumatic history were 0.245 times more at risk for fungal infection than patients with no trauma. On this regard, other studies in North Karnathaka [17], in South India [45] supported our finding. However, there was no statistically significant association between bacterial and fungal prevalence and other possible risk factors assessed. In our study, there was no study participant who had the history of contact eye lens wearing. It may be due to the socioeconomic status of the population studied since contact lenses are highly expensive. In contrast, other studies reported contact lens wearing as an important predisposing factor for both bacterial and fungal infections of the eye [44, 45]. But it depends on the care of contact lens and environmental factors.

Since the treatment of ocular infection is on empirical basis with first line broad spectrum antibiotics and the increasing of drug resistance among pathogens causing external ocular infection, continuously updated data on antimicrobial susceptibility patterns would be beneficial for the trend of empirical therapy. In the current study, Gentamycin (96.2%), Tobramycin (88.6%), Ciprofloxacin (85%) and chloramphenicol (77.4%) were effective against overall bacterial isolates. This is in line with other previous studies elsewhere in India [5], Iran [25], Uganda [30] and in Navodaya [1] with minor differences. Studies in Ethiopia like in Hawassa

[19], Jimma [4], and Addis Ababa [46] also reported the same pattern with our finding. A study in Gondar also showed that 74.2% of overall bacterial isolates were sensitive for ciprofloxacin, tetracycline, chloramphenicol, co-trimoxazole [32]. However, another study in Gondar showed that most of bacterial isolates were resistant for gentamycin (45.2%), penicillin (71%), trimethoprim-sulphamethoxazole (58.1%), and tetracycline (64.6%) [31]. In addition, a study in Borumeda showed lower coverage of tetracycline, norfloxacin, ceftriaxone and ciprofloxacin against gram negative bacteria [6].

On the other hand, gram positive cocci were sensitive for erythromycin (79.5%), clindamycin (85.2%) and vancomycin (77.4%) while gram negative isolates were 100% sensitive for Ceftazidime, Meropenim and Amikacin. However, 73.9% of gram positive cocci were resistant for penicillin especially *Staphylococci* (94.7%) and tetracycline (47.7%) and most gram negative isolates were resistant for ampicillin and amoxicillin 81.5% and 59.3% respectively and also 26.7% of gram negative bacteria were resistant for tetracycline and chloramphenicol. This is similar with other studies conducted in Navodaya, Iran, Gondar, Jimma and Hawassa [1, 4, 19, 26, 38]. This may be due to these antibiotics are more or less cheap and easily purchased in most pharmacies without physicians prescription and this may lead to misuse of antibiotics [4, 38].

S. aureus, the most prevalent bacteria in our study which was highly sensitive for Tobramycin 32(100%), Gentamycin 31(96.9%), Clindamycin 30(93.8%), and Ciprofloxacin 29 (90.6%) however, Penicillin 31(96.9%) and Tetracycline 18(56.2%) were showed lower coverage. This finding is consistent with previous studies such as in Jimma and Gondar [4, 32].

The result of this study showed that 12.3% of *Staphylococci* were oxacilin resistant. Of this 12.5% and 12% of *S. aureus* and CoNS were Oxacilin resistant respectively while *S. pneumoniae* was sensitive for oxacilin (94.4%). Study in Uganda reported comparatively higher percentage 31.9% and 27.6% of Methicillin resistant *S. aureus* and CoNS respectively [30]. However a study in Cambodia reported 95.7% of *S. aureus* was sensitive for oxacilin [22]. A review paper in United States reported that from 3% to 64% of ocular staphylococcal infections were due to Methicillin-resistant *S aureus* [47]. And this condition is becoming more common and the organisms are resistant to many antibiotics [48].

In this study, the prevalence of multidrug resistant was 71.2%. This is in agreement with previous works in Hawassa 69.9% [19], Gondar 77.1% [38]. However, another study in Gondar reported the higher prevalence of MDR 87.1% [31]. This difference may be due to the difference in type of drugs tested and time study done. Resistance among ocular pathogens seems to be increasing in consonance with the increase of resistance among bacteria associated with systemic infections. The factors contributing to the development of drug resistance among ocular bacterial isolates may include overuse of antibiotics for systemic infection as well as overuse of topical antibiotics in the eye with or without physician prescription, improper dosing regimen, misuse of antibiotics for viral infections, extended duration of therapy and due to lack of microbiology laboratory in most health institutions clinicians' advocated use of empirical first line broad spectrum therapy. Therefore these factors may result in increasing of drug resistance development and it needs continuous assessment and measures to be taken by the concerned body [4, 19, 49].

7. Limitation of the study

- ✓ We couldn't isolate anaerobic bacteria pathogens because of lack of facilities needed
- ✓ Due to need of additional special laboratory techniques, we couldn't isolate *Chlamydia trachomatis*

8. Conclusion

The prevalence of bacterial and fungal pathogens from external ocular infections was found to be higher. Conjunctivitis cases were the most commonly identified type of external ocular infection. The emergence of drug resistance and multiple antimicrobial resistances were also high. High prevalence of bacterial and fungal external ocular infection high lights the need for nationwide study on bacterial and fungal external ocular infections and precise identification of causative agents and periodic evaluation drug susceptibility pattern of bacterial pathogens associated with external ocular infection.

9. Recommendation

- Clinicians need to give a due attention and extending their suspicion for fungal infections of external ocular. If it is possible laboratory confirmation become very important.
- The first-line empirical antibiotics therapy like Ampicilin, Tetracycline and Penicillin showed resistance for the commonly isolated bacteria. Hence, reconsideration of the effectiveness of these antimicrobial agents would be important
- We also recommend large scale researches that can assess wide geographical area with large population

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11. Annexes

11.1. Annex I: Informed consent (in English and Amharic)

I undersigned to confirm that, as I give consent to participate after a clear understanding of the objectives and conditions of the study & with recognition of my right to withdraw from the study if I change my mind.

Ido interestingly give consent to Mr.to include me in the proposed research. The proposal has been explained to me in the language I understand.

Name of Participant: _____

Participant's signature: _____

Name of data collector: _____

Signature of data collector: _____

Date: _____

11.2. Patients consent form (Amharic version)

የተሳታፊው ስም

ስለጥናቱ አስፈላጊ የሆኑትን መረጃዎች እንብቤ ወይም ተነቦልኝ አላማውም ከወጫዊ የዓይን ክፍል በሽታዎች የሚገኙት ተዋስኖን ማለትም ባክቴሪያዎችን እና ፈንገሶችን መለየት እና የሚያድኑት መድሀኒቶች ማወቅ ሲሆን ለጥናቱ አስፈላጊ የሆኑትን መረጃዎች እና ናሙና እንደምስጥ ተረድቻለሁ። ከጥናቱ ጋር የተያያዙ ጥያቄዎችንም ጠይቄ ማብራሪያዎች ተሰጥተዋል።

እኔ.....የተባልኩ የተጠየኩትን ጥያቄ እና ናሙና ለተመራማሪዬ ለመስጠት ፈቃደኝነቴን ገልጫለሁ.

