ASSESSMENT OF OCULAR BACTERIAL FLORA AMONG UNIVERSITY OF BENIN UNDERGRADUATES

BY

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DEPARTMENT OF OPTOMETRY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

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CERTIFICATION

This is to certify that the project work **ASSESSMENT OF OCULAR BACTERIAL FLORA AMONG UNIVVERSITY OF BENIN UNDERGRADUATE** was carried out by **IMASUA BIBIAN ERUEMULOR** in the Department of Optometry, Faculty of life sciences, University of Benin, Benin City.

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DEDICATION

This project is dedicated to the All-Wise God for his direction and wisdom all through the course of the work. I also dedicate this work to my dad, Late Imasua Stephen and mum, Mrs. Imasua Abigail, my beloved siblings and friends for their efforts in my life.

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ABSTRACT

External eye infections occur when harmful microorganisms invade the eyes thereby inflicting harm. Infection in the eyes occurs in three main parts: eyelid, cornea and conjunctiva. The study was carried out to assess bacterial associated with ocular infections. A questionnaire was used to obtain participants demographics and basic general information related to the work. Thereafter samples from 50 healthy students in university of Benin was obtained from the lower cul-de-sac by using a sterile swap stick. Within 4-6 hours of collection, the samples were transported under aseptic condition to the laboratory for analysis. On MacConkey agar, nutrient agar, and mannitol salt agar, the samples were inoculated. The microbes isolated were determined using typical microbial and biological procedures. Antimicrobial test was carried out to ascertain the sensitivity or resistant status of each isolate. The commonest flora isolated was *Escherichia Coli* in 96% of participants. The total mean CFU for males and females was 3.145 and 2.660 respectively ($p=0.519$). There was a significant relationship between history of contact lens use with prevalence of *Escherichia Coli (p*=0.009), also a significant relationship between history of contact lens use with percentage of occurrence of *Pseudomonas aeuriginosa* ($p=<0.001$). There was also a relationship between history of visit to clinic and percentage occurrence of *Pseudomonas aeuriginosa* ($p=0.010$) and history of visit to clinic with percentage occurrence of *Escherichia Coli* (0.023) and also a significant relationship was seen between the listed names of the eyedrop with percentage of occurrence of *Staphylococcus aureus* ($p=0.054$). This study showed that lifestyles factors such as contact lens use and eye drop use can affect the normal flora of a person.

KEYWORDS: *Escherichia Coli, Pseudomonas aeuriginosa, Staphylococcus aureus,* Normal flora, eye infections.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND INFORMATION

1.1.1 THE EYE

The human eyes are specialised sense organs that can receive visual images and transmit them to the brain. The human eye is a round spherical in shape. Light passes via translucent front and stimulates receptor cells on the retina (rods for black-and-white vision in dim light and cones for colour vision), which in turn sends signals to the brain via the optic nerve (Perkins and Davson, 2023). It has epithelia, connective tissue, smooth muscle, vascular, and neuronal tissue, the eye is a complex sensory organ made up of a variety of tissue types. While some eye structures must be highly transparent to allow light to pass through unhindered, other tissues are distinguished by their thick pigmentation, which serves to absorb light and therefore regulate the flow of light through the ocular structure. The eye which is the main visual organ has photoreceptors which enables it to perceive things. It concentrates light from an object onto the retina, the area of the eye that is sensitive to light. Changes in the retina's sensitive neurons cause nerve action potentials, which are then transmitted to the brain by the optic nerve. The orbit, which houses the eye and defends it from mechanical harm. The orbit is a bony protective socket where the eyes are located. Six extraocular muscle move the eye up, down, and side to side as well as rotate it to keep it in place (Boyd and Turbert, 2023). The cornea which is the eyeball's front surface must stay wet at all times. The eyelids help with this by regularly spreading the lacrimal apparatus and other gland secretions over the surface during active hours and covering the eyes

during sleeping to keep it moisture and avoid evaporation. The lids also provide the additional purpose of protecting the eyes from foreign body destruction by activating the blink reflex.

The ciliary body, which produces aqueous humour, is part of the anterior segment of the eye's circulation. The retinal circulation is similar to the brain circulation but lacks autonomic innervation. The choroidal vasculature has fenestrated capillaries and the highest density of autonomic innervations known in the body and the optic nerve head (ONH) is part of the fourth segment (Flammer *et al*., 2013).

1.1.2 OCULAR OF MICROBIOTA

Microbiota also known as ocular bacterial flora refers to the population of microbes that live in a healthy normal individual's eye. It is used to describe the countless bacteria that live permanently in the eye, including the conjunctiva and eyelid margins. Ocular bacteria flora has two opposing role in ocular health. They are the defense organisms for the prevention against microorganisms and they can be the source of ocular infections if compromised. The organisms present in the conjunctiva and eyelids that make up the ocular bacterial flora aren't pathogenic. The eyes are sterile at birth, but later on different microorganisms cause infections in them. The majority of the ocular flora's organisms are gram-positive. *Staphylococcus epidermidis, diphtheroids,* and *Staphylococcus aureus* are all present in the normal flora of the human ocular surface. *Coagulation-negative Staphylococci* which are found in 20–80% of conjunctival swabs and 30–100% of lid swabs, are the most abundant ocular surface bacteria (Jing *et al*., 2020). Ocular surface flora's composition can change according to a number of causes such as: Patients in the intensive care unit (ICU) often have impaired ocular defense mechanisms as a result of metabolic disturbances, mechanical ventilation, sedation, paralysis, and reduced level of

consciousness. These factors include dry eye, immunosuppressive medication, age, medical conditions like diabetes mellitus (Ramani *et al*., 2021).

1.1.3 DEFENCE OF THE EYE

The defence of the eye is complex, the complex system employs a number of defence mechanisms to shield the eye from environmental dangers, diseases, and foreign objects. The following are some of the main eye defence mechanisms:

a) Tear Film: The tear film defends the eye's surface and helps maintain the stability of the ocular environment. It has proteins with antibacterial characteristics and enzymes that help prevent infections. Tears also flush out waste and foreign objects, lowering the risk of infection.
b) Eyelids and Eyelashes: The eyelids and eyelashes function as a physical barrier to keep foreign objects from getting close to the eye therefore initiating a fast blink.
c) Conjunctiva and Cornea: The mucous membrane known as the conjunctiva lines the inside of the eyelids and the front of the eye. It has immune cells that are able to fight infections. Since there are no blood vessels in the cornea the clear there is less chance of an infection spreading through the blood.
d) The blink reflex is a defence system that prompts the eyelids to quickly close in the presence of potential dangers. This spreads tears to keep the ocular surface wet and discourages foreign things from getting inside the eye.
e) Immune cell: White blood cells and other immune cells are found in the eye and can fight off infections. The conjunctiva and other eye tissues contains these cells.

f) Corneal reflex: When the cornea is touched, the corneal reflex a protective action to close the eye from any harm. The surface of the eye is protected by this reflex.

g) Blood-Aqueous Barrier and Blood-Retinal Barrier: The blood-aqueous and blood-retinal barriers control the flow of substance across the bloodstream and the inside of the eye, preventing the introduction of germs and toxins that can be harmful.

h) Inflammatory response: The eye can mount an inflammatory response to fight against a threat in the event of an infection or injury. But excessive inflammation can be harmful, therefore the immune system of the eye must maintain a balance.

Numerous bacteria constantly thrive on the ocular surface. To keep the cornea transparent to preserve vision, the outer ocular system's capacity to identify infections as foreign and destroy them is essential. In order preserve the outer eye, a variety of mechanical, anatomical, and immunological defence mechanisms have developed (Akpek and Gottsch, 2003).

1.1.3 MECHANISM OF MICROBIOTA

In order to keep the ocular surface's equilibrium and prevent the colonisation of pathogenic species, the ocular microbiota plays a crucial function. The "tear film" that spreads throughout the ocular surface by the blink reflex is known as "tear film," and it is crucial because it shields the eye from external objects that are infected with microorganisms. The ocular surface mucosa is directly exposed to the environment, it serves as a defense against potentially pathogenic microorganisms (Aragon *et al.,* 2021). Tears has variety of antimicrobial substances, including lysozyme, lactoferrin, and defensins, which aid in preventing the colonisation of tissues by infections. Mucins also make the clearance of microorganisms from the surface of the eye easier. Reflex is crucial because it shields the eye from external objects that are contaminated with bacteria by distributing tears as a film called "tear film" over the ocular surface. In truth, tears

include a variety of antimicrobial substances, including lactoferrin, defensins, and lysozyme, which aid in avoiding bacterial colonization (Petrillo *et al.,* 2020). Mucins also make it easier to remove microorganisms from the surface of the eye. For resources and space on the ocular surface, beneficial microbes in the ocular microbiota may compete with pathogens that could be harmful. Beneficial bacteria can stop the colonisation of infectious microbes and lower the risk of illnesses by filling these niches.

Bacteria found in the ocular surface

Haemophilus influenzae, Corynebacterium, pseudomonas aeruginosa, staphylococcus epidermis, and *staphylococcus aureus* are all component of the normal human eye surface flora. Several factors which can affect the composition of ocular flora are medical illness such as diabetes, immunosuppressive medications, and dry eye. These healthy flora maintain connections with other microorganisms, and they carry out an immune-modulating role (Gome *et al*., 2020). It is crucial to remember that the presence of germs on the ocular surface does not always indicate an infection or sickness. The ocular surface has natural defense mechanisms such as tears and blinking which helps prevent bacterial growth and maintain balance of microorganisms (Wang *et al.*, 2022).

Bacteria causing eye infections

Due to their virulence and low host resistances, pathogenic microorganisms can cause eye infections in specific conditions like poor living conditions, poor personal cleanliness, low socioeconomic status, low immunity status, and others (Alshamahi *et al.,* 2020). The most common bacteria linked to eye infections include *staphylococcus, streptococcus pneumoniae, bacillus, pseudomonas aeruginosa, enterobacteriaceae, neisseria gonorrhoea, morradella spp.,*

and Haemophilus influenza (Ayehubizu *et al*., 2021). Bacterial infections are the common cause of ocular surface diseases and this occur when microbes overwhelm the host or when the eye has been compromised giving way for organisms to enter and cause harm (Ramesh *et al.,* 2010).

1.1.5 ABNORMALITY OF MICROBIOTA

The eye is a complicated organ that is responsible for seeing. Ocular infections is any disease of the eye that is caused by harmful microorganisms as a result of direct exposure to diseased people, environment or contaminated object can result in the spread of infection or disease. A study done by Zhang *et al*., 2021 demonstrated that the ocular surface microbial composition was more intricate and thorough in the patients with diabetic mellitus suffering from dry eye disease. External ocular infections are mainly caused by virulence of the pathogen, poor living conditions, weakened immune systems, poor personal hygiene, trauma, surgery, persistent nasolacrimal duct obstruction, and systemic disorders. When this microorganisms enter the eyes it result in visual disruption. Redness and pain are the symptoms of infection affecting the cornea and conjunctiva. Eye infections are a common presenting problem in primary care today. Patients often seek care service when they experience itching, redness, discharge, pain and problem with vision which may often present as blurry vision. According to Chin *et al* (2019) “Eye infections are a frequent cause of presentations to clinic and can vary in severity from self-resolving to sight-endangering”. Although most eye infections that present to be self-limiting, so it can resolve on its own without specific treatment. Some may cause corneal scarring or permanent blindness as a result of the complications that occured during its course of resolving. Ocular infection is a public health problem because they can cause a lot of motility if treated wrongly and this could result in vision loss. Due to the potential risk to vision, it is considered a public health burden. Ayehubizu *el al*., (2021) contributed that ocular infections can affect the structures of the eye,

causing blindness and vision loss if left untreated. Ocular infections may be endogenic or exogenic. The endogenic occurs when the blood stream is infected so the infection passes through the blood as a means to reach other site. While exogenic occurs from contamination from the environment which could be direct or indirect means. The loss or impairment of visual function brought on by an eye infection can cause severe disability (Ubani, 2009).

Bacteria

Bacteria are described as the smallest, most independently reproducing, unicellular, and free-living organisms. Bacteria are prokaryotes it is the smallest, simplest, oldest cell on earth, and the size is 1-5μm (Stewart, 2012). It has a genetic material in the form of DNA which is located in the cytoplasm. The cell wall gives them shape, structure and protection. These bacteria must be grown in the lab in order to fully comprehend their physiology and the functions they carry out in the ecosystem, host health, and the creation of natural products. On Earth, bacteria inhabit a wide ecological niches. They play a vital part in organic degradation of matters, and symbiotic relationship with other species. Bacteria are found in different environments such as water, soil, and the human body where they can form complex microbial communities. They also play an important role in microbial biology on surface fluid and interactions between surfaces, which are two characteristics that are pervasive and vital in the natural environment. Chemical signalling molecules serve as the words used by bacteria for interacting with one another. They specifically release these molecules, which are known as autoinducers, detect them, and react to them (Schauder and Bassier, 2001). According to their basic morphologies, bacteria are divided into five groups: cocci, spiral-shaped spirilla, rod-shaped bacilli, comma-shaped vibrios, and spirochaetes. They can be grouped in varieties of ways like: chains, cluster and pairs which makes the identification easier.

Etiology external ocular infections and pathophysiology

Infections of the eyes are mainly caused by bacteria worldwide. Contact lenses, trauma, surgery, age, dry eye condition, chronic nasolacrimal duct obstruction, and prior ocular infections are just a few of the several factors that might cause an infection to be mono- or poly-microbial. Some bacterial produce harmful substances that can damage and cause inflammation. For example toxin produced by *staphylococcus aureus* can cause tissue destruction and cell death. 285 million people around the world are estimated by the World Health Organization to be impaired (Ayehuibizu *et al* 2021). Numerous eye conditions including conjunctivitis, keratitis, endophthalmitis, blepharitis, as well as symptoms of orbital cellulitis and dacryocystitis, are often caused by bacteria. When bacteria, fungi, or viruses infect an eye surface area or surrounding tissue, an infection can result. It is important to encourage eye cleanliness and wellness at all times. Contact with infected eyelid margins, infected fingers, and surrounding skin spreads the disease most especially from the nasopharynx via the nasolacrimal duct also can occur from contaminated eye drops, or infected contact lenses, and less frequently from the genital through the bloodstream (Mohammed *et al*., 2020).

Risk factors

I. Age
II. Poor hygiene
III. Contact lenses
IV. Race

Diagnosis

I. Patient history

II. Slit lamp examination

III. Laboratory test

Treatment

The main treatment is determining the pathogen causing the ocular infection and using the appropriate eye drop also prophylactic treatment can be applied too.

Ocular surface diseases

Beginning in 2008, the Human Microbiome Project (HMP) was the first to illustrate the microbiota (microorganisms) from 5 different bodily regions, including the gastrointestinal system, skin, nasal passages, oral cavity and urogenital tract. The second objective was to evaluate how these microbes impacted on human health and cause disease (Zilliox *et al.,* 2020). The conjunctiva, which serves as a protection to the white part of the eye and the cornea, which covers the colourful iris and dark pupil, make up the eye's surface layers. In order to protect and lubricate the eye, ocular surface epithelial cells produce and secrete mucins, which together form a hydrophilic barrier this helps safeguard the eye from damage. High molecular weight, extensively glycosylated membrane-associated mucins (MAMs), such as MUC1, MUC4, and MUC16 make up the glycocalyx which is the barrier that surrounds the body. Through interactions with galectin-3, these mucins extend into the tear film from the corneal and conjunctiva anterior surfaces and stop the effect of the pathogens (Ablamowiaz *et al.,* 2016).

The surface layers of the eyes are damaged by ocular surface diseases. Ocular surface diseases come in many forms. Although neurotrophic keratitis, blepharitis, Meibomian gland dysfunction and ocular rosacea are all included with dry eye been the most prevalent. Ocular surface disease disrupt the defense mechanism of the eye. It is crucial to remember that the disruption often occur by underlying issues or outside influences rather than being natural function of the ocular surface. It is important to know the influence of microbiome has on the eye surface will help to modulate this microbiome and reduce pathogenesis (Paiva *et al., 2022).* Although several symptoms of ocular surface diseases (OSDs) including dry eyes and other forms of OSD has its own unique symptoms that it presents with.

Symptoms of dry eyes may include:

I. Watery eyes
II. Stinging or burning feeling in your eyes
III. Eye redness
IV. Blurred vision
V. Stringy mucus in or around your eyes
VI. Difficulty driving
VII. Difficulty wearing contact lenses (Vallie, 2022).

Risk factors

I. Dry eye
II. Blepharitis
III. Female sex
IV. Older age

Diagnosis

Using a slit lamp ocular surface illness can be identified and its effects quantified. Damage to the cornea can be found using fluorescein dye when applied to the bulbar conjunctiva and viewed under a blue cobalt filer. On slit-lamp examination, the following dry eye symptoms appear: superficial corneal erosions, insufficient tear lake volume, early tear film break-up time, conjunctival hyperemia, dissimilar conjunctival surface, and meibomian gland dysfunction (Zeev *et al*., 2022).

Management

I. Eliminate exacerbating factor
II. Support ocular lubricant
III. Consider therapeutic contact lenses
IV. Hypo osmolar drugs
V. Silicon hydrogel lens (Buckley, 2018).

1.1.6 FACTORS THAT CAN AFFECT MICROBIOTA

Individual differences exist in the exact makeup of the ocular flora, which may also be affected by things like:

1 Age: The ocular bacterial flora can vary with age. Infants and young children have different ocular microbiome compared to adults. The ocular microbiome gradually evolves and stabilizes over time, and older individuals may have a different bacteria, leading to an imbalance in the ocular microbiome. During childhood, the microbiota tends to grow and change. The composition of the microbiota can be influenced by dietary changes, interactions with other people, and exposure to many external factors. As one age, diet, lifestyle

influences the microbiota. Microbiota of aged people show reduced diversity due to the presence of bacteria. Age-related health problems like inflammation, metabolic alterations, and gastrointestinal diseases may be worsened by these changes.

2 Environmental factor: External environment plays an important role in shaping the ocular bacterial flora. Exposure to different environment such as urban or rural setting. Can introduce different types of bacterial into the eyes. Other factors include climate, humidity, and pollution can introduce bacterial into the eye and alter the natural balance of microbiome.

3 Antibiotics use: Topical antibiotics usage can impact the ocular bacterial flora. Application of topical antibiotics can eliminate both pathological and beneficial bacteria leading to an imbalance. Antibiotics have the potential to disrupt the microbiota's in the eye affecting the balance and allowing bacteria to gain entry and cause infection.

4 Personal hygiene: Individual practice hygiene can affect the ocular bacterial flora. Constant hand eye contact, not washing hand, and improper handling of contact lens can introduce bacteria to the eye. Poor hygiene can cause micro-organisms to grow faster in the eye. Maintaining a good cleansing habit is essential because excess soap can interfere with the microbiome of the eye.

5 Immune system: This plays a vital role in ocular bacterial flora. A proper immune system helps to maintain the balance of microbiome and prevent over growth of pathogenic bacterial. A disorder can impact the composition and diversity of ocular bacteria.

6 Ocular surface disease: Certain ocular surface diseases like dry eye, blepharitis can alter ocular bacterial flora.

7 Contact lens wearers: It has been found that contact lens user have a different ocular microbiome compared to non-contact lens users. Contact lens use alter the ocular

environment, and promote bacterial adhesion to the lens surface. Thereby increasing the risk of bacterial infections.

8 Food: Diet significantly influences the types of bacteria that exist in the colon. Each individual's microbiome is distinct due to these characteristics. Particularly, a high-fiber diet has an impact on the variety and quantity of microbiota in the intestines. Dietary fibre can only be digested and degraded by enzymes found in the colon's microbiota.

Age, gender, personal habits, contact lens use, disease states, antibiotic use, and infections, among other factors, can all have an impact on the ocular surface microbiota. The knowledge of what influences the intraocular microbiome is still in its infancy. It is logical to assume that since the intraocular region is a bit separated from the outside world, the intraocular microbiota is more likely to be connected to host variables (Jing *et al.,* 2020).

1.1.7 CONJUNCTIVA

The eye is protected from damage by a slender, transparent membrane known as the conjunctiva. It gives protection to the sclera, the white part of the eye, and the lower and upper eyelid. It has three parts which are:

I. Palpebral conjunctiva: This is the part that lines the eyelids.
II. Bulbar conjunctiva: It serves as a lining to the eyeball and protects the white part of the eye.
III. Conjunctiva fornix: It is the portion that connects the palpebral and bulbar conjunctiva together.

The long ciliary nerve which supplies the cornea also supplies the conjunctiva's circumcorneal zone. The rest part of the conjunctiva is supplied by the lacrimal, infratrochlear, supratrochlear, supraorbital, and frontal nerves (Khurana, 2007).

The conjunctiva contributes to the tear film and shields the eye from infection and other harmful objects. It protects the immune system and stops microbes from getting inside the eye. It has several lymphatic vessels that is highly vascularized. The epithelial layer of the conjunctiva is made up of stratified squamous and stratified columnar epithelium, it is non-keratinized with scattered goblet cells. It provide immunological barriers which protect the ocular surface of the eye (Diebold and Garcia-Posada, 2021). The conjunctiva epithelium ranges in thickness from three to five cell layers. The epithelium's cuboidal basal cells grow more flattened as they go nearer to the surface.

Structure of the conjunctiva

The conjunctiva has three layers namely:

1. Epithelium
2. Adenoid layer
3. Fibrous layer

1. **Epithelium:** Conjunctiva's layer of epithelial cells differs from location to region and in its various portions as follows:
    a) Squamous epithelium of the marginal conjunctiva is composed of five layers.
    b) Tarsal conjunctiva: The two-layered epithelium of the tarsal conjunctiva includes a superficial layer of cylindrical cells.
    c) Deep layer of flat cells.

d) Fornix and bulbar conjunctiva: It have 3-layered epithelium: a deep layer of cuboidal cells, middle layer of polyhedral cells and a superficial layer of cylindrical cells.

e) Limbal conjunctiva is made up of five to six stratified squamous epithelium.

2. **Adenoid layer:** It is also known as the lymphoid layer and is made up of small connective tissue reticulum in which lymphocytes are embedded. The fornices are where this stratum is most developed.
3. **Fibrous layer:** Collagenous and elastic fibers are woven together to form a mesh. Except for the area of the tarsal conjunctiva where it is quite thin, it is thicker than the adenoid layer. Conjunctiva nerves and vessels are seen in this layer where the bulbar conjunctiva is located. It melds with the underlying Tenon's capsule (Khurana, 2007).

1.1.8 CONJUNCTIVA MICROBIOTA IN HEALTHY EYES

The eye is continually in contact with germs while a person is awake. Many of the microorganisms discovered in the ocular microbiota, such as *streptococci* and *coryneforms*, are known to live on the skin. However, not all skin bacteria are discovered in the eye, indicating that only a subset of skin microbes are chosen for and present within the conjunctiva microbiome. The microbiota that reside on the conjunctiva is made up of the following: *Hemophilus*, *Diptheroids*, like Gram-negative *bacilli*, Non-hemolytic *streptococci, Neisseria, Staphylococci.* Tears contain beta-lysine, lysozymes, and lactoferrin, immunoglobulin G (IgG), and bacteria are destroyed by the synergistic activities of these components. Humans blink about 12 times per minute, which protects the eye from microbial contamination by providing a "tear film" over the surface of the eye. Patients with Bell's palsy, for instance, who are unable to blink and remove bacteria from the cornea frequently develop bacterial conjunctivitis, which is indicative of tears' antibacterial qualities. Conjunctiva's cultivable microbiota was examined shortly after birth and

it was found to contain the same bacteria as the cervix that is predominantly *viridans streptococci, Gardnerella sp., Haemophilus vaginalis, Micrococcus sp, S. epidermidis, Bacillus sp., diphtheroids, Bacteriodes sp., Propionibacterium acnes, Peptococcus sp., Peptostreptococcus sp., Lactobacillus sp., Escherichia coli, S. aureus, Candida sp and Bifidobacteria sp*., (Willcox, 2013). Commensal bacteria are important to humans for a variety of physiological, developmental processes, nutritional, and defense. Despite the fact that the tear film is an essential physical barrier separating the eye from its surroundings, it is important to remember that commensal bacteria on the ocular surface also contribute to both adaptive and innate immunity. Indeed, commensal microorganisms on the ocular surface and the eye have coexisted peacefully (José, 2020). Understanding the pathogenesis of diverse ophthalmic diseases requires investigation of the composition of the ocular microbiome. In this case, compared to what was subsequently discovered utilizing molecular-based methods, the initial research which used microbiological culture techniques, indicated a small diversified profile of the ocular microbiota.

Studies have shown that age has an effect in conjunctiva microbiota. The microbiota in infant and young children differ from adult. Sex and age collectively shape the conjunctiva microbiome, and may alter the immune homeostasis of the ocular surface through change of its microbiome (Wen *et al.,* 2017). Sex hormones may affect the health of the ocular surface, according to mounting research. The two major sex hormones which are estrogen and testosterone as well as progesterone has a major role to the change in the ocular surface. Changes in the levels of the steroid hormones frequently lead to immune-associated diseases such as ocular allergies and dry eye. The typical flora that lives on the eyelids and conjunctiva is made up primarily of bacteria and sometimes fungi that come from the outside world. Microbiome secretes antibiotics and

chemical mediators to maintain surface homeostasis and immunoregulation, this play a significant role in preserving normal bodily functions and health. Additionally, they fight harmful bacteria for nutrients, which slows down the growth of the latter (Sthapit *et al.,* 2014). Thus, understanding the complexity of ocular microflora not only throw light on their critical role towards normal function of the eye, but also educate on certain visual urgency (Deepthi *et al.,* 2020).