የተሳታፊው ስም

የተሳታፊው ፊርማ ቀን

የመረጃ ሰብሳቢው ስም

የመረጃ ሰብሳቢው ፊርማ ቀን

11.3. Annex II Parental Consent Form (English version)

I _____ parent, after being fully informed about the purpose of this study,
Study title: The bacterial and fungal pathogens from external ocular infection at St. Paul Hospital
Millennium Medical College, Addis Ababa, Ethiopia.

I, the undersigned, have been told about the present study. My child has to say to choose if I want to be in the study. I have been informed there is no harm except little discomfort during sample collections. I have been informed that other people will not know my child results as it coded with number rather than writing name. I understand that there may be no benefit to me personally apart from clinical service I get from these results. I have been encouraged to ask questions and have had my questions answered. I have been told that participation in this study is voluntary and I may refuse to be in the study. I know my participation will also be approved by my child. By signing below I agree to let my child to participate in this research study.

Name of adult patients

Signature

____/____/____
Day/month/year

Name of the researcher

Signature

____/____/____
Day/month/year

Witness (Illiterate)

Signature

____/____/____
Day/month/year

11.4. Parental Consent Form (Amharic Version)

የስነምግባራዊ ምርመራ ቅጽ

እኔ-----የልጄ አስታሚ ስሆን የዚህን ጥናት አላማ በወል ተረድቻለሁ። የጥናቱ ርዕስ በቅዱስ ዳዌሎስ ሆስፒታል ሚሊኒየም ሜዲካል ኮሌጅ የአይን ክሊኒክ ታካሚዎች መሀከል የወጪኛው የአይን ኢንፎርሽን ተህዋስን የሚያመጣውን ህመም እና የተህዋስን መድሃኒት የመቋቋም ያለውን ስርጭት በአይን ህመማችን ላይ ምን ያህል እንደሆነ ለማውቅ ሲሆን በጥናቱ ልጄ እንዲሳተፍ ምርጫው የእኔ መሆኑን ነግረውኛል። ናሙና ሲወሰድ ከትንሽ የህመም ስሜት ውጪ ምንም አይነት ጉዳት ልጄ ላይ እንደሌለው ተነግሮኛል። በጥናቱ ወቅትም የልጄ መረጃዎች በሚስጥር ስለሚያዝ በሌላ ሰው ዘንድ እንደማይታወቅ ተረድቻለሁ። በውጤቱ ከሚገኘው የህክምና አገልግሎት በቀር ሌላ ልጄ በግሉ የሚያገኘው ጥቅም እንደሌለ ተረድቻለሁ። ጥያቄ እንደጠይቅ ዕድል ተሰጥቶኝ ለጥያቄዎቼም በቂ ምላሽ አግኝቻለሁ። የልጄ በጥናቱ መሳተፍ በእኔ ፍላጎት ብቻ እንደሆነ እና በጥናቱም አለመሳተፍ ምንም አይነት ተፅዕኖ በልጄ ላይ እንደማያስከትል ተረድቻለሁ። በከዚህ ባሻገር የልጄ በጥናቱ ውስጥ ለመካተት የእኔ የወላጅ አሳዳጊ ፈቃድ እንደሚያስፈልገው ተረድቻለሁ። በእኔ ፍቃድኝነት ልጄ በጥናቱ እንደሚሳተፍ ከዚህ በታች በፊርማዬ አረጋግጣለሁ።

የተሳታፊው ሥም

ፊርማ

____/____/____
ቀን/ወር/ዓ.ም

ምስክር (ማንበብና መጻፍ ለማይቻሉ)

ፊርማ

ቀን / ወር / ዓ.ም

11.5. Annex IV: Patient Information Sheet (In English and Amharic)

1. Purpose

To assess bacterial and fungal pathogens from external ocular infection St. Paul hospital millennium medical college suspected with external ocular infections.

2. Procedures to be carry out

- Conjunctival, eyelid margin swabs and corneal scraping can also be collected as required.

3. There might be very little discomfort but no risk at all while collecting sample.

4. Expected benefit of the study

- The participants can be diagnosed for external ocular infections
- The study gives information about the overall prevalence of bacterial and fungal infections and the drug susceptibility pattern at the study area

5. Confidentiality of your information records are kept confidential

- You are not mentioned by name.
- Your specimens are used only for this study purpose.

6. Voluntary participation

- Your participation is by willingness.
- The participant can withdraw study at any time and he/she can't be asked the reason.
- Withdrawal from study doesn't have impact on participant health service.

7. Contact address

Addis Ababa University, Faculty of Medicine department of medical laboratory sciences.

Addis Aklilu Tel: 0912718569 E-mail: addaklilu@gmail.com

ለጥናቱ ተሳታፊዎች

1. የጥናቱ አላማ

የዉጫዊ የዓይን ክፍል በሽታ የሚያመጡ ተዋስኖንን መለየት እና የተህዋስያኑ መድሃኒት የመቋቋም ያለዉን ስርጭት በአይን ህሙማን ለይ ምን ያህል እንደሆነ ለማውቅ ነው።

2. የተሳትፎ ሁኔታ

- በጽሁፍ ለሚቀርቡ ጥቁዎች ምላሽ መስጠት
- ከዓይን ላይ ለሚወሰዱ ናሙናዎች ፍቃደኛ መሆንና በሚወሰዱበት ጊዜ ትብብር ማድረግ

3. ሊከሰቱ የሚችሉ ስጋቶች

- ናሙና በሚወሰዱበት ወቅት እጅግ በጣም ዝቅተኛ የሆነ የህመም ስሜት ሊሰማ ይችላል። ነገር ግን ይህ ነው የሚባል ችግር የሚያስከትል አይደለም።

4. ጥቅሞች

- በጥናቱ ተሳታፊዎች ዓይን ላይ የባክቴሪያ ወይም የፈንገስ በሽታ መኖሩን ማረጋገጥ እና ህክምና ማግኘት።
- በጥናቱ ተሳታፊዎች ላይ በሽታ ከተገኘባቸው ተገቢዉን ህክምና እንዲያገኙ ማድረግ።

- የጥናቱን ውጤት ለሆስፒታሉ በመስጠት ተገቢውን መረጃ አግኝቶ የአገልግሎት ማሻሻያ ለማድረግ ያስችላዋል።

5. ሚስጥር ስለመጠበቅ

- የተሳታፊ ስም አይገለጽም
- የሰጡት ናሙና ለተናቱ አላማ ብቻ የሚያገለግል ይሆናል

6. በጥናቱ ያለመሳተፍ ወይም ራስን የማግለል መብት

- የርስዎ ተሳትፎ በፈቃደኝነት ላይ የተመሰረተ ነው።
- እርስዎ በጥናቱ ላይ መሳተፍዎን ለመሰረዝ ከፈለጉ በማንኛውም ሰዓት መሰረዝ ይችላሉ። ይህንንም ከወሰኑ ማንም ምክንያቱን እንዲገልጹ ሊያስገድድዎት አይችልም።
- በጥናቱ ዉስጥ አለመሳተፍ በጤና እንክብካቤዎ ላይ ምንም አይነት ተጽእኖ አይኖረውም።