Understanding how potentially toxic organisms may penetrate and colonize the anterior eye prior to the onset of infection requires a complete knowledge of conjunctiva flora and lens storage case during contact lens usage.

1.1.9 CONJUNCTIVITIS

Conjunctivitis is a typical condition that causes dilation of the conjunctiva blood vessels leading to inflammation. It is clearly defined as conjunctiva hyperemia linked with discharge which may be purulent or mucopurulent, mucoid, watery (Khurana, 2007). It is a common condition that affects the eye of all ages. A relatively frequent and self-limiting infection is bacterial conjunctivitis. It is majorly caused by *staphylococcus aureus* and *Staphylococcus epidermidis.* Conjunctivitis comes in three primary varieties: allergic, infectious, and chemical. Depending on the type, conjunctivitis has a variety of causes.

1. **Allergic conjunctivitis**

a) **Allergic conjunctivitis:** In recent years, the occurrence of allergic illnesses has rapidly increased in number. In clinical practice, one of the most common ocular disorders is ocular allergies. When allergens interact with IgE that is bound to sensitized mast cells, allergic conjunctivitis is the outcome. The clinical manifestation of this allergy is seen in

the eyes. Vernal keratoconjunctivitis (VKC), atopic keratoconjunctivitis (AKC), seasonal allergic conjunctivitis (SAC) and perennial allergic conjunctivitis (PAC) are all included under the umbrella name of allergic conjunctivitis. On the other hand, compared to AKC and VKC, SAC and PAC, have quite different clinical and pathophysiology aspects (La Rosa *et al*., 2013).

b) **Giant conjunctivitis:** It is typically linked to contact lenses or ocular prostheses. It is a type of allergy to the eye. It should not be thought of as true allergy diseases but rather as chronic ocular micro trauma related disorders that need to be properly treated by ophthalmologists in collaboration with contact lenses specialists (La Rosa *et al* 2013).

2. **Chemical conjunctivitis:** It is caused by putting chemicals, liquids, fumes, or smoke in the eyes. The hazardous chemical or liquid must be removed immediately by rapidly flushing or irrigation of the eye with running water or normal saline. This condition can be very painful and it result from things like irritants, occupational hazards and allergens. Many chemical put in the eye can cause several ocular problems so a thorough history is necessary (Senaratne G., and Gilberlt, 2005).
3. **Infectious conjunctivitis:** It is caused by microorganisms. It is caused by various infectious agents. The diagnosis of conjunctivitis caused by infections may be difficult so it is important to take a proper history, noting the clinical signs and symptoms and using the appropriate diagnostic test can help in determining the cause (Yue and Hauswirth, 2022).

Regardless of the type of conjunctivitis, it is important to maintain proper hygiene practices, such as handwriting, avoiding touching the eyes sleeping with clean pillow.

Common bacterial ocular infections

a) Bacterial conjunctivitis

Inflammation of the conjunctiva caused by a bacterial infection is known as bacterium conjunctivitis. *Streptococcus pneumonia, staphylococcus* species, and gram-negative bacteria are primarily cause. It is frequently caused by *moraxella catarrhlis, hemophilus influenza* in children (Hutnik *et al.,* 2010). The condition affect both sexes, all ages and all races, it occurs as a result poor hygiene, infection and injury to the eye. It presents with thick or sticky discharge, crusting of the eyelid. It is generally considered to be self-limiting disorder (Sheikh *et al.,* 2001*)*. Due to the presence of bacterial pathogen inhibiting the ocular surface, it was found that bacteria are the most common cause of eye infections globally (Ogu *et al.,* 2022).

Patients of all ages can get bacterial conjunctivitis, which is prevalent. Common symptoms include purulent discharge that lasts all day long and inflamed eyes. Topical antibiotics are a treatment option (Tarabishy *et al.,* 2008).

The main bacteria that infect children include S*treptococcus pneumoniae*, or *Moraxella catarrhlis, Haemophilus influenza.* Some species that result in bacterial conjunctivitis include: *Streptococci pneumonia, Chlamydia trachomatis, Moraxella lacunata, Staphylococcus aureus, Haemophilus influenza.*

Although it has a significant societal impact due to its high prevalence. Antibiotics are frequently used to help patients go back to their normal routines faster and to stop the spread of disease since they can speed up the symptoms and the eradication of microbes. The current standard of care for treating bacterial conjunctivitis involves giving patients multiple applications of antibiotic eye drops, which is tedious and often uncooperative. New antibiotics with better

efficacy against the bacterial species that are most frequently linked with disease have been discovered due to the rising risk of bacterial resistance. The first chloroflouroquinolone designed for ocular use is besifloxacin (Deschenes *et al*., 2015).

Risk factors

It spread from:

I. Contaminated objects.
II. Poor contact lens hygiene.
III. Ocular diseases like blepharitis, dry eye anatomic abnormalities of the ocular surface and lids.
IV. Crowded living or social conditions.
V. Recent ocular surgery, exposed sutures or ocular foreign bodies.
VI. Immune compromise
VII. Chronic use of topical medications.

Treatment

Most frequently used antibiotics for bacterial conjunctivitis are as follows:

1st generation: Fluoroquinolones:

2nd generation: Ofloxacin 0.3% drops, and Ciprofloxacin 0.3% drops or ointment,

3rd generation: Levofloxacin 0.5% drops

4th generation: Gatifloxacin 0.5% drops, or Besifloxacin 0.6% drops, Moxifloxacin 0.5% drops.

Chloramphenicol is the most commonly prescribed ophthalmic antibiotic outside of the United States across a large portion of the world. However, this medication is not offered for topical use in the United States because chloramphenicol use systemically is linked to a side effect that could be fatal (aplastic anemia).

Treatment with antibiotics will decrease the symptoms and remove the microorganisms from the conjunctival surface. This is so because it may help reduce the length of infection, decrease complications, and reduce the spread of the infection to others. Of all the commonly prescribed antibiotics, fluoroquinolone, gatifloxacin and ofloxacin stood out as the most effective options for treating bacterial eye infections (Ramesh *et al*., 2010).

b) Bacterial keratitis

Infection of the cornea by bacteria is known as bacterial keratitis. The cornea is exposed to the environment therefore it is prone to get infected quickly. The most common form of infectious keratitis is called bacterial keratitis (BK). Identification and eradication of the microorganisms are essential because a bacterial ulcer is a sight-threatening condition (Zang, 2022). Since a bacterial ulcer poses a threat to vision, identifying and eradicating the microorganisms is crucial. Infectious keratitis, also known as corneal ulcer, is an emergency that needs to be treated right away. If in severe case it doesn't respond to initial treatment, hospitalization and intravenous antibiotics is needed. When a patient presents with symptoms of infectious keratitis, a clinical history and thorough clinical examination will show if the patient falls into the high-risk or low-risk category (Wong *et al*., 2012).

Recent treatments for infectious keratitis include "gatifloxacin," "fourth generation fluoroquinolones", "collagen cross-linking" and "moxifloxacin". The mainstay of treatment for

bacterial keratitis has been broad spectrum antibiotics, however with the increase of bacterial resistance, there is a need for innovative antimicrobial drugs and methods of treatment. Among the new treatments which are corneal collagen cross-linking and fourth-generation fluoroquinolones (Wong *et al*., 2012). The common cause is *staphylococcus (CoNS), streptococcus pneumonia pseudomonas and staphylococcus aureus* (Green *et al*., 2008).

Eyedrop use could vary from four times per day to once every 30 minutes, even at night, depending on the severity of the disease. As an addition, oral antibiotics are occasionally used and systemic pain relievers can be given if patient is having serious discomfort. One drop of fortified antibiotics, like vancomycin (50 mg/mL) or fortified cefazolin (50 mg/mL), are alternated with tobramycin (14 mg/mL) every hour is considered the standard therapy for bacterial keratitis (Jean, 2023).

c) Neonatal conjunctivitis

The risky bacterial conjunctivitis known as neonatal conjunctivitis affects babies. The most usual causes of neonatal eye discharge are congenital nasolacrimal duct obstruction and either chemical or infectious conjunctivitis. The first four weeks of life are when neonatal conjunctivitis, also known as ophthalmia neonatorum frequently appears. The most common eye disease in newborns is an infection that usually develops after delivery. The cause include:

For the first 24 hours of life: Chemical factors is the cause.

For 24 to 48 hours of life: *Neisseria gonorrhoeae* and *staphylococcus aureus* are most likely the cause of bacterial infection.

For 5 to 14 days of life: It is caused by *chlamydia trachomatis*

For 6 to 14 days of life: It is caused by herpes keratoconjunctivitis

For 5 to 18 days: It is caused by *pseudomonas aeruginosa* (Makker, *et al*., 2017).

Also infants whose mother have sexually transmitted disease have a high risk of developing it. If preventive measure is not taken it may result in permanent visual impairment (Moore *et al., 2015).* The use of antibiotics ointment or eye drops as a prophylactic treatment for neonatal ophthalmia is a common practice in health care settings. It is mainly caused by various infectious agents and less commonly by microorganisms.

d) Gonococcal conjunctivitis

Neisseria gonorrhoeae (NG), a gram-negative *diplococcus*, is the causing factor, and it belongs to the category of extragenital gonococcal infections. It is a medical emergency because it can lead to serious complication and serious vision loss if left untreated. There are two main groups:

i) Gonococcal ophthalmia neonatotum: It is acquired from an infected mother during birth and affects 30% to 50% of neonates who are perinatally exposed.

ii) Non neonate: Autoinoculation or the inoculation of genital fluids from a sexual partner are the two main causes of infection (Belga *et al.,* 2019).

e) Stye

A stye (sty) is a red, uncomfortable lump that can look like a boil or a pimple and appears close to the edge of your eyelid. An inflammation of the oil glands of the eyelid is what causes styes. Stye is brought on by a person's lack of personal hygiene. It provoke activities of bacteria majorly *streptococcus* and *staphylococcus* infections of the eyes. It is associated with small papule formation in either lower or upper eyelid (Sreeremya, 2018). It is caused by bacteria infection of the eyelash follicle. There are two types:

I. Internal hordeolum: This results from disruption to the meimbomain gland in the tarsal plate. This develop when the infection affect the small gland within the eyelid.

II. External hordeolum: This occurs in the base of the eyelash follicle or in the small oil glands of the eyelids. It occurs from damage to the Zeiss or Moll glands associated with the eyelash follicles (Cheng *et al*., 2017).

Molone *et al* (2020) suggested that a conventional nonsurgical treatment (warm compresses and oral or topical antibiotics) should be used for internal hordeolum treatment.

f) Blepharitis

Inflammation of the eyelids is known as blepharitis. It occurs when the base of the eyelids have a large number of microorganisms. The outer margins of both eyes are affected. It happens when microscopic oil glands near the end of the eyelashes become clogged, causing irritation and redness. In clinical practise, patients frequently develop blepharitis which is a persistent inflammatory disease of the eyelids. It can be divided into two types: posterior blepharitis, which is characterised by meibomian gland dysfunction, and anterior blepharitis which involves the anterior lid edge and eyelashes (Stephen *et al.,* 2014). Seborrheic dermatitis or acne rosacea are often seen in conjunction with primary meibomitis, which does not appear to be primarily an infectious condition but rather a sign of generalized sebaceous gland dysfunction (McCulley *et al*., 2017).

Although blepharitis does not endanger vision, it has the potential to lead to keratopathy, corneal neovascularization, ulceration, and chronic ulceration of the eyelid morphology if untreated (Christopher, 2016).

Symptoms according to Bernardes *et al., (*2010) include:

I. Tearing
II. Burning
III. Photophobia
IV. Irritation
V. Red eyes
VI. Blurred vision

Diagnosis is through eye exam which is based on the findings from slit lamp examination and additional tests. Blepharitis can cause dry eye, damage to the cornea, chronic red eyes, chalazion and style.

Corneal ulcer

Corneal ulcer is an injury to the eye from bacteria, fungi or viral infection. It is the most common cause of blindness worldwide. The open sore or erosion is due to infection caused by any underlying conditions that can affect the normal structure of the eye and injury. Corneal infections are responsible for a large portion of scaring resulting to poor vision. Symptoms of corneal ulcer are eye pain, discharge, red eyes, photophobia, swollen lids, a white or grey spot on the cornea, and sandy sensation. The most common cause of evisceration or enucleation is as a result of corneal ulcer (AlMahahmoud *et al*., 2019). There is risk of bacterial infection and ulcer development when the cornea is damaged by foreign particles (Katara *et al.,* 2013).

Risk factors of corneal ulcer

I. Contact lenses wearer
II. Dry eyes
III. Long use of steroid
IV. Injury prior to eye surgery
V. Trauma

To correctly diagnose keratitis, the management should be determined by the severity of the clinical presentation. Moxifloxacin (55.4%) and board spectrum antibiotics (62.7%) are the two most common drugs for treating less serious ulcer and serious ulcer respectively (Park *et al*., 2015).

Others infectious ocular infections

a) **Viral conjunctivitis:** Infectious conjunctivitis is primarily caused by viral conjunctivitis, which is responsible for up to 80% of cases (Keen, 2018). It is highly contagious, symptoms includes redness, tearing, blood vessel engorgement, ocular discharge, discomfort, photophobia, and pseudo membranes are classical symptoms of viral conjunctivitis. Adenoviruses are the most common cause of viral conjunctivitis. The adenovirus is a nonenveloped, double-stranded DNA virus that belong to the Adenoviridae family (Solano *et al*., 2022).
b) **Herpes simplex keratitis:** It occur from an infection with herpes simplex virus type 1 which is also known as human herpes virus type. It is known to be the major cause of visual morbidity (Kaye *et al*., 2006).

1.2 AIM AND OBJECTIVES OF STUDY

1.2.1 Aim of Study

The aim of this study is the assessment of ocular bacterial flora among university of Benin undergraduates.

1.2.2 Objective of Study

1. To investigate the ocular bacterial flora in university of Benin undergraduates.
2. To investigate if there is relationship between lifestyle and ocular bacterial flora.
3. To determine the difference between male and female ocular bacterial flora.
4. To determine the antibiotics susceptible of the ocular bacterial flora.

1.3 STATEMENT OF PROBLEM

All human organ are susceptible to bacterial infection. This bacterial infections have high impact in public health. There have been significant increase of bacteria causing ocular infection. The eye has flora when there is an imbalance or when opportunistic organisms enter it lead to various eye infections. The assessment of bacterial flora involves identifying the types of bacteria present in the eye, their quantities and their susceptibility to antibiotics. This study is therefore aimed to determine the assessment of ocular bacterial flora among university of Benin undergraduates

1.4 SIGNIFICANCE OF STUDY

1. This study will assist eye care practitioner in investigating the bacterial causing ocular infections.
2. This study will give the eye care practitioner a broader knowledge of bacteria associated with ocular infections.
3. It will help to enhance prophylactic management of ocular bacterial infections.
4. It will help determine antimicrobial susceptibility and guild in formation of antibiotics policy.

CHAPTER TWO

2.0 LITERATURE REVIEW

Capriotti *et al.,* (2009) conducted a study on the normal ocular flora in healthy eyes from a rural Sierra Leonean population. 276 individuals of Masungbo, Sierra Leone's was used to carry out the work and the conjunctiva swaps were taken from the right eyes of healthy individuals. Conjunctiva samples were then analyzed for microbial growth. It was found that the conjunctiva swabs from healthy eyes contained *coagulase-negative Staphylococcus* (28.6%), fungi (26.0%), *Staphylococcus aureus* (19.9%), Gram-negatives other than *Pseudomonas/Haemophilus* (9.8%), *Nocardia/Actinomyces* (6.5%), and *Pseudomonas aeruginosa* (6.2%) were isolated. A rural population of Sierra Leone shows a high prevalence of bacterial and fungal colonization of healthy eyes.

Graham *et al.,* (2007) worked on ocular pathogen or commensal: a polymerase chain reaction (PCR) based study of surface bacterial flora in normal and dry eyes. Using the tear break-up time, McMonnies survey, goblet cell density, and meibomian gland assessment, 91 subjects were categorized as normal (n= 57) or dry eye (n= 34). For bacterial identification, conventional bacterial culture, broad-range 16S rDNA PCR, cloning, and DNA sequencing were used. Over the course of three months, a selection of the participants underwent repeated sampling. In a subset of the participants, the relationship among goblet cell loss and bacterial counts was evaluated. While molecular techniques revealed a significant number of new bacteria, most of the bacteria detected by culture were *coagulase-negative staphylococci*. In situations of overt inflammation on the normal eye surface, atypical ocular surface bacteria such *Rhodococcus erythropolis, Klebsiella oxytoca, and Erwinia sp.* were found to be on the ocular surface.

Ogu *et al.,* (2021) carried out a study on prevalence and type of bacteria associated with ocular infections of patients visiting the optometry clinic of federal university of technology Owerri. Using sterile swab sticks, fifty (50) samples were obtained from out-patients with various eye diseases. It was done to isolate and identify the microbes present in the eye. For the purpose of isolating the organisms the spread plate culture method was used. The isolate was described and identified. Individuals with ocular infections were chosen, of which 14 men and 36 women. A total of 57 bacterial isolates were found from the fifty (50) samples that were cultivated. A summary of the biochemical, morphological, and cultural traits of the bacteria isolated from eye illnesses was provided. Gram positive 35 (61.4%) and Gram negative 22 (38.6%) bacteria were the most frequently isolated types. A short overview of the biochemical, morphological, and cultural traits of the bacteria isolated from eye conditions was provided.

Khorazo *et al*., (1935) carried out a prospective cultures on 1,122 non inflamed conjunctivae. The predominant bacteria identified in a healthy conjunctiva were *diphtheroids* and *staphylocci.* Only a small percentage of occasions involve the presence of other organisms. As people get older, they are far more likely to encounter *diphtheroid* germs which is common between 30 to 50 years. It was determined that children under the age of five are more commonly affected by *pneumococci* and green *streptococci*.

Alshamahi, *et al.,* (2020) carried out a study on prevalence of *staphylococcus aureus* in external ocular infection and the occurrence of Mrsa in isolates. A total of 197 individuals with external eye infections were examined in a study which lasted a year from September 2016 to October 2017. Samples were collected and sent to the Sana'a-based National Centre of Public Laboratories (NCPHL). Standard laboratory practices have been used to isolate and identify potential bacterial pathogens. MRSA was identified using the disc diffusion method with 5

micrograms of methycillin disc and 1 microgram of oxacillin disc. Selected medications were tested for antimicrobial sensitivity using the disc diffusion method. Conjunctivitis, keratitis, blepharitis, and blepharo-conjunctivitis are among the ocular illnesses. Only 146 out of a total of 197 cultured swabs resulted in a positive culture (74.1%). *Staphylococcus aureus* was the primary pathogen (30.1%) in 52.1% of the total isolates made from gram-positive bacteria, MRSA rates of infection were 34.1% high. MRSA isolates had a higher rate of tested antibiotic resistance compared to MSSA isolates which had a lower rate of resistance. it was concluded that *S. aureus* was the most prevalent bacterial isolate in external ocular infections, and the high rate of MRSA, as well as the emergence of *S. aureus* isolates resistant to a wide range of antibiotics, have caused MRSA in ocular infections to become a multi-drug-resistant strain, increasing its danger in ocular infections.

Deepthi *et al.,* (2020) carried out a research on bacterial infection: pathogenesis and diagnosis. It was done to know the various human microbiota their pathogenicity, and their impact on human physiology. The succession of genome-based methods through identification based on the 16S rRNA gene greatly expanded our knowledge of the variety of ocular surface bacteria. Evidence like this suggests that while few bacteria are involved in maintaining normal eye functions, many bacteria are actively involved in the pathophysiology of ocular disorders. Therefore, knowing the complex nature of ocular microflora sheds insight on specific visual requirements as well as their crucial role in maintaining normal operation of the eye. It was found that the sta*phylococcus, streptococcus, pseudomonas, corynebacterium, and propionibacterium genera* are the most frequently encountered on the ocular surface. Systematic microbiological analyses confirm the infectious nature. The primary genera for the ocular surface have been found to be staphylococcus, streptococcus, pseudomonas, corynebacterium, and propionibacterium.

Sthapit *et al*., (2014) conducted a work on conjunctival flora of normal human eye. A cross-sectional descriptive study which took place from March to July 2011 in the microbiology and ophthalmology regions of Dhulikhel Hospital, Kathmandu University Hospital. Based on bacteriological investigations of patients healthy conjunctiva who visited the outpatient eye department. It was conducted on 200 normal eyes (each right and left eye separately) from 100 healthy participants representing a range of age groups, sexes, and geographic distributions made up the study population. The swab was collected and then brought to the lab. After inoculating the swab onto blood, chocolate, and Brucella blood agar, it was analyzed after 24, 48, and 72 hours. Following this, the colonies from Gram staining were categorized into cocci, rods, pairs, tetrads, and chains. To discriminate between *staphylococcus* (+ve catalase) and (-ve catalase), a catalase test was conducted. Based on the findings, out of 200 samples 78% of the right eye and 79% of the left eye had bacterial growth. *Coagulase negative streptococcus* was the most prevalent flora isolate, present in 50% of right eyes and 52% of left eyes.

Abadi *et al.,* (2022) carried out a retrospective study. 345 articles were retrieved 60 articles were left for full text assessment after 285 were disqualified based on the index and an examination of the titles and abstracts. 33 papers were removed after full-text screening, leaving 27 studies that met the requirements. According to the results of the current systematic study *S. epidermidis* accounted for 19.1% of all bacterial infections of the eyes in Iran. 6.9% of eye-related infections were caused by *P. aeruginosa* isolates, whereas 6.7% and 3.3% of infections reported to be caused by *S. aureus* and *streptococci* of the viridans group, respectively.

Ayehubizu *et al.,* (2021) carried out a study on common bacterial causes of external ocular infections, associated risk factors and antibiotic resistance among patients at ophthalmology unit of Felege Hiwot Referral Hospital, Northwest Ethiopia: a cross-sectional study which started

from 1st February and lasted to 30th April 2019. Patients with EOIs were sequentially accepted. Face-to-face interviews with a structured questionnaire were used to gather the data. 360 patients in all participated in the trial, with 64.7% of them being men. Participants in the study had a median age of 59.5 years. Overall, 208 individuals (57.8%; 95% CI: 52.6–62.8%) developed bacterial EOIs that were verified by culture. Conjunctivitis cases had a percentage of culture-confirmed EOIs of 60.4%, while blepharitis cases had a percentage of 55.8%. Significant correlations between EOIs and ocular trauma (P 0.001), ocular illness (P 0.001), and having an eye allergy (P = 0.027) were found. It was found that ampicillin was not effective against gram-negative isolates (87.5%). Tobramycin and piperacillin resistance rates for *P. aeruginosa* isolates were both 50% and 85.3% respectively. The overall percentage of isolates with multiple medication resistance was 45.2%. 80% of the samples of Enterobacter and 64.3% of the isolates of *K. pneumoniae* had multi-drug resistance. Penicillin, ampicillin, tetracycline, and piperacillin resistance are common in bacterial external ocular infections as well as multi-drug resistance. As a result, it is necessary to regularly test isolates for antimicrobial susceptibility in order to evaluate empirical treatment for eye infections in the study area.

A study was carried out by Ramesh *et al.,* (2010) on Prevalence of bacterial pathogens causing ocular infections in South India. A retrospective analysis was done on all patients who had clinically identified bacterial ocular infections between January 2005 and December 2005, including blepharitis, suppurative scleritis, conjunctivitis, internal and external hordeolum, canaliculitis, dacryocystitis, keratitis, preseptal cellulitis, panophthalmitis and endophthalmitis. Samples from the extra- and intra-ocular regions were gathered, analyzed under a microscope directly, and cultured. A total of 756 individuals with bacterial ocular infections were examined; of these, 462 (61%) had infections of the adnexa, 217 (28.7%) of the cornea, 6 (0.8%) of the

sclera, and the rest 71 (9.39%) of the intraocular tissues. *S. pneumoniae* (169 of 776; 21.78%), *coagulase negative staphylococci* (142 of 776; 18.3%), and *S. aureus* were the most abundant bacterial species found (195 of 776; 25%). The largest number of gram-positive isolates were susceptible to cefazolin (545 of 624; 87.34%), chloramphenicol (522 of 624; 83.65%) and gatifloxacin (511 of 624; 81.89%) and gram-negative isolates were to amikacin (127 of 136; 93.38%), gatifloxacin (125 of 136; 91.91%) and ofloxacin (119 of 136; 87.5%), while aerobic actinomycetes were to amikacin (100%), gatifloxacin (14 of 16; 87.5%), chloramphenicol (14 of 16; 87.5%) and ofloxacin (13 of 16; 81.25%). It was determined that *S. aureus* frequently causes infections of the eyelids and conjunctiva*, S. pneumoniae* regularly infects the cornea and lacrimal apparatus, and *coagulase negative staphylococci* frequently infect the intraocular cavity.