7. መረጃ ስለማግኘት

የተመራማሪው አድራሻ..... ስልክ: 0912718569 ፣ ኢ-ሜይል:

addaklilu@gmail.com

አዲስ አበባ ዩኒቨርሲቲ ህክምና ፋክልቲ የህክምና ላቦራቶሪ ሳይንስ ት/ ቤት

11.6. Annex V: Questionnaire

Addis Ababa University, Faculty Of Medicine, College Of Health Sciences, Department Of Medical Laboratory Sciences, Clinical Laboratory Masters Program In Diagnostic And Public Health Microbiology Specialty

The structured questionnaire used to collect the demographic data of patients and clinical presentations and other associated risk factors. Place the “X” in the box options.

Patient card No.....

Date / /

Code

1. Patient Full Name

2. Sex

12. Face washing habit?

1. Less frequently 2. Frequently
3. More frequently

13. Do you make surgery of eye?

1. Yes 2. No

14. Having been hospitalized for long period of times?

1. Yes 2. No

Signature of Data collector

Signature of study participant

11.7. Annex VI: Standard Operative Procedures

1. Specimen collection

A. Conjunctiva and lid margin swab for bacterial and fungal culture

Sample will be collected at bedside by an ophthalmologist prior to administering antibiotics or topical medications. After detailed ocular examinations, external ocular sample were collected by swabbing the purulent conjunctivitis. Patient was requested to look up, lower eyelid was pulled down and then samples 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 pre-moistened with sterile saline. The sterile normal saline moistened swab was rubbed over the lower Conjunctival sac from medial to lateral side and back again.

1) Roll a sterile, pre-moistened cotton swab, using a new swab for each of the following body sites:

2) Inoculate the following media.

- a) 5% sheep's blood agar plate or blood agar simply
- b) Chocolate agar plate
- c) MacConkey's agar
- d) Sabouraud's Dextrose plate – for fungal culture only

B. Dacryocystitis

- 1) Cleanse skin with alcohol and tincture of iodine or iodophor
- 2) Collect a specimen of purulent discharge by using a swab like conjunctivitis collection
- 3) Do not perform a needle aspiration of the lacrimal gland.

C. Corneal scrapings

It should be collected beside by an ophthalmologist and usually submitted concurrently with Conjunctival samples

- 1) Instill 1 or 2 drops 0.5% proparacaine hydrochloride, if not already administered
- 2) Using a sterile gauge 21 disposable syringe needles, use short firm strokes in one direction to obtain corneal scrapings from the advancing edge of the ulcer. Obtain multiple areas.
- 3) Obtain 3-4 scrapings per cornea
- 4) Inoculate a 5% sheep's blood agar plate and a chocolate agar plate making one row of "C" formations for each scraping.

2. Microscopy

Gram stain

Gram reaction principle

Differences in Gram reaction between bacteria is thought to be due to differences in the permeability of the cell wall of Gram positive and Gram negative organisms during the staining process. Following staining with a triphenyl methane basic dye such as crystal violet and treatment with iodine, the dye-iodine complex is easily removed from the more permeable cell wall of Gram negative bacteria but not from the less permeable cell wall of Gram positive bacteria. Retention of crystal violet by Gram positive organisms may also be due in part to the more acidic protoplasm of these organisms binding to the basic dye (helped by the iodine).

Procedure

- 1. Prepare smear on clean slide then air-dry and heat-fix specimen using a Bunsen burner or spirit lamp

2. Allow slide to cool on staining rack
3. Flood slide with crystal violet; leave for 1 minute
4. Rinse slide in clean running water
5. Flood slide with Gram's iodine; leave for 1 minute
6. Rinse slide in clean running water
7. Apply acetone and rinse immediately under running water (exposure to acetone 5 seconds)
8. Counter-stain with carbol fuschin/safranin for 1 minute
9. Rinse in clean running water then dry with blotting paper
10. View specimen with 10x objective
11. Place a drop of immersion oil on the slide and view with 100x oil-immersion objective.

Fungal examination

Lactophenol cotton blue (LPCB) or potassium hydroxide (KOH) wet mount preparations are used to visualize fungi.

- 1 Add a drop of lactophenol cotton blue/KOH mounting to the slide.
- 2 Holding the cover slip between your forefinger and thumb, touch one edge of the drop of mountant with the cover slip edge, the lower it gently, avoiding air bubbles. The preparation is now ready.
- 3 Initial observation should be made using the low power objective (10x), switching to the higher power (40x) objective for a more detailed examination.

3. General protocol of Culture media preparation

1. Weighing and dissolving of culture media
2. Sterilization
3. Addition of heat sensitive ingredients
4. p^H testing of culture media
5. Dispensing of the culture media
6. Sterility testing
7. Quality assurance of culture media
8. Storage of culture media

Prepare media made from dehydrated products in as damp-free an environment as possible. To prevent the risk of inhaling fine particles of dehydrated media, wear a dust mask while handling dehydrated media, powder or use granulated media

- Wash the hands immediately after preparing media.
- Once the ingredients are weighed, do not delay in making up the medium. Follow exactly the manufacturer's instructions.
- Use completely clean glassware, plastic or stainless steel equipment that has been rinsed in pure water. The container in which the medium is prepared should have a capacity of at least twice the volume of the medium being prepared.
- Use distilled water from a glass still. Deionized water can also be used providing the exchange resins do not contain substances inhibitory to bacteria. Water containing chlorine, lead, copper, or detergents must not be used. Besides containing substances harmful to bacteria, impure water can alter the pH of a medium or cause a precipitate to form.
- Add the powdered or granular ingredients to the water and stir to dissolve. Do not shake a medium but mix by stirring or by rotating the container.
- When heating is required to dissolve the medium, stir while heating and control the heat to prevent boiling and foaming which can be dangerous and damage the medium, Overheating a medium can alter its nutritional and gelling properties, and also its pH.
- Autoclave a medium only when the ingredients are completely dissolved. Always autoclave at the correct temperature and for the time specified.
- Dispense medium in bottles or tubes in amounts convenient for use. Know the length of time prepared media can be stored without deteriorating (take into account storage temperature).

4. Dispensing sterile media into petri dishes

1. Lay out the sterile petri dishes on a level surface.
2. Mix the medium gently by rotating the flask or bottle. Avoid forming air bubbles. Flame sterilize the neck of the flask or bottle and pour 15–20 ml of medium into each dish (90–100 mm diameter). air bubbles enter while pouring, rapidly flame the surface of the medium before gelling occurs. Rotate the dish on the surface of the bench to ensure an even layer of agar.
3. When the medium has gelled and cooled, stack the plates and seal them in plastic bags to prevent loss of moisture and reduce the risk of contamination. Do not leave the plates exposed to bright light especially sunlight.
4. Store at 2–8 °C.

Note: Agar plates should be of an even depth (not less than 4 mm) and of a firm gel. The surface of the medium should be smooth and free from bubbles.

Each Media Preparation

I). Preparation of 5%Solid Blood Agar

Blood agar is used with Nutritious agar and sterile defibrinated blood for the isolation and differentiation of many external ocular infection bacteria.

Formula / Liter Supplements

To make about 35 blood agar plates:

Blood agar base.....	40 g
Distilled water.....	1000 ml
Defibrinated blood	50 ml

1. Prepare the agar medium as instructed by the manufacturer. Sterilize by autoclaving at 121 °C for 15 minutes. Transfer to a 50 °C water bath.
2. When the agar has cooled to 50 °C, add aseptically the sterile blood and mix gently but well.
3. Avoid forming air bubbles.

Important: The blood must be allowed to warm to room temperature before being added to the molten agar.

4. Dispense aseptically 12-15 ml of blood agar amounts in sterile Petridish.
5. Date the medium and give it a batch number.
6. Store the plates at 2–8 °C. Preferably in sealed plastic bags to prevent loss of moisture.

II. Chocolate (Heated Blood) Agar

When blood agar is heated, the red cells are lyzed and the medium becomes brown in colour. It is referred to as chocolate agar and supplies the factors required for the growth of *H. influenzae*. It is also used to culture nutritionally demanding pathogens such as *N. meningitidis* and *S. pneumoniae*.

- 1 Prepare as described for blood agar except after adding the blood, heat the medium in a 70 °C water bath until it becomes brown in colour. This takes about 10–15 minutes during which time the medium should be mixed gently several times.
- 2 Allow the medium to cool to about 45°C, remix and dispense in sterile petri dishes as described for blood agar.

Important: Care must be taken not to overheat or prolong the heating of the medium because this will cause it to become granular and unfit for use.

3 Date the medium and give it a batch number. Store the plates as described for blood agar.

III. MacConkey Agar

Intended Use

MacConkey Agar is selective for Gram negative organisms, and helps to differentiate lactose fermenting gram negative rods from Non lactose fermenting gram negative rods. It is primarily used for detection and isolation of members of family enterobacteriaceae and *Pseudomonas* spp.

Principles of the Procedure

Enzymatic Digest of Gelatin, Enzymatic Digest of Casein, and Enzymatic Digest of Animal Tissue are the nitrogen and vitamin sources in MacConkey Agar. Lactose is the fermentable carbohydrate. During Lactose fermentation a local pH drop around the colony causes a color change in the pH indicator, Neutral Red, and bile precipitation. Bile Salts Mixture and Crystal Violet are the selective agents, inhibiting Gram-positive cocci and allowing Gram negative organisms to grow. Sodium Chloride maintains the osmotic environment. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Gelatin	17 g
Enzymatic Digest of Casein	1.5 g
Enzymatic Digest of Animal Tissue.....	1.5 g
Lactose.....	10 g
Bile Salts Mixture	1.5 g
Sodium Chloride.....	5 g
Neutral Red.....	0.03 g
Crystal Violet.....	0.001 g
Agar.....	13.5 g

C^{ph} Final pH: 7.1 ± 0.2 at 25

Precaution: i. for Laboratory Use.

ii. Irritant

Directions

1. Suspend 50 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121C for 15 minutes.

IV). Mueller Hinton Agar

Intended Use

Mueller Hinton Agar is used in antimicrobial susceptibility testing by the disk diffusion method. This formula conforms to Clinical and Laboratory Standard Institute (CLSI), formerly National Committee for Clinical Laboratory Standards (NCCLS).

Principles of the Procedure

Beef Extract and Acid Hydro lysate of Casein provide nitrogen, vitamins, carbon, and amino acids in Mueller Hinton Agar. Starch is added to absorb any toxic metabolites produced. Agar is the solidifying agent. A suitable medium is essential for testing the susceptibility of microorganisms to sulfonamides and trimethoprim. Antagonism to sulfonamide activity is demonstrated by para-amino benzoic acid (PABA) and its analogs. Reduced activity of trimethoprim, resulting in smaller growth inhibition zones and inner zonal growth, is demonstrated on medium possessing high levels of thymide. The PABA and thymine/thymidine content of Mueller Hinton Agar are reduced to a minimum, reducing the inactivation of sulfonamides and trimethoprim.

Formula / Liter

Beef Extract	2 g
Acid Hydro lysate of Casein.....	17.5 g
Starch	1.5 g
Agar	17 g

Final pH 7.3 ± 0.1 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution: For Laboratory Use.

Directions

1. Suspend 38 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.

4. OPTIONAL: Supplement as appropriate. Pour cooled Mueller Hinton Agar into sterile petri dishes on a level, horizontal surface to give uniform depth. Allow to cool to room temperature.
5. Check prepared Mueller Hinton Agar to ensure the final pH is 7.3 ± 0.1 at 25 °C.

5. How to inoculate culture media

Immediately before inoculating a culture medium check the medium for visual contamination or any change in its appearance which may indicate deterioration of the medium, e.g. darkening in color. When inoculating, or seeding, culture media an aseptic (sterile) technique must be used.

This will:

- prevent contamination of cultures and specimens,
- prevent infection of the laboratory worker and the environment.

Aseptic techniques

1. Flame sterilizes wire loops, straight wires, and metal forceps before and after use.
Whenever possible, use a Bunsen burner with a protective tube, e.g. *Bactiburner* to avoid particles being dispersed when flame sterilizing wire loops
2. Flame the necks of specimen bottles, culture bottles, and tubes after removing and before replacing caps, bungs, or plugs.
3. When inoculating, do not let the tops or caps of bottles and tubes touch an unsterile surface. This can be avoided by holding the top or cap in the hand. Always use racks to hold tubes and bottles containing specimens or culture media.
4. Make slide preparations from specimens after inoculating the culture media.
5. Decontaminate the work bench before starting the day's work and after finishing.
6. Use a safety cabinet when working with hazardous pathogens.
7. Wear protective clothing; wash the hands after handling infected material.

6. Antimicrobial susceptibility tests

Disc diffusion susceptibility tests

Disc diffusion techniques are used by most laboratories to test routinely for antimicrobial susceptibility. A disc of blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial, and this is placed on a plate of susceptibility testing agar uniformly inoculated with the test organism. The antimicrobial diffuses from the disc into the medium and the growth of the test organism is inhibited at a distance from the disc that is related (among other factors) to the susceptibility of the organism. Strains susceptible to the antimicrobial are inhibited at a distance from the disc whereas resistant strains have smaller

zones of inhibition or grow up to edge of the disc. For clinical and surveillance purposes and to promote reproducibility and comparability of results between laboratories, WHO recommends the (NCCLS) modified Kirby-Bauer disc diffusion technique.