Vola *et al* (2013). Carried out a study on prevalence and antibiotic susceptibility of methicillin-resistant *Staphylococcus aureus* in ocular infections. The Universidade Federal de São Paulo's ocular microbiology lab's electronic records were retrospectively evaluated. Every case of conjunctivitis, keratitis, and endophthalmitis with a positive *S. aureus* culture were identified throughout a ten-year period (between January 2000 and December 2009). Using the Kirby-Bauer disc diffusion method, antibiotic susceptibility was identified. 56 (9.9%) of the 536 *S. aureus* isolates that were found to be resistant to methicillin. Infections with *Staphylococcus aureus* developed significantly during the course of the 10-year period, rising from 7.55% to 16.18% overall (p=0.001) and from 3.7% to 13.16% in conjunctivitis (p=0.001). On the other hand, we found that the tendency did not exist in people with keratitis (p=0.38). Comparing *S. aureus* isolates to *Staphylococcus aureus* isolates, it was demonstrated that the latter had greater rates of resistance to tobramycin, gentamicin, ciprofloxacin, gatifloxacin, and moxifloxacin (p 0.001). Vancomycin worked on all of the patients. *Staphylococcus aureus* ocular infections are

becoming more common generally, and they have statistically significant higher rates of resistance to common antibiotics than other strains of the bacteria.

Rhumaid *et al.,* (2022) conducted a study on the prevalence and antibiotic susceptibility of pathogenic bacteria associated with ocular infections. According to age, sex, and location in rural and urban areas, the study was aimed at determining the incidence of bacterial isolates and their role in patients with external ocular infections. It also sought to look into the patterns of antibiotic sensitivity and resistance that were frequently used to treat these patients in Babylon Governorate, Iraq. In this study, 200 individuals with clinical signs of external ocular infections aged 20 to 68 and of both sexes were included. To differentiate between viral and bacterial isolates, eye swabs were taken and cultured. A total of 105 isolates (52.5%) were determined to be positive bacterial cultures and included in the study, while 95 (47.5%) were negative bacterial cultures. The findings showed that ocular infections mostly affected individuals between the ages of 20 and 49 years, and most of the patients (61.9%) were males over the age of 65 years. Conjunctivitis which infected 45.7% of patients, was the most frequent eye infection. Blepharitis was next affecting 21.9% of patients followed by blepharoconjunctivitis which affected 14.3% of patients, dacryocystitis which affected 12.4% of patients, and keratitis 5.7% was recorded. Also, the results indicated that the most common bacteria implicated in ocular infections are *Staphylococcus aureus* (37.1%), followed by *Coagulase negative Staphylococci*(CoNS)(26.7%), *Haemophilusinfluenzae*(21.9%), *Streptococcuspneumoniae* (6.7%), *Klebsiella pneumonia* (3.8%), *Streptococcus pyogenes* (1.9%), *Pseudomonas aeruginosa* (1.9%). To show how well some antibiotics worked against these harmful bacterial isolates, tests were conducted on them. It was discovered that while most bacterial isolates were responsive to ciprofloxacin, gentamycin, and chloramphenicol they were resistant to ampicillin, penicillin, and tetracycline.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 RESEARCH DESIGN

This study is a cross sectional observational study.

3.2 SAMPLING TECHNIQUE

Multi staged random sampling technique was used in this study.

3.3 STUDY LOCATION

This research was done in the selected 2 faculties (Faculty of Medicine and Faculty of Education) in University of Benin Ugbowo and the laboratory work done in Microbiology laboratory, University of Benin.

3.4 SAMPLE POPULATION

The study was drawn from undergraduate healthy participants in University of Benin who meets up with the criteria and are eager to take part in the research.

3.5 STUDY DURATION

This study took place within a period of two months.

3.6 SAMPLE SIZE

This was determined using Fischer's Formula considering the prevalence of bacterial infections to be 2.2% (Malu 2014). The expected margin of error (d) was 0.05, and interval (z) was 95%.

$n= Z^2P (1-P)\backslash d^2$

Where,

n= sample size

Z = statistical level of confidence of 95% (1.96)

P= maximum reported prevalence of 2·2% (Malu 2014)

d=Precision desired (5%, d=0.05)

Therefore,

$n= 1.96^2 \times 0{\cdot}022\ (1-0.022)\backslash 0.05^2$

n= 33·06

Attrition factor is 10% of n

Therefore, 10% of 33 is 3·3

Adding the attrition factor we have (33·06+3·3) = 36.36

Therefore, 50 participants was used for the study.

3.7 INCLUSION CRITERIA

I. Subjects that filled the consent form to participate in the research.
II. Undergraduates in University of Benin.
III. Subjects with no complaint of discharge.
IV. Individuals within an age range of 17- 35 years
V. Individuals who are not on any systemic or topical antibiotics
VI. Individuals who have not worn contact lens in the past 2 weeks

3.8 EXCLUSION CRITERIA

I. Subjects who are not in University of Benin.

II. Subjects that graduated from University of Benin.

III. Subjects that are not student.

IV. Subjects with complain of discharge

V. Individuals below age 17 or above 35 years.

VI. Individuals that are on systemic or topical antibiotics

VII. Individuals who have worn contact lens in the past 2 weeks.

3.9 ETHNICAL CONSIDERATION

I. Ethnical clearance was obtained from the Departmental Research and Ethics Committee of the department of Optometry University of Benin, Benin City in accordance with the tenet of the declaration of Helsinki. This is to ensure that all procedures that will be performed on each subject will not be against public interest or inflicting unnecessary harm.

II. Informed consent of all subjects will be obtained before any procedure will be done to ensure full cooperation.

3.10 RESEARCH MATERIALS

1) Swab stick
2) Sanitizer and disposable gloves
3) Microscope
4) Normal saline
5) Mannitol salt agar

6) Incubator
7) Masking tape
8) Colony counter
9) Marker pen
10) Nutrient agar
11) Convex lens
12) Cooler bags
13) Test tubes
14) Safranin
15) Crystal violet
16) Ethyl alcohol
17) Gram iodine
18) Tryptic soy broth
19) Slides
20) Heat
21) Recording book and pen

3.11 EXPERIMENTAL PROCEDURES

1. Administration of bio data/consent form.
2. Collection of samples.
3. Grouping of the samples.
4. Isolation and identification of bacteria and other microorganisms.
5. Antibiotics Susceptibility Test

3.11.1 ADMINISTRATION OF BIODATA FORM OR CONSENT FORM

After explaining the procedure to the subject, a consent form that contains the bio data and general questions was provided.

3.11.2 COLLECTIONS OF SAMPLES

The sample was collected using sterile swap stick for every subject that meets up with the criteria and then taken to the laboratory for analysis to know the bacterial present. The samples was obtained aseptically only from one eye by following standard procedures. I scrubbed and sanitize my both hands before wearing a pair of disposable gloves and face mask. Thereafter the sample from the conjunctiva of the healthy eye was taken by lowering the eyelid exposing the palpebral conjunctiva and the cul-de-sac and careful using of the sterile swap moistened in a saline solution to wipe the conjunctiva (Lee *et al,* 1989). The swab stick was labelled indicating the serial number and date of collection. The specimens for detection of bacterial was kept in a cooler bag and transported to the laboratory for the analysis to be done.

3.11.3 GROUPING OF SAMPLES

Obtained samples were grouped into male and female.

3.11.4 ISOLATION AND IDENTIFICATION OF BACTERIA

The samples collected were transported to the microbiology laboratory, within four to six (4-6) hours of collection for isolation, identification and antibiotics sensitivity testing. The microbial analysis was performed on the samples according to standard procedures.

3.11.5 ENUMERATION OF ISOLATES

Standard plate count of bacterial swabs soaked in 10ml of tryptic soy broth was prepared using 0.1ml of broth. The duplicate plates were incubated overnight and colonies of the bacteria were counted and enumerated using the formula.

CFU/ml = number of colonies x dilution / volume of inoculum

Where CFU= Colony Forming Unit

1) MORPHOLOGICAL TEST

Gram Staining: This recognises and categorises gram-positive and gram-negative bacteria. Each distinct colony was collected using a sterile swab stick, and then a drop of distilled water was added to it before spreading it on a slide. After being air-dried, the slide was heated up over a flame. Gram's iodine solution was added and allowed to stand for around 30 seconds before washing off the Gram's iodine solution. The resulting smear was treated with 95% ethyl alcohol for about 30 seconds before being rapidly rinsed with distilled water to remove the colour. Using Safranin for 30 seconds, secondary staining was achieved. The slide was blotted dry after cleaning with distilled water. With a 40X objective and an oil immersion objective, the dispersion of the stained organisms could be observed on the slide. Gram positive organisms were denoted by the colour purple, whereas gram negative organisms were identified by the colour pink.

2) BIOCHEMICAL TEST

This was tested to see if a specific bacterial isolate is capable of producing enzymes like catalase, oxidase, coagulase, and citrate. Additional motility tests including lactose, motility, and indole tests were performed.

a) CATALASE TEST

A test for catalase was conducted to help detect its presence. The enzyme causes an effervescence by catalysing the release of oxygen from hydrogen peroxide. Harmful hydrogen peroxide (H2O2) is converted into non-toxic oxygen and water by catalase. Since it is made by aerobic organisms, it is highly helpful in identifying which organisms are aerobic and which are anaerobic.

Procedure: On a clean, grease-free slide, a drop of 3 hydrogen peroxide was applied to the spot. Effervescent behaviour indicated active coagulase, but a lack of effervescence indicated inactive coagulase.

b) OXIDASE TEST

To find cytochrome C oxidase, which might reduce oxygen and synthetic electron acceptors, an oxidase test was performed. Tetramethyl-p-phenylene-diamine is a redox dye that is dependent on the presence of certain oxidases in bacteria that catalyse the passage of electrons between the bacterium's electron donors and the dye.

Procedure: Tetramethlyphenylene diamine hydrochloride was 1% solution was applied to whatman filter paper. On the impregnated filter paper the test isolate was cultured for a period of 24 hours. The appearance of the colour purple indicated an excellent result.

c) CITRATE TEST

This experiment was designed to assess an organism's ability to utilise citrate as its only carbon source. The medium has ammonium dihydrogen phosphate as its sole source of nitrogen and sodium citrate as the only source of carbon. Bromothymol blue serves as the indicator. The bacteria isolates to be tested were cultured in a medium that was set up as a slant in a test tube, and then left to stand for a period of 24 hours in an incubator. Citrate will cause the medium to turn blue when it act positively, while citrate will cause the medium to remain green when it do not react.

d) COAGULASE TEST

The *staphylococcus* species that produce the coagulase enzyme, such as *Staphylococcus aureus,* can be distinguished from those that do not using this test. On two separate slides, a drop of physiological saline was put. The isolated colony was separated into two thick suspensions by emulsifying a piece of it in each drop using a loop. Within 10 seconds, the organisms began to cluster close as a result. To differentiate between the organism's possible granular appearance and genuine coagulase clumping, no plasma is added to the second solution.

e) MOTILITY TEST

To ascertain if the isolates were motile or not, this test was carried out. A semi-solid nutritional agar in a test tube was used to conduct the motility test. The isolate was stabbed into the agar around the centre of the tube using a sterilised needle. At 37 °C, the test tube was incubated for up to 7 days, the experiment tube was monitored daily. Non-motile isolates had growth that was restricted to the stab line, whereas motile isolates had more varied growth regardless of the stab line.

f) INDOLE TEST

Sterilised test tubes with 4 ml of tryptophan broth were used for this experiment. Aseptically inoculating the tube with the isolates was done. A 48-hour period of incubation at 37°C was used with the test tubes. 0.5 ml of Kovac's reagent was instilled to the broth culture. The cherry red ring was used as the standard for classifying things as positive or negative. For the test, isolates with the cherry red ring were labelled as positive while isolates without the ring were categorized as negative.

3.11.6 ANTIBIOTICS SUSCEPTIBILITY TEST

Kirby-Bauer disc diffusion technique was used. The antibiotics discs used for this study contained the following antibiotics:

Antibiotics to test gram-positive bacteria

1. Cefuroxime (30µg)
2. Gentamycin (10µg)
3. Ceftriaxone (30µg)
4. Erythromycin (5µg)
5. Cloxacillin (5µg)
6. Ofloxacin (5µg)
7. Ceftazidime (30µg)

Antibiotics for gram-negative bacteria

1. Ciprofloxacin (5µg)
2. Cefuroxime (30µg)
3. Gentamycin (10µg)
4. Cefixime (5µg)

5. Ofloxacin (5µg)

6. Augmentin (30µg)

7. Nitroflurantoin (300µg)

8. Ceftazidime (30µg)

In order to create a bacterial lawn on a plate of Mueller-Hinton agar, a sterile swab was placed into the broth culture of a specific microbe. For about 5 minutes, the plate was left to dry. The forceps flame was applied on the forceps to sterilize it before being used to place the discs with the relevant antibiotics on the plate. The discs was gently pressed on the agar to make sure they adhered to the agar. A 37°C incubation temperature was used for the plate overnight incubation (Davis & Brown, 2016). After the incubation phase (zone of inhibition), a space was observed around the disc. The Clinical and Laboratory Standard Institute's established standards was used to categorize each drug's zone diameter into sensitive, intermediate, and resistant categories (Reller *et al*., 2009). The multiple antibiotic resistance (MAR) Index for resistant bacteria was determined by dividing the number of antibiotics that an isolate demonstrated resistance to by the total number of antibiotics the isolate has been subjected to.