Preparation of turbidity standard

1. Prepare a 1% v/v solution of sulphuric acid by adding 1 ml of concentrated sulphuric acid to 99 ml of water. Mix well.

Caution: Concentrated sulphuric acid is hygroscopic and highly corrosive, therefore do not mouth pipette, and never add the water to the acid.

2. Prepare a 1% w/v solution of barium chloride by dissolving 0.5 g of dehydrate barium chloride ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) in 50 ml of distilled water.

3. Add 0.6 ml of the barium chloride solution to 99.4 ml of the sulphuric acid solution, and mix.

4. Transfer a small volume of the turbid solution to a capped tube or screw-cap bottle of the same type as used for preparing the test and control inocula.

Procedure

1. Using a sterile loop, touch 3–5 well-isolated colonies of similar appearance to the test organism and emulsify in 3–4 ml of sterile physiological saline or nutrient broth.

2. In a good light match the turbidity of the suspension to the turbidity standard (mix the standard immediately before use). When comparing turbidities it is easier to view against a printed card or sheet of paper

3. Using a sterile swab inoculate a plate of Mueller Hinton agar. Remove excess fluid by pressing and rotating the swab against the side of the tube above the level of the suspension. Streak the swab evenly over the surface of the medium in three directions, rotating the plate approximately 60° to ensure even distribution.

4. With the Petri-dish lid in place, allow 3–5 minutes (no longer than 15 minutes) for the surface of the agar to dry.

5. Using sterile forceps, needle mounted in a holder, or a multidisc dispenser, place the appropriate antimicrobial discs, evenly distributed on the inoculated plate ensure the discs are correctly placed.

Note: The discs should be about 15 mm from the edge of the plate and no closer than about 25 mm from disc to disc. No more than 6 discs should be applied (90 mm dish). Each disc should be lightly pressed down to ensure its contact with the agar. It should not be moved once in place.

6. Within 30 minutes of applying the discs, invert the plate and incubate it aerobically at 35°C for 16–18 h (temperatures over 35°C invalidate results for oxacillin).

7. After overnight incubation, examine the control and test plates to ensure the growth is confluent or near confluent. By using a ruler on the underside of the plate measure the diameter of each zone of inhibition in mm. The endpoint of inhibition is where growth starts. The sensitivity and resistance is evaluated based on the WHO standard for each bacteria species [32].

7. Biochemical tests

1. Catalase test

This test is used to differentiate those bacteria that produce the enzyme catalase, such as staphylococci, from non-catalase producing bacteria such as streptococci.

Principle

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer. The culture should not be more than 24 hours old.

Procedure

1. Pour 2–3 ml of the hydrogen peroxide solution into a test tube.
2. Using a sterile wooden stick or a glass rod (not a nichrome wire loop), remove several colonies of the test organism and immerse in the hydrogen peroxide solution.

Important: Care must be taken when testing an organism cultured on a medium containing blood because catalase is present in red cells. If any of the blood agar is removed with the organism, a false positive reaction may occur.

3. Look for immediate bubbling as shown in Plat.

2. Coagulase tests

This test is used to identify *S. aureus* which produces the enzyme coagulase.

Principle

Coagulase causes plasma to clot by converting fibrinogen to fibrin. Two types of coagulase are produced by most strains of *S. aureus*: Free coagulase which converts fibrinogen to fibrin by activating a coagulase-reacting factor present in plasma. Free coagulase is detected by clotting in the tube test. Bound coagulase (clumping factor) which converts fibrinogen directly to fibrin without requiring a coagulase reacting factor. It can be detected by the clumping of bacterial cells in the rapid slide test.

Slide test method (detects bound coagulase)

- 1 Place a drop of distilled water on each end of a slide or on two separate slides.
- 2 Emulsify a colony of the test organism (previously checked by Gram staining) in each of the drops to make two thick suspensions.

Note: Colonies from a mannitol salt agar culture are not suitable for coagulase testing. The organism must first be cultured on nutrient agar or blood agar.

- 3 Add a loop full (not more) of plasma to one of the suspensions, and mix gently. Look for clumping of the organisms within 10 seconds. *No* plasma is added to the second suspension.

This is used to differentiate any granular appearance of the organism from true coagulase clumping.

Test tube method (detects free coagulase)

1. Take three small test tubes and label:

T _ Test organism (18–24 h broth culture)*

Pos _ Positive control (18–24 h *S. aureus* broth culture)*

Neg _ Negative control (sterile broth)*

*Nutrient broth is suitable. Do not use glucose broth.

2. Pipette 0.2 ml of plasma into each tube.
3. Add 0.8 ml of the test broth culture to tube T. Add 0.8 ml of the *S. aureus* culture to the tube labeled 'Pos'. Add 0.8 ml of sterile broth to the tube labeled 'Neg'.
4. After mixing gently, incubate the three tubes at 35–37°C. Examine for clotting after 1 hour. If no clotting has occurred, examine after 3 hours. If the test is still negative, leave the tube at room temperature overnight and examine again.

Note: When looking for clotting, tilt each tube gently.

Results

Clotting of tube contents *S. aureus*

No clotting or fibrin clot Negative test

Note: There should be no clotting in the negative control tube fibrin clot in tube

3. Indole

Testing for indole production is important in the identification of enterobacteria. Most strains of *E. coli*, *P. vulgaris*, *P. rettgeri*, *M. morgani*, and *Providencia* species break down the amino acid tryptophan with the release of indole.

Principle

The test organism is cultured in a medium which contains tryptophan. Indole production is detected by Kovac’s or Ehrlich’s reagent which contains 4 (p)-dimethylaminobenzaldehyde. This reacts with the indole to produce a red colored compound. Kovac’s reagent is recommended in preference to Ehrlich’s reagent for the detection of indole from enterobacteria.

Procedure

Detecting indole using tryptone water

1. Inoculate the test organism in a bijou bottle containing 3 ml of sterile tryptone water.
2. Incubate at 35–37°C for up to 48 h.
3. Test for indole by adding 0.5 ml of Kovac’s reagent. Shake gently. Examine for a red color in the surface layer within 10 minutes.

Results

Red surface layer Positive indole test

No red surface layer Negative indole test

4. Citrate utilization

This test is one of several techniques used occasionally to assist in the identification of enterobacteria. The test is based on the ability of an organism to use citrate as its only source of carbon.

Ways of performing a citrate test

- Using a Rosco citrate identification tablet. This is the most economical method when only a few tests are performed. The tablets have a long shelf-life and good stability in tropical climates.
- Using Simmon's citrate agar but the dehydrated medium is only available in 500 g pack size from manufacturers. After being opened the medium does not have good stability in tropical climates.

Citrate utilization using a Simmon's citrate agar

Principle and interpretation

The medium contains citrate, ammonium ions, and other inorganic ions needed for growth. It also contains Bromothymol blue, a pH indicator. Bromothymol blue turns blue at a pH of 7.6 or greater. When an organism catabolizes citrate, it produces alkaline waste products, causing the medium to turn blue. Furthermore, only an organism that can utilize citrate will produce visible growth on the citrate slant.

Result

Positive: Blue colored growth

Negative: No growth/ no color change

5. Oxidase test

The oxidase test is used to assist in the identification of *Pseudomonas*, *Neisseria*, *Vibrio*, *Brucella*, and *Pasteurella* species, all of which produce the enzyme cytochrome oxidase.

Principle

A piece of filter paper is soaked with a few drops of oxidase reagent. A colony of the test organism is then smeared on the filter paper. Alternatively an oxidase reagent strip can be used. When the organism is oxidase-producing, the phenylenediamine in the reagent will be oxidized to a deep purple colour. Occasionally the test is performed by flooding the culture plate with oxidase reagent but this technique is not recommended for routine use because the reagent rapidly kills bacteria. It can however be useful when attempting to isolate *N. gonorrhoeae* colonies from mixed cultures in the absence of a selective medium. The oxidase positive colonies *must* be removed and subcultured within 30 seconds of flooding the plate.

Procedure

- 1 Place a piece of filter paper in a clean petridish and add 2 or 3 drops of *freshly* prepared oxidase reagent.
- 2 Using a piece of stick or glass rod (not an oxidized wire loop), remove a colony of the test organism and smear it on the filter paper.
3. Look for the development of a blue-purple colour within a few seconds.

6. Urease test

Testing for urease enzyme activity is important in differentiating enterobacteria. *Proteus* strains are strong urease producers. *Y. enterocolitica* also shows urease activity (weakly at 35–37 °C). Salmonellae and shigellae do not produce urease.

Principle

The test organism is cultured in a medium which contains urea and the indicator phenol red. When the strain is Urease producing, the enzyme will break down the urea (by hydrolysis) to give ammonia and carbon dioxide. With the release of ammonia, the medium becomes alkaline as shown by a change in colour of the indicator to pink-red.

Urease test using Christensen's (modified) urea

- 1 Inoculate heavily the test organism in a sterile Christensen's modified urea
- 2 Incubate at 35–37 °C for 18 hrs
- 3 Look for a pink colour in the medium

Results

Pink colour Positive urease test

No pink colour Negative urease test

7. Motility test medium

This medium is used for checking the motility of organisms. Low agar concentration allows free movement of bacteria.

Principle and interpretation

Bacteria are motile by means of flagella. This test is done to determine whether an organism is motile or non-motile.

Procedure

The test isolate is inoculated by stabbing in the center of media in the tube with straight wire.

Result

- 1) Non motile-- growth restricted to stab line
- 2) Motile-- Diffused growth or swarm extends as a zone of turbidity from the stab line.

8. Triple sugar iron agar

Triple sugar iron agar is used for the differentiation of enteric pathogens by ability to determine carbohydrate fermentation and hydrogen sulphide production.

Results

Butt colour Slant colour Interpretation

Yellow Red Glucose only fermented

Yellow Yellow Glucose fermented, also lactose and/or sucrose

Red Red No action on glucose, lactose or sucrose

Bubbles or cracks present: gas production

Black precipitates present: hydrogen sulphide gas production

9. Lysine Iron agar

Principle

Lysine Iron agar is a solid medium useful in the identification of Family *Enterobacteriaceae*. Lysine Iron agar is used in the qualitative determination of lysine decarboxylation, lysine deamination, and hydrogen sulfide production. The medium is not as sensitive as other media in the determination of hydrogen sulfide production. The medium can only be used with organisms that are capable of glucose fermentation.

Lysine Iron agar contains dextrose as fermentable carbohydrate, lysine as an amino acid, bromocresol purple as a pH indicator, and ferric ammonium citrate and sodium thiosulfate as sulfur source and hydrogen sulfide indicator. Initially the organism ferments glucose, causing a production of acid and changing of the pH indicator in the butt to yellow.

If an organism produces decarboxylase enzymes, the organism will decarboxylate lysine to produce cadaverine, an alkaline product. The production of cadaverine will cause the pH indicator to change back to purple.

If the organism is able to deaminate lysine, the amine converts to alpha-ketocarboxylic acid and the slant turns red.

If the organism is not able to deaminate or decarboxylate the lysine the butt will remain yellow, and the slant will remain purple.

Procedure

1. Inoculate the medium using a single well-isolated colony from an 18-24 hour pure culture growing on solid medium.
2. Stab the butt of the agar twice, and streak back and forth on the slant.
3. Incubate tubes, with caps loosened in ambient air at 35-37°C for 18-24 hours.
4. Observe for lysine deamination or decarboxylation and hydrogen sulfide production.

Interpretation of Test

Uninoculated Lysine iron agar appears purple.

Lysine decarboxylation (detected in the butt)

Positive test – purple slant/purple butt (alkaline) K/K

Negative test – purple slant/yellow butt (acid) K/A (fermentation of glucose only)

Lysine deamination (detected in the slant)

Positive test – red slant

Negative slant – no color change (slant remains purple)

10. Bile solubility test

This helps to differentiate *S. pneumoniae*, which is soluble in bile and bile salts, from other *alpha* hemolytic *streptococci* (*viridans streptococci*) which are insoluble.

Principle

A heavy inoculum of the test organism is emulsified in physiological saline and the bile salt sodium deoxycholate is added. This dissolves *S. pneumoniae* as shown by a clearing of the turbidity within 10–15 minutes. *Viridans* and other *streptococci* are not dissolved and therefore there is no clearing of the turbidity.

Tube method

Although the bile solubility test can be performed by testing colonies directly on a culture plate or on a slide a tube technique is recommended because the results are easier to read.

1. Emulsify several colonies of the test organism in a tube containing 2 ml sterile physiological saline, to give a turbid suspension.
2. Add two drops of bile salt sodium deoxycholate
3. Look for clearing of turbidity

11. Satellitism test

Satellites test for *Haemophilus influenzae* identification

Staphylococcus aureus produce NAD as a metabolic byproduct when grow in a culture media containing blood. Therefore, *Haemophilus* species may grow on sheep blood agar very close to the colonies of *Staphylococcus aureus* (as it produces NAD-factor V); this phenomenon is known as satellite.

Why *Haemophilus* needs X and V Factor?

Haemophilus influenzae uses factor X to produce essential respiratory enzymes such as cytochrome, catalase and peroxidase. Factor V is used as an electron carrier in the organism's oxidation-reduction system.