3.12 DATA ANALYSIS

The statistical programme for social science software package version 22.0, was used to analyze the results. It was presented using descriptive statistics, such as frequency and percentages. Data analysis was performed with paired T tests and other statistical methods like Analysis of Variance (ANOVA).

CHAPTER FOUR

4.0 RESULT AND ANALYSIS

4.1 RESULT PRESENTSTION

Table 4.1: Shows descriptive statistics for age

Variable	Range	Mean	Standard deviation	Standard error	Variance
Age	17-33	21.06	3.133	0.443	9.813

This is a descriptive statistical table of age showing the range, mean, standard deviation, standard error and variance. Individuals between age 17-33 was used for the study.

Table 4.2: Showing age, mean CFU and occurrence of bacterial isolates between males and females

VARIABLES	MALE (n=25)	FEMALE (n=25)	TOTAL (N=50)	P. VALUE
Age	21.320±3.540	20. 800±2.708	21.060±3.133	0.563
CFU	3.145±3.051	2.660±2.150	2.902±2.624	0.190
% Occurrence of Pseudomonas aeruginosa	9.000±19.940	5.088±7.640	7.044±15.074	0.364
% Occurrence of Staphylococcus aureus	8.1896±9.384	17. 011±20.431	12.600±16.354	0.056
%Occurrence of E Coli	82.811±20.606	77.899±22.721	80.355±21.610	0.427

Mean ± Standard deviation

It shows age, mean CFU and mean occurrence of bacteria isolates between males and females. It also shows the standard deviation and P. values of the variables. The mean ± standard deviation

in both males and female were 3.145±3.051 and 2.660±2.150 respectively. Age, CFU, % occurrence of pseudomonas aeruginosa, % occurrence of staphylococcus aureus and % occurrence of E Coli show no significant relationship.

Table 4.3: Showing Total Bacteria Count for Males at 37°C for 24 Hours

SAMPLES	DILUTION	BACTERIA COUNT (CFU)	CFU MOL (10^3)
M 01	10^1	13	1.30
M 02	10^1	1	0.10
M 03	10^1	35	3.50
M 04	10^1	120	12.00
M 05	10^1	1	0.10
M 06	10^1	20	2.00
M 07	10^1	19	1.90
M 08	10^1	33	3.30
M 09	10^1	36	3.60
M 10	10^1	11	1.10
M 11	10^1	14	1.40
M 12	10^1	34	3.40
M 13	10^1	25	2.50
M 14	10^1	6	0.60
M 15	10^1	112	11.20
M 16	10^1	73	7.30
M 17	10^1	6	0.60
M 18	10^1	28	2.80
M 19	10^1	23	2.30
M 20	10^1	38	3.80
M 21	10^1	31	3.10
M 22	10^1	21	2.10
M 23	10^1	11	1.10
M 24	10^1	7	0.70
M 25	10^1	14	1.40

M= Male, CFU= colony forming unit, CFU MOL= Colony forming unit per mol

M01-M25 represents samples of participants that were collected for males

It shows the total counts of bacteria which was collected from 25 male participants. It was then cultured on nutrient agar and MacConkey agar at 37°C after 24 hours with a dilution factor of $10^{1.}$

Table 4.4: Showing Total Occurrence or Prevalence of Bacteria Isolates in Males

Samples	Pseudomonas aeruginosa	Staphylococcus aureus	Escherichia Coli
M 01	−	+	+
M 02	−	−	−
M 03	+	+	+
M 04	+	+	+
M 05	−	−	+
M 06	+	+	+
M 07	+	−	+
M 08	−	−	+
M 09	+	+	+
M 10	+	+	+
M 11	+	−	+
M 12	−	+	+
M 13	+	+	+
M 14	−	−	+
M 15	+	+	+
M 16	+	+	+
M 17	+	+	+
M 18	+	+	+
M 19	−	−	+
M 20	−	+	+
M 21	−	+	+
M 22	+	+	+
M 23	−	+	+
M 24	+	+	+
M 25	+	+	+

+ =Present, − =Absent

M01-M25 represents samples of participants that were collected for males

It shows the total occurrence or prevalence of bacteria isolates in males. +ve indicates the presence of the bacteria and −ve indicate the absence of the bacteria. Of the cultured samples, 3 bacteria was isolated of which each participants had +ve in one or two or all (3) and −ve in all the 3 isolates.

Table 4.5: Showing Percentage of Occurrence of Bacteria Isolates in Males

Samples	Pseudomonas aeruginosa (%)	Staphylococcus aureus (%)	Escherichia Coli (%)
M 01	0.00	23.08	76.92
M02	100.00	0.00	0.00
M 03	5.71	14.29	80.00
M 04	1.67	4.17	94.16
M 05	0.00	0.00	100.00
M 06	10.000	5.00	100.00
M 07	15.79	0.00	84.21
M 08	0.00	0.00	100.00
M 09	2.78	8.33	88.89
M 10	9.09	9.09	81.82
M 11	21.43	0.00	78.57
M 12	0.00	2.94	97.06
M 13	4.00	8.00	88.00
M 14	0.00	0.00	100.00
M 15	1.79	3.57	94.64
M 16	2.74	6.85	90.41
M 17	16.67	16.67	66.66
M 18	7.14	3.57	89.29
M 19	0.00	0.00	100.00
M 20	0.00	7.89	92.11
M 21	0.00	9.68	90.32
M 22	4.76	9.53	85.71
M 23	0.00	36.36	63.64
M 24	14.29	28.57	57.14
M 25	7.14	7.14	85.72

M 01-M25 represents samples of participants that were collected for males

It shows the percentage of occurrence of bacteria isolates in males. From the bacteria cultured there was high occurrence of *pseudomonas aeruginosa*, *staphylococcus aureus* and *Escherichia Coli*.

Table 4.6: Showing Total Bacteria Count for Females at 37°C for 24 Hours

SAMPLES	DILUTION	CFU	CFU MOL(10)^2
F 01	10^1	18	1.80
F 02	10^1	72	7.20
F 03	10^1	30	3.00
F 04	10^1	108	1.08
F 05	10^1	27	2.70
F 06	10^1	20	2.00
F 07	10^1	18	1.80
F 08	10^1	13	1.30
F 09	10^1	26	2.60
F 10	10^1	22	2.20
F 11	10^1	35	3.50
F 12	10^1	16	1.60
F 13	10^1	21	2.10
F 14	10^1	7	0.70
F 15	10^1	32	3.20
F 16	10^1	14	1.40
F 17	10^1	20	2.00
F 18	10^1	18	1.80

F 19	10^1	5	0.50
F 20	10^1	26	2.60
F 21	10^1	35	3.50
F 22	10^1	20	2.00
F 23	10^1	12	1.20
F 24	10^1	37	3.70
F 25	10^1	13	1.30

F= Female, CFU= colony forming unit, CFU MOL= Colony forming unit per mol

It shows the total counts of bacteria which was collected from 25 female participants. It was then cultured on nutrient agar and MacConkey agar at 37°C after 24 hours with a dilution factor of 10^1.

Table 4.7: Showing Total Occurrence or Prevalence of Bacteria Isolates in Females

Samples	Pseudomonas aeruginosa (%)	Staphylococcus aureus (%)	Escherichia Coli (%)
F 01	+	+	+
F 02	+	−	+
F 03	+	+	+
F 04	−	+	+
F 05	−	+	+
F 06	−	+	+
F 07	+	−	+
F 08	−	+	+
F 09	−	+	+
F 10	−	−	+
F 11	−	−	+
F 12	+	−	+
F 13	−	+	+
F 14	+	+	+
F 15	−	+	+
F 16	+	+	+
F 17	+	+	+
F 18	−	+	+
F 19	−	+	−

F 20	−	+	+
F 21	−	+	+
F 22	+	+	+
F 23	+	+	+
F 24	−	+	+
F 25	+	+	+

+ =Present, − =Absent.

F 01-F25 represents samples of participants that were collected for females

It shows the total occurrence or prevalence of bacteria isolates in females. +ve indicates the presence of the bacteria and −ve indicate the absence of the bacteria. Of the cultured samples, 3 bacteria was isolated of which each participants had +ve in one or two or all (3) and −ve in all the 3 isolates. The most occurring bacteria was *Escherichia Coli.*

Table 4.8: Showing Percentage of Occurrence of Bacteria Isolates in Females

Samples	Pseudomonas aeruginosa (%)	Staphylococcus aureus (%)	Escherichia Coli (%)
F 01	11.11	16.67	72.22
F 02	1.39	0.00	98.61
F 03	3.33	6.67	90.00
F 04	0.00	6.48	93.52
F 05	0.00	14.81	85.19
F 06	0.00	20.00	80.00
F 07	11.11	0.00	88.89
F 08	0.00	30.77	69.23
F 09	0.00	7.692	92.31
F 10	0.00	0.00	100.00
F 11	0.00	0.00	100.00
F 12	12.50	0.00	87.50
F 13	0.00	19.05	80.95
F 14	28.57	42.86	28.57
F 15	0.00	12.50	87.50
F 16	7.14	14.29	78.57
F 17	15.00	10.00	75.00
F 18	0.00	16.67	83.33
F 19	0.00	100.00	0.00
F 20	0.00	19.23	80.77
F 21	0.00	11.43	88.57

F 22	5.00	15.00	85.00
F 23	16.67	25.00	58.33
F 24	0.00	5.41	94.59
F 25	15.39	30.77	53.84

F 01-F25 represents samples of participants that were collected for females

It shows the percentage of occurrence of bacteria isolates in females. *Escherichia Coli* remained the most prevalent of all bacterial isolate. From the bacteria cultured, there was high occurrence of *pseudomonas aeruginosa, staphylococcus aureus* and *Escherichia Coli*.

Table 4.9: Showing Antibiotics Susceptibility Test

Antibiotics	**Potency(ug)**	**Pseudomonas aeruginosa (mm)**	**Staphylococcus aureus (mm)**	**Escherichia Coli (mm)**
Gentamicin (GEN)	10	26(S)	20(S)	25(R)
Cefixime (CXM)	5	0(R)	0(R)	30(S)
Ofloxacin (OFL)	5	30(S)	30(S)	30(S)
Augmentin (AUG)	30	30(S)	0(R)	30(S)
Nitrofurantion (NIT)	300	30(S)	30(S)	30(S)
Ciprofloxacin (CPR)	5	30(S)	30(S)	30(S)
Ceftaziaime (CAZ)	30	0(R)	0(R)	0(R)

Cefuroxime (CRX)	30	30(S)	0(R)	30(S)

Key words: S=Sensitivity, R= Resistance, I= Intermidate

It shows the antibiotic susceptibility test for 25 males and 25 females. The antibiotics test was carried out using Gentamicin (GEN), Cefixime (CXM), Ofloxacin (OFL), Augmentin (AUG), Nitroflurantion (NIT), Ciprofloxacin (CPR), Ceftaziaime (CAZ), Cefuroxime (CPX) on all the isolates (*pseudomonas aeruginosa, staphylococcus aureus* and *Escherichia Coli*) using the Abtek Biological Limited Sensitivity rings.

Morphological Characteristics

Table 4.10: Showing Gram Stain Results for Isolates

Isolates	**Gram Reaction**	**Cell Type**	**Cell Arrangement**
Pseudomonas aeruginosa	−VE (pink colour)	Short rod	Cluster
Staphylococcus aureus	+VE (purple colour)	Coci	Cluster

Escherichia Coli	−VE (pink colour)	rod	Chain

−VE= Negative, +VE= Positive

It shows the morphological characteristics of the isolates. It shows the gram stain results for the isolates which was observed under the microscope. *Pseudomonas aeruginosa* and *Escherichia Coli* were observed to be negative (pink colour), while *Staphylococcus aureus* was positive (purple colour).

Table 4.11: Showing Biochemical Test Results for Isolates

Bacteria	Catalase	Citrate	Indole	Oxidase	Motility	Coagulase
Pseudomonas aeruginosa	+	+	−	+	+	−
Staphylococcus aureus	+	+	−	−	−	+

Escherichia Coli	+	−	+	−	+	−

−VE= Negative, +VE= Positive

This shows the biochemical test results for the isolates. This test was done to further characterize the isolates. These test are catalase, citrate, indole, oxidase, motility, and coagulase test.

Tables 4.12: Showing Cultural Characteristics of Isolates

Bacterial isolates	Shape	Colour	Size(mm)	Elevation	Margin	Opacity
Pseudomonas aeruginosa	Irregular	Green	10	Raised	Rough	Opaque
Staphylococcus aureus	Round	Yellow	5	Raised	Rough	Opaque

Escherichia Coli	Round	Pink	1	Raised	Rough	Opaque

This shows the cultural characteristics of bacteria. The isolates were observed to have irregular shape in *Pseudomonas aeruginosa*, while *Staphylococcus aureus* and *Escherichia Coli* were seen to have a round shape. The colours were green for *Pseudomonas aeruginosa*, yellow for *Staphylococcus aureus* and pink for *Escherichia Coli*.

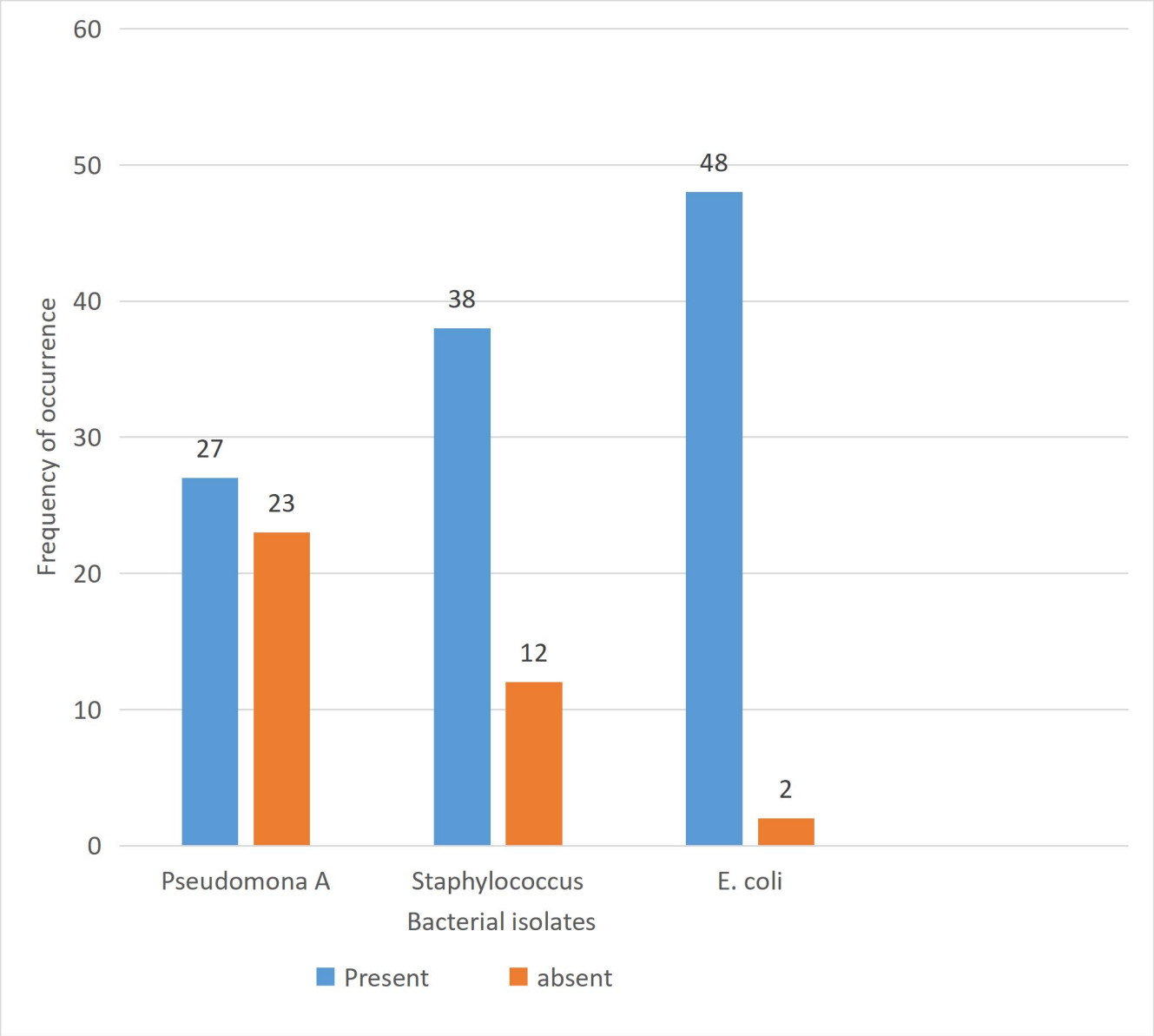

Figure 4.1

This is a bar chart showing the frequency of occurrence of isolated bacteria. The isolated bacteria are *Pseudomonas aeuriginosa*, *Staphylococcus aureus* and *Escherichia Coli*. Blue bar indicates present of the bacterial while orange bar indicates absent of the bacterial.

Table 4.13a: Showing variables and the Chi square values

Variables	History of eye infection	History of contact lens	History of contact lens use	History of contact lens use in past 2weeks	Do you stay in an environment that exposes your eyes to dust, smoke, chemicals or pollutants
CFU	0.572	0.938	0.955	0.806	0.265
Prevalence of Pseudomonas Aeuriginosa	0.824	0.292	0.126	0.287	0.313
Prevalence of Staphylococcus Aureus	0.350	0.282	0.226	0.426	0.819
Prevalence of Escherichia Coli	0.431	0.953	**0.009***	0.921	0.515
Percentage Occurrence of Pseudomonas Aeuriginosa	0.291	0.999	**<0.001***	0.958	0.371
Percentage Occurrence of Staphylococcus Aureus	0.742	0.959	0.654	0.156	0.688
Percentage occurrence of Escherichia Coli	0.388	0.870	0.084	0.606	0.294

*= Significant (p >0.05)

This shows variables and the chi square value for history of infection, history of contact lens, history of contact lens use, history of contact lens use in the past 2 weeks, do you stay in an environment that exposes your eyes to dust or smoke, chemicals or pollutants. On Chi square, specific variables showed significant while the rest were non-significant.

Table 4.13b: Showing variables and the Chi square values

Variables	Medical or allergy history	History of visit to clinic because of allergy	Indicate the name of the eyedrop	History of any eyedrop use
CFU	0.616	0.699	0.669	0.789
Prevalence of Pseudomonas Aeuriginosa	0.354	0.578	0.363	0.713
Prevalence of Staphylococcus Aureus	0.087	0.655	0.173	0.426
Prevalence of Escherichia Coli (E Coli)	0.699	0.808	0.958	0.921
Percentage Occurrence of Pseudomonas Aeuriginosa	0.578	**0.010***	0.999	0.958
Percentage Occurrence of Staphylococcus Aureus	0.655	0.061	**0.054***	0.156
Percentage Occurrence of Escherichia Coli (E Coli)	0.808	**0.023***	0.469	0.606

*= Significant (P >0.05)

It shows variables and the chi square value for medical or allergy history, history of visit to clinic, indicate the name of the eyedrop and history of eyedrop use. On Chi square, specific variables showed significant while the rest were non-significant.

CHAPTER FIVE

5.0 DISCUSSION

Healthy eyes most times are invaded by bacteria. There may be a connection between the local and the normal flora and the development of eye diseases **(**Capriotti, 2009). It has been reported that these normal flora varies in individuals due to difference in genetics, race, nutrition, immunosuppression, age, contact lens wear, surgery and antibiotics therapy (Sthapit and Tuladhar, 2009).

This study helped to identify the bacterial flora in the conjunctival of males and females undergraduate's in university of Benin and their antibiotics susceptibility**.** Table 4.1 is a descriptive statistical table of age showing the range, mean, standard deviation, standard error and variance. Individuals between age 17-33 was used for the study.

Table 4.2 shows age, mean CFU and mean occurrence of bacteria isolates between males and females. It also shows the standard deviation and P. values of the variables. The mean ± standard deviation in both males and female were 3.145±3.051 and 2.660±2.150 respectively but do not have a significant relationship (p=0.190). There was no significant relationship between % occurrences of *pseudomonas aeruginisa*, and *E coli whereas* % occurrences of *staphylococcus* has a P value of 0.056. The mean CFU observed in males was higher than that in female which was also seen in table 4.3 and table 4.6. The result showed a higher CFU in males which was not consistent with a study done by Ogu *et al.,* (2021).

Table 4.3 shows the total counts of bacteria which was collected from 25 male participants. It was then cultured on nutrient agar and MacConkey agar at 37°C after 24 hours with a dilution factor of $10^{1.}$ The mean CFU of bacteria isolates from male undergraduates in university of Benin was (3.1445). The minimum CFU of bacteria isolated was (1.00) while the maximum CFU was

(120). The increase in bacterial load could be due to factors from the environment which affects their life styles.

Table 4.4 shows the total occurrence or prevalence of bacteria isolates in males. M1-M25 represents males, +ve indicates the presence of the bacteria and −ve indicate the absence of the bacteria. Of the cultured samples, 3 bacteria was isolated of which each participants had +ve in one or two or all (3) and −ve in all the 3 isolates. The most occurring bacteria was *Escherichia Coli* which was present in 24 participants, 18 participants had *staphylococcus aureus* present and 15 participants had *pseudomonas aeruginosa* present.

Tables 4.5 shows the percentage of occurrence of bacteria isolates in males. From the bacteria cultured there was high occurrence of *pseudomonas aeruginosa*, *staphylococcus aureus* and *Escherichia Coli*. These 3 bacteria were the most common bacteria found in the cultured samples. The minimum percentage occurrence of *pseudomonas aeruginosa* was (0.00) and the maximum occurrence was (100). *Staphylococcus aureus* percentage of minimum occurrence was (0.00) and maximum value of (38.75), while *Escherichia Coli* was seen to have a percentage minimum occurrence of (0.00) and a maximum of (100).

Table 4.6 shows the total counts of bacteria which was collected from 25 female participants. It was then cultured on nutrient agar and MacConkey agar at 37°C after 24 hours with a dilution factor of 10^1. The mean CFU of bacteria isolates from female undergraduates in university of Benin was (2.660). The minimum CFU of bacteria isolated collected was (5.00) while the maximum CFU was (108.00). The high CFU could be due to external factors like dust, smoke or use of cosmetics.

Table 4.7 shows the total occurrence or prevalence of bacteria isolates in females. F1-F25 represents females, +ve indicates the presence of the bacteria and −ve indicate the absence of the bacteria. Of the cultured samples, 3 bacteria was isolated of which each participants had +ve in one or two or all (3) and −ve in all the 3 isolates. The most occurring bacteria was *Escherichia Coli* which was present in the 24 participants, 20 participants had *staphylococcus aureus* present and 11 participants had *pseudomonas aeruginosa* present. The most prevalent bacteria in this study as seen in tables 4.4 and 4.7 showed the most occurring bacteria was *Escherichia Coli* which do not agree with the work done by Jing *et al.,* (2020) which showed that the prevalent bacterial was *Coagulase* negative *staphylococci*.

Table 4.8 shows the percentage of occurrence of bacteria isolates in females. *Escherichia Coli* remained the most prevalent of all bacterial isolate. From the bacteria cultured, there was high occurrence of *pseudomonas aeruginosa, staphylococcus aureus* and *Escherichia Coli*. These 3 bacteria was the most common bacteria found in the cultured samples. The minimum percentage occurrence of *pseudomonas aeruginosa* was (0.00) and the maximum occurrence was (28.57). *Staphylococcus aureus* percentage of minimum occurrence was (0.00) and maximum occurrence of (100.00), while *Escherichia Coli* was seen to have a percentage minimum occurrence of (0.00) and a maximum of (100). In table 4.4 and 4.8 *Escherichia Coli* was the most frequent isolated bacteria which agree with the work done by Dadashi and Dehghanzadeh, (2016).

Tables 4.9 shows the antibiotic susceptibility test for 25 males and 25 females. The antibiotics test was carried out using Gentamicin (GEN), Cefixime (CXM), Ofloxacin (OFL), Augmentin (AUG), Nitroflurantion (NIT), Ciprofloxacin (CPR), Ceftaziaime (CAZ), Cefuroxime (CPX) on all the isolates (*pseudomonas aeruginosa, staphylococcus aureus* and *Escherichia Coli*) using the Abtek Biological Limited Sensitivity rings (positive and negative rings) using Kirby-Bauer disc

diffusion method. *Pseudomonas aeruginosa* was sensitive(S) to all antibiotics but resistant(R) to Cefixime and Ceftaziaime. *Escherichia Coli* was seen to be resistance to Gentamicin and Ceftaziaime, and sensitive to the rest antibiotics. *Staphylococcus aureus* was sensitive to 4 antibiotics and resistance to 4 antibiotics (Cefixime, Augmentin, Ceftaziaime, and Cefuroxime) which do not agree to a work done by Alshamahi *et al*., (2020) which showed that *staphylococcus aureus* has a high risk of resistance to antibiotics.