Procedure of Satellitism test to identify *Haemophilus influenzae*

1. Mix a loopful of suspected colonies of *Haemophilus* colonies in about 2 ml of sterile physiological saline (or sterile peptone water).
2. Using a sterile swab, inoculate the organism suspension on:-
 - a. Plate of nutrient agar or tryptic soya agar
 - b. a plate of blood agar
3. Streak a pure culture of *S. aureus* across each of the inoculated plates
4. Incubate both plates in a carbon dioxide enriched atmosphere at 35 to 37°C for 18-24 hours.
5. Examine the culture plates for growth and satellite colonies

12. Identification of *Candida* species

Principle

CHROM agar *Candida* Medium is a selective and differential medium for the isolation of fungi. With the inclusion of chromogenic substrates in the medium, the colonies of *C. albicans*, *C. tropicalis* and *C. krusei* produce different colors, thus allowing the direct detection of these yeast species on the isolation plate. Colonies of *C. albicans* appear light to medium green, *C. tropicalis* colonies appear blue-greenish to metallic-blue, and *C. krusei* colonies appear light rose with a whitish border. Other yeast species may develop either their natural color (cream) or appear rose or light to dark mauve. An additional advantage of the medium is the easy detection of mixed yeast cultures due to their colony appearance in different colors. The proprietary chromogen mix consists of artificial substrates (chromogens), which release differently colored compounds upon degradation by specific enzymes. This permits the differentiation of certain species, or the detection of certain groups of organisms, with only a minimum of confirmatory tests. Chloramphenicol inhibits most bacterial contaminants.

Test Procedure

Streak the specimen for isolation onto the surface of the medium. If the specimen is cultured from a swab, roll the swab gently over a small area of the surface at the edge, then streak from this area with a loop. Incubate plates aerobically at 35 ± 2 °C for 20 to 48 hours in an inverted position. An incubation time of 42 hours is required for full color development of *Candida* colonies. Minimize exposure to light both before and during incubation. Occasional isolates, such as *Cryptococcus neoformans* and filamentous fungi, will require a longer incubation time and possibly a lower incubation temperature for optimal growth. Therefore, a plate with a second fungal medium, e.g. BD Sabouraud's Agar with Gentamycin and Chloramphenicol should be inoculated and incubated at 20 to 25° C if fungi other than *Candida* species are expected.

Results

After incubation, plates from specimens containing fungi will show growth. It is recommended to read the plates on a white background. If *Candida* species are present, colonies will appear light to medium green (*C. albicans*), light rose to pink with a whitish border (*C. krusei*), or blue greenish to metallic blue with or without violet halos (*C. tropicalis*). Other *Candida* species and

other yeasts appear light to dark mauve (rose to violet) or, if none of the chromogenic substrates is utilized, will assume their natural colony color (cream to white).

NOTE: - Data from various studies indicate that further identification tests are not necessary for *Candida albicans*, *C. tropicalis*, and *C. krusei*.

13. Quality control

- As quality control, sterility of sheep blood agar, MacConkey agar, mannitol salt agar and Mueller Hinton Agar will be checked by incubating overnight at 35-37 °C without specimen inoculation.
- The proficiency of catalase reagent (hydrogen peroxide) will be checked by known *S. aureus* (positive control) and *S. pyogenes* (negative control).
- For Gram staining reagents *S. aureus* (Gram positive) and *E. coli* (Gram negative) were used as quality control.
- For bile solubility test Positive Control: *S. pneumonia* negative Control: *S. mitis*.
- Before use of any reagents and culture media any physical change like cracks, excess moisture, color, hemolysis, dehydration, & contamination were assessed and expiration date was also checked. Temperature of incubator and refrigerator was monitored daily. *S. aureus* (ATCC 6538), *S. pneumonia* (NCTC 12977), *S. mitis* (NCTC 10712), *E. coli* (ATCC 8739), *S. aureus* (ATCC 25923) and *S. pyogenes* (ATCC 19615) was used as a quality control throughout the study for culture and antimicrobial susceptibility testing.

Important: suspensions of suspected *N. meningitides* must be prepared in the BSLII safety cabinet

11.8. Annex VII: Assurance of Principal Investigator

I, the undersigned, declare that this is my original work and has not been presented in this and any other University and all sources of materials used for this thesis have been duly acknowledged.

Name: -----

Signature: _____

Date: _____

This thesis has been submitted for examination with my approval as University Advisor.

Name: - Dr. Adane Bitew, Associate Professor

Signature: _____

Date: _____

This thesis has been submitted for examination with my approval as External Advisor.

Name: - Mr. Nega Asaminew

Signature: _____

Date: _____

11.9. Annex VIII: Curriculum vitae of External advisors

1. Nega Asaminew

PERSONAL INFORMATION

Name : Negga Asamene Abera (BSc, MSc, MPH)

Profession: Medical laboratory Technologist and Field Epidemiologist

Sex: Male

Place of Birth: North Wollo

Date of Birth: 23/09/1978G.C

Nationality: Ethiopian

Marital Status: Married

Address: Gulele sub city kebele 10/11

P.O.BOX: 1242, Addis Ababa -Ethiopia

Mobile : 0911- 70 44 59

negaasamene@gmail.com

EDUCATIONAL BACKGROUND

Period	Institute	Qualification
2011-2012	Addis Ababa University	MSC

2012-2013	Addis Ababa University	MPH in field Epidemiology
2005-2007	<i>Addis Ababa University</i>	<i>B.Sc.</i>
2002-2004	<i>Addis Ababa University</i>	<i>Diploma</i>
1989-2001	Tadagiwa Ethiopia. Senior secondary school	Certificate

OTHER TRAINING

1. On quality assurance and SOP Development in lab-----Certificate
2. External quality assessment, -----Certificate
3. Lab. procedure & development & control of Document -----Certificate
4. Laboratory management system-----Certificate
5. On diagnosis & surveillance epidemic prone diseases----- Certificate
6. On assessment of drinking water quality----- Certificate
7. On antimicrobial susceptibilities-----Certificate
8. On Humastar 80 Chemistry analyzer-----Certificate
9. On advance Vitek2 compact machine -----Certificate
10. On School- based surveys-----Certificate
- 11 Polytech laboratory information systems -----Certificate
12. Mycology advance training on Identification of molds and yeasts-----Certificate
13. HIV basic course -----Certificate
14. Diarrhea disease course -----Certificate

15. Logistics for health Commodities ----- Certificate
16. Health research methods and Ethics----- Certificate
17. Trans- Isolate media preparation----- Certificate
18. National EPI coverage survey coordination----- Certificate
19. ArcGIS and SPSS training-----certificate

WORK EXPERIENCE

- From October 1996 – 1999 as Research technician at Ethiopian Health and Nutrition Research Institute (EPHI) Department of Infectious and Other Diseases Research (DIO DR) Clinical Bacteriology and mycology Unit
- From 2005 up till now as researcher and Medical Laboratory Technologist at EPHI
Major duties and Responsibilities
 - Surviving as coordinator in National EPI surveys
 - Involving in research projects supported by CDC-Atlanta and NIPH ,FMOH and other partners
 - Outbreak investigation both Epidemiologically and Laboratory level
 - Surveillance system evaluation
 - Surveillance data analysis and presenting for decision making
 - Early warning and response for public health important diseases
 - Planning and writing specification of different laboratory reagents and equipment for annual consumption and procurement respectively.
 - Distributing proficiency test (EQA) of HIV, TB and Malaria, samples to regional and Hospital laboratories of the country.
 - Performing of EQAsamples coming from south Africa and UK NEQAS
 - Diagnoses of clinical specimen from referred the patients. Recoding and reporting patient results.
 - Working in different department of the laboratory using manual and automation methods especially in Microbiology, HIV/TB, Hematology, and Clinical chemistry laboratory.