Table 4.10 shows the morphological characteristics of the isolates. It shows the gram stain results for the isolates which was observed under the microscope. *Pseudomonas aeruginosa* and *Escherichia Coli* were observed to be negative (pink colour), while *Staphylococcus aureus* was positive (purple colour). It also showed that *Pseudomonas aeruginosa* was short rod, Staphylococcus aureus was coci and *Escherichia Coli* was rod. *Pseudomonas aeruginosa* and *Staphylococcus aureus* showed a cluster cell arrangement while *Escherichia Coli* showed chain cell arrangement.

Table 4.11 shows the biochemical test results for the isolates. This test was done to further characterize the isolates. These test are catalase, citrate, indole, oxidase, motility, and coagulase test. *Pseudomonas aeruginosa* was positive to catalase, citrate, oxidase, motility and negative to indole and coagulase. *Staphylococcus aureus* was positive to catalase, citrate, motility, coagulase test and negative to indole, oxidase and motility. *Escherichia Coli* was positive to catalase, indole, motility and negative to citrate, oxidase and coagulase.

Table 4.12 shows the cultural characteristics of bacteria. The isolates were observed to have irregular shape in *Pseudomonas aeruginosa*, while *Staphylococcus aureus* and *Escherichia Coli* were seen to have a round shape. The colours were green for *Pseudomonas aeruginosa*, yellow

for *Staphylococcus aureus* and pink for *Escherichia Coli*. *Staphylococcus aureus* size was observed to be 10mm, *Staphylococcus aureus* was 5mm and *Escherichia Coli* was 1mm. The three bacteria isolated were observed to be raised, rough and opaque.

Figure 4.1 is a bar chart showing the frequency of occurrence of isolated bacteria. The isolated bacteria are *Pseudomonas aeuriginosa*, *Staphylococcus aureus* and *Escherichia Coli*. The blue bar represent that the bacteria is present while the orange bar represent that the bacteria is absent. The most occurring bacterial was *Escherichia Coli* which had a frequency of occurrence of 48, the next was *Staphylococcus aureus* which had a frequency of occurrence as 38 and the least bacteria isolated was *Pseudomonas aeurihinosa* which had a frequency of occurrence of 27**.**

Table 4.13a shows variables and the chi square value for history of infection, history of contact lens, history of contact lens use, history of contact lens use in the past 2 weeks, do you stay in an environment that exposes your eyes to dust or smoke, chemicals or pollutants. On Chi square, specific variables showed significant while the rest were non-significant. There was a significant relationship between history of contact lens use with prevalence of *Escherichia Coli* (p=0.009), also a significant relationship was seen between history of contact lens use with percentage of occurrence of pseudomonas aeuriginosa (p=<0.001).

Table 4.13b shows variables and the chi square value for medical or allergy history**,** history of visit to clinic, indicate the name of the eyedrop and history of eyedrop use. On Chi square, specific variables showed significant while the rest were non-significant. There was a significant relationship between history of visit to clinic because of allergy and percentage occurrence of *pseudomonas aeuriginosa* (p=0.010), also a significant relationship was seen between indicate the name of the eyedrop with percentage of occurrence of *staphylococcus aureus* (p=0.054) and

history of visit to clinic with percentage of occurrence of *Escherichia Coli*. There was significant relationship in percentage occurrence of *staphylococcus aureus* which agree with a work done by Vola *et al.,* (2013) which had p=0.001.

CHAPTER SIX

6.1 CONCLUSION

The result shows presence of *Pseudomonas aeuriginosa*, *Staphylococcus aureus* and *E coli* to be present in participants used for the study. The mean CFU for male and female was 3.145 and 2.660 respectively also the colony unit count for males were higher than in females. There was no significant difference in the CFU of males and females. It also shows that *Escherichia Coli* was the most prevalent of all isolated bacteria while *pseudomonas aeuriginisa* was the less prevalent. *Pseudomonas aeuriginisa* and *Staphylococcus aureus* show high resistance to Ceftaziame and cefixime. There is high degree of sensitivity of bacterial to Ofloxacin, Gentamicin, Augmentin, Nitrofurantion , Ciprofloxacin and Cefuroxime. This study showed that lifestyles factors such as contact lens use and eye drop use can affect the normal flora of a person.

This study also provides answers to bacterial causing ocular infections also will help broaden practitioners knowledge of bacteria associated with ocular infections, the prophylactic management of ocular bacterial infections and determine antimicrobial susceptibility.

6.2 RECOMMENDATION

1. Further study should be carried out in Nigeria to find out the best antibiotics for patients who are immunocompromised.
2. Government and media should advise the public on the importance of good hygiene and the importance of maintaining a clean environment.
3. Eye care practitioner should enlighten patients on the importance of early visit to clinic as a result of eye infections or trauma.

4. Institutions should enforce strict sanitation rules and regulations in hostels and learning environment.

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APPENDIX 1

CONSENT FORM AND QUESTIONAIRE ON ASSESSMENT OF OCULAR BACTERIAL FLORA AMONG UNIVERSITY OF BENIN UNDERGRADUATES.

Dear Sir/Ma

I am a final year student of the Department of Optometry, University of Benin. I am doing a project work on "Assessment of ocular bacterial flora among University of Benin undergraduates". The procedure is safe, data collected will be treated with utmost confidentiality and every protocol regarding health and safety will be maintained.

I confirm that I have read and understand the subject information of the study and I agree to participate.

Participant signature:

Participant code:

Please kindly provide answers to the following questions. Thank you.

Kindly tick the appropriate boxes in the following:

SECTION A: BIO DATA

• 1 Sex: Male [ ] Female [ ]

• 2 Age (in years):

• 3 Marital status: Single [ ] Married [ ]

SECTION B: General Questions

Please tick ( ) the appropriate answer to questions below

1. Have you experienced an eye infections or inflammation in the past? Yes ( ) No ( )

2. Do you wear contact lens? Everytime ( ) Rarely ( ) Sometimes ( ) Never ( )

3. If yes, how often do you replace them? Everytime ( ) Rarely( ) Sometimes( ) Never( )

4. Have you worn contact lens in the past 2 weeks? Yes ( ) No ( )

5. Do you stay in an environment that exposes your eyes to dust, smoke, chemical or other pollutant? Everytime ( ) Rarely ( ) Sometimes ( ) Never ( )

6. Do you have any medical allergy or condition that affects your eyes? Yes ( ) No ( )

7. If Yes, specify the allergy

Itching ( ), Redness ( ), Dryness ( ), Discharge ( ), Burning sensation ( ) Grittiness ( )

8. Have you visited an eye clinic because of allergy? Yes ( ) No ( )

9. Are you currently on any eye drop? Yes ( ) No ( )

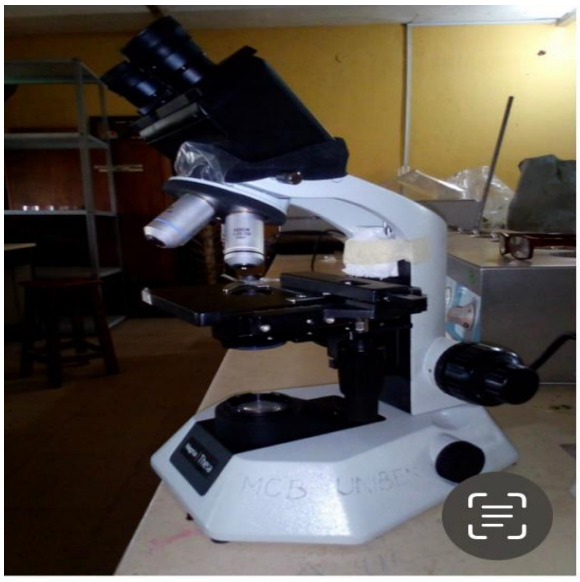
10. If yes to no. 8 indicate the name of the eye drop

APPENDIX 2

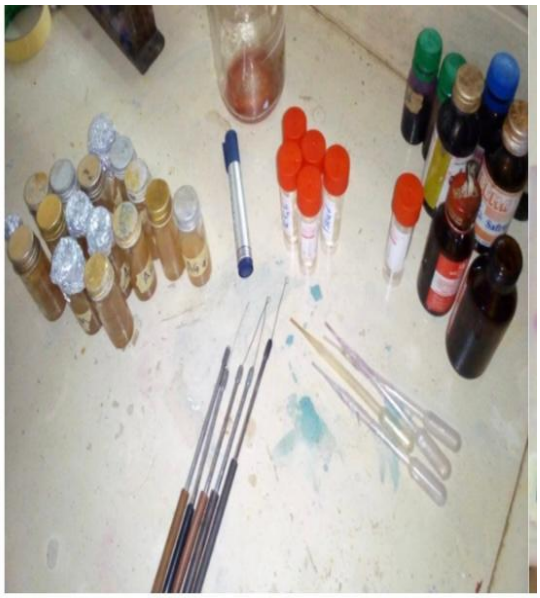
Collection of samples

Instillation of antibiotics eyedrop

Labeling of samples



Microscope



Bacterial for gram staining procedure

Antimicrobial sensitivity disc (Abtek biological limited)

APPENDIX 3

SPSS RAW DATA

Descriptives

CFU/ml

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Male	25	3.1448	3.05191	.61038	1.8850	4.4046	.10	12.00
Female	25	2.6600	2.14942	.42988	1.7728	3.5472	.50	10.80
Total	50	2.9024	2.62390	.37108	2.1567	3.6481	.10	12.00

ANOVA

CFU/ml

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.938	1	2.938	.422	.519
Within Groups	334.420	48	6.967		
Total	337.358	49			

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
% of pseudomonas_aeruginosa	Male	25	9.0000	19.94026	3.98805	.7691	17.2309	.00	100.00
	Female	25	5.0883	7.63963	1.52793	1.9348	8.2418	.00	28.57
	Total	50	7.0441	15.07447	2.13185	2.7600	11.3283	.00	100.00
% of staphylococcus aureus	Male	25	8.1896	9.38463	1.87693	4.3158	12.0634	.00	36.36
	Female	25	17.0119	20.43195	4.08639	8.5780	25.4458	.00	100.00
	Total	50	12.6007	16.35435	2.31285	7.9529	17.2486	.00	100.00
% of E coli	Male	25	82.8108	20.60572	4.12114	74.3052	91.3164	.00	100.00
	Female	25	77.8996	22.72139	4.54428	68.5207	87.2785	.00	100.00
	Total	50	80.3552	21.60975	3.05608	74.2138	86.4966	.00	100.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
% of pseudomonas_aeruginosa	Between Groups	191.269	1	191.269	.839	.364
	Within Groups	10943.476	48	227.989		
	Total	11134.745	49			
% of staphylococcus aureus	Between Groups	972.912	1	972.912	3.849	.056
	Within Groups	12132.859	48	252.768		
	Total	13105.772	49			
% of E coli	Between Groups	301.499	1	301.499	.641	.427
	Within Groups	22580.575	48	470.429		
	Total	22882.074	49			

Prevalence of pseudomonas aeruginosa

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	-	23	46.0	46.0	46.0
	+	27	54.0	54.0	100.0
	Total	50	100.0	100.0	

Prevalence of staphylococcus aureus

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	-	12	24.0	24.0	24.0
	+	38	76.0	76.0	100.0
	Total	50	100.0	100.0	

Prevalence of E coli

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	-	2	4.0	4.0	4.0
	+	48	96.0	96.0	100.0
	Total	50	100.0	100.0	

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	11.529[a]	3	.009
Likelihood Ratio	4.476	3	.214
Linear-by-Linear Association	3.630	1	.057
N of Valid Cases	50		

a. 7 cells (87.5%) have expected count less than 5. The minimum expected count is .04.

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	24.598[a]	6	.000
Likelihood Ratio	7.249	6	.298
Linear-by-Linear Association	7.604	1	.006
N of Valid Cases	50		

a. 11 cells (91.7%) have expected count less than 5. The minimum expected count is .02.

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	9.259[a]	2	.010
Likelihood Ratio	4.972	2	.083
Linear-by-Linear Association	.405	1	.525
N of Valid Cases	50		

a. 5 cells (83.3%) have expected count less than 5. The minimum expected count is .10.

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	9.285[a]	4	.054
Likelihood Ratio	5.018	4	.286
N of Valid Cases	50		

a. 8 cells (88.9%) have expected count less than 5. The minimum expected count is .02.

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	9.509[a]	3	.023
Likelihood Ratio	5.327	3	.149
Linear-by-Linear Association	.858	1	.354
N of Valid Cases	50		

a. 6 cells (75.0%) have expected count less than 5. The minimum expected count is .10.

Crosstab

Count

		Prevalence of pseudomonas aeruginosa		
		-	+	Total
Indicate the name of the eyedrop	None	22	26	48
	Unknown	0	1	1
	Zalatan	1	0	1
Total		23	27	50