- Working in different Private laboratory using manual and automation methods especially Parasitology, Hematology, serology and urinalysis during week ends and night.
- Writing proposals on different diseases prioritized by MOH
- Diagnosis of Unknown etiology outbreaks using Molecular techniques
-

Key Skills

- Language skill very good in both spoken and written Amharic, Tigrigna and English
- Good on listing and spoken of Oromifa and Geez

Computer skills Efficiently utilize Ms-Word, Excel & SPSS, EPI-info, and Arc-GIS power point

- Research: I have a good Knowledge of conducting research both in group and individually
- Negotiation: I like to discuss and resolve problematic issues and I can resist any problematic situations during traveling of field since I grow up by walking on foot in rural part of the country.
- I like to participate in different social development activities. And I very enthusiastic person who like to work with any one without any problem.

Research papers

1. Prevalence and Drug Susceptibility Pattern of Extended-Spectrum Beta-Lactamase Producing Bacteria, Ethiopia, 2011-2012
2. Otitis Meda In Ethiopia Bacterial Profile and Antimicrobial susceptibility Pattern
3. Prevalence of Antibiotic resistant salmonella Species and selected Internal protozoa parasites in Harar Hiwot Fana Hospital ,Ethiopia
4. Prevalence and anti biotic susceptibility Pattern of common uropathogens isolated from pregnant women attending antenatal care clinics of St.paul hospital

5. An outbreak of acute febrile illness caused by Sand fly Fever Sicilian Virus in the Afar region of Ethiopia, 2011
6. Streptococcus Pyogen under five children 2009
7. Ciprofloxacin resistant of Neisseria gonorrhoea isolates obtained from genital samples at the Ethiopian Public Health Institute within the period of 2007 and 2014

Future Plan

Work on Antimicrobial Resistance with Global Health security agenda

Social Activities

Serving as Young Ambassador of ASM to Ethiopia since 2013 till now

And work on organizing of workshops on Biosafety, Biosecurity and due use of research in different Universities of Ethiopia during these three years over 150 professionals of Laboratory and University Instructors were attend this work shop

and in ASM young Ambassadors net work on Young Leaders circle which serve as a voice of young/early career scientists in the world.

Current Running Projects

1. **Enhanced sentinel site bacterial Meningitis surveillance with CDC and Norwegian Institute of Public Health (NIPH)**
2. **Sexually transmitted infections in selected Health Facility of Ethiopia with CDC**
3. **Pediatric Bacterial Meningitis surveillance Net work with WHO**
4. **Epidemiology and Antimicrobial resistance of common Bacterial Pathogens in Ethiopia in Global Health Security agenda (GSHA)**

4. References

Dr. Lucy Boulanger (Ethiopian field epidemiology training program resident Advisor)

Base at CDC Ethiopi boulangerl@et.cdc.gov

Dr Zegeye Hailemariam (Ethiopian field epidemiology training program resident Advisor based at Ethiopian public Health Institute

Dr. Adamu Adisie Addis Ababa University EFETP academic Advisor

zegeyehailemariam@yahoo.com Tel 251- 911665648

Dr. Alemayehu Worku Addis Ababa University School of Public Heal

2. Dr. Elias Hailu

CURRICULUM VITAE:

Personal information

Name Dr. Elias Hailu Gebreab, MD

Sex male

Age 49

Marital status married

Contact address: eliashailu2012@gmail.com

Mobile +251910989686

Academic title: Assistant professor, department of Ophthalmology, St. Paul Hospital Millennium Medical College

Educational background:

1986-1993 Addis Ababa University, Medical Faculty (MD)

1998-2002 Addis Ababa University, Medical Faculty (Residency in Ophthalmology Certificate in ophthalmology)

2007 Storm eye institute, University of South Carolina, sub specialty in anterior segment

2008 Aravind Eye care system, India: Management priorities in Eye care Delivery

2013 Tilganga Institute of Ophthalmology, Nepal: phacoemulsification training

License, Certification:

Expert Medical Specialist (Ophthalmology), Cornea & external eye diseases

Registration number EMSOCED=176/2007 by FMHACA

Short course attended

- DSAEK [Descemets stripping and automated corneal transplant hands on training April 10-13/2016
- COECSA Leadership & mentoring program 17-20 february 2014 Dar Es Salaam/ Tanzania
- Attended Fifth annual Toronto Cataract course @ University of Toronto 2007
- Fundamentals of assessment course organized by SPHMMC & Open University 22March-23 March/2011
- Attended 103 DOG Congress/ German ophthalmological society 25-29 September 2005
- Effective Teaching Skills Training certificate organized by jhpiego, CDC, USAID & FMOH August 3-7/2009
- Participate in the 8th General Assembly of IAPB Buenos Aires, Argentina, August 25-28/2008
- Participate in ASCARS annual conference in 2014 in Boston, USA
- Short course on refractive surgeries, LASIK in Moran institute at the university of UTAH/ USA

Position held:

2008-Present Assistant professor of Ophthalmology, SPHMMC

2008-Present Medical staff WGGGA Vision Privet Company

2002-2007 Assistant professor of Ophthalmology, Jimma University

Professional Activity:

2012- 2015, President, Ophthalmological Society of Ethiopia

2002-2007 Head department of Ophthalmology at Jimma University

2011-2013 Head department of Ophthalmology at SPHMMC

2013-2014 Medical Director of WGGGA Vision privet clinic

2002-2007 teach under graduate & cataract surgeon & postgraduate residents at Jimma University

2008-present Teach undergraduate medical students

2002-present perform over 30,000 cataract surgeries all over Ethiopia in various eye camps

Outreach & Campaigns

Conducted under Jimma University in south west Ethiopia in the following Hospitals & Health centers

Limmu Genet Hospital

Agaro Hospital

Gera Health center

Gathira Health center

Aman Hospital

Bedele Hospital

Metu Karl Hospital

Conducted along IRC

Sherkole Sudanese refugee camps

Yarenja Sudanese refugee camps

Shimelba Ertreanrefugeecamps

Kebribeya Somali refugee camps

Conducted along RaDO

Dolo Ado Somali refugee camp

Kebribeya, & other two Somali refugee camps

Gambella Jewi, Kule & Terkedi refugee camps

Research & publications:

1. Prevalence study of pseudoexfoliation syndrome among south west Ethiopians
2. Prevalence study of diabetic retinopathies among ophthalmic patients at Menelik II Hospital

3. A case report on Brain stem anesthesia following retro bulbar block for cataract surgery.