Enhancing the management of blepharitis.

Wisdom Chukwudi Iwumune

A thesis submitted in partial fulfilment of the requirement of Liverpool John Moores University for the degree of Master of Philosophy.

February 2021.

ACKNOWLEDGEMENT

I wish to thank all who participated in this project right from Nigeria to here at LJMU especially Dr Ismini and Dr Zouganelis. I also use this opportunity to express my profound gratitude to my family for sponsoring my education till date.

I dedicate this work to the loving memory of my beloved late mother, Mrs Theresa Iwumune.

Wisdom

List of figures.

List of Tables.

Contents:

Page No.

ABSTRACT

Blepharitis is a chronic eye infection affecting the eyelid commonly result from exacerbation of normal microbial flora on the skin and occasionally due to meibomian gland dysfunction.

Aim: Identifying organisms responsible for blepharitis with a view to enhancing its management.
Method: Investigations of these patients were carried out with the use of slit lamp biomicroscopy after recording their habitual visual acuity. Patients were initially screened out at the GP department before eye examination. Samples were collected with swaps and pure strains were isolated. Samples' DNA were extracted and amplified through polymerase chain reaction before sequencing.
Result: Various strain of Staphylococcal organisms and *Pseudomonas aeruginosa* were the common organisms identified by 16S rRNA sequencing. Susceptibility tests were performed with most of the organisms found to be insensitive to the antibiotics tested.
Conclusion: Blepharitis is known as one of the anterior ocular infection often encountered in clinical practice. It has been noted that clinical presentation does not provide enough diagnostic evidence in management thereby prompting the need for further microbial analysis. Proper identification of causative organisms with the help of more sophisticated and enhanced technique of molecular identification is believed to proffer better outcome in clinical practice.

Chapter 1. INTRODUCTION.

1.1.0. Definition.

Blepharitis is an inflammatory condition of the eyelid and the surrounding structures characterized by: swollen lids, irritation, itching around the lid, and presence of crust, flakes or scars around the eyelid border (Putnam, 2016). There are also signs of thickened meibomian glands, ulceration, trichiasis, madarosis and hypertrophy along the lid margins especially in chronic blepharitis (Freitas et al, 2010). These common signs of infection of the skin around the eye and consequent involvement of other structures have contributed in most cases to misdiagnoses of blepharitis, underreporting of cases and divisions in the treatment of blepharitis infection (Putnam, 2016). In a bid to solve this problem, a method of culture-independent analysis relied on the 16S RNA gene sequences was developed and used to investigate the microbial community in human body to enhance the management of ocular infections like blepharitis (Costello et al., 2009 and Fierer et al., 2008).

1.1.1. Aetiology

Blepharitis was first described in 1946 by Phillip Thygeson, as the chronic inflammation of the lid border and was later characterized into two types, namely; squamous and ulcerative blepharitis (Phillip Thygeson, 1946). Further classification was into acute and chronic, with chronic as the most commonly diagnosed (Eberhardt and Rammohan, 2017).

Clinically, the disease condition can be differentiated into anterior blepharitis affecting the anterior lamella of the eyelid including the eyelashes and the posterior blepharitis primarily causing meibomian gland dysfunction (Craig et al., 2017). It can be bacterial mostly staphylococcal resulting from exotoxin reactions in staphylococcal infection or in situations of responses to staphylococcal antigen. Blepharitis can also say to be seborrheic which result from the ciliary portion of sebaceous glands of Zeis. The inflammatory processes are often associated with the anterior and posterior eyelid lamellae and the periocular skin (Duncan et al., 2015). The lypolic enzyme also found on the lid may trigger inflammatory responses and disrupt the tear film homoeostasis by degrading the lipid layer constituents in posterior blepharitis {Dougherty, 1991). Although the pathophysiology of blepharitis is multifactorial and is yet to be established

(Pflugfelder et al., 2014). This was collaborated in a study by Bruce Jackson (Bruce Jackson, 2016) whose investigation also revealed the complexity in tracing the pathogenesis of blepharitis from the multiplicity of factors involved in the disease process; such as the microbial infection leading to lid abnormalities. However, ocular surface inflammation and infection has been attributed to overcolonisation of the periorbital region (Pflugfelder et al., 2014), by bacterial such as *Staphylococcus aureus, Staphylococcus epidermidis and Pseudomonas aeruginosa (*Lee et al., 2012, Watters et al., 2017). Also, there are noticeable association of *Demodex* infestation and blepharitis (Kabataş, 2017). Notably of recent is the increasing case of keratitis and endophthalmitis resulting from coagulase-negative Staphylococcal infection (Chirinos-Saldana, 2013).

Ocular infection results from the inversion of living tissue by microorganisms, which live within the body tissues for survival and multiplication thereby compromising the integrity of the host system leading to a diseased condition in the affected area or organ (Tobinick, 2003). Pathogenic agents found in human including but not limited to bacteria, virus, protozoa, fungi and numerous parasitic agents that can get access into human system. Most active infection may not present with noticeable symptoms or subclinical signs while inactive or dormant infections are described as latent infection (Tuller et al., 2005). Some eye infections are said to be acute when microbes present within short period of time during which tissues are infected or chronic in an infection that has presented for long period of time. There are few trends involved in infection process. Each of these processes represents stages during the development of the infection which follow a sequential order in disease development (Tobinick, 2003). The causative organisms required a reservoir where they are domiciled to thrive and replicate its number while releasing toxins in the host tissues. Causative agents must be adequate and virulence enough to be able to effect damages in the host system. Some part of the human eyes that serves as a reservoir includes the eyelid margin, cornea and the conjunctiva. The invasion of microorganisms into a host system required a medium through which it can get access into the host system. However, for infection to occur, the host system must be inhabitable for the pathogenic agent to cause disease. Host tissue respond to invading agents through the formation of antibodies and subsequently inflammation resulting from the activities exacted by the pathogens especially in situation of low

immunity. Although there are occasions where host immune system overreacts, causing damages to the host system by a process called autoimmune reaction (Tobinick, 2003).

Bacterial insensitivity or resistance to antibiotics is of high increase, which raises alarm on the future of antibacterial agents as likely to be short of need in the near future (Wencewicz et al., 2013). Extended use of antibiotics therapy has been identified to be an enabling factor towards the adaptation of microorganisms and development of certain cross-resistance characteristics although more studies is required unravel the trend in ocular infections (Chirinos-Saldana et al., 2013). Microbiome accounts for 1-3% of the human body mass. Bacterial species are the most common organisms found on the skin. They occasionally attack the host especially when exacerbated by poor hygiene or in immune-compromised individuals. Blepharitis is one of the eye related diseases associated with the breakdown in skin bacterial flora. Although the pathophysiology remains unknown however, it's most commonly caused by *Staphylococcus* species and *P. aeruginosa* and their ability to form biofilms contributes to their increased virulence (Lynch and Robertson, 2008, Lee et al., 2012). Although topical antibiotics being used in the management of blepharitis has recorded success in reducing microbial colonization, palliative treatment such as the recommendation for eye hygiene and warm compress are still recommended in most cases (Geerling et al., 2011). Furthermore, prolonged use of corticosteroid is contraindicated not just for its non-antibiotic ability but for the risk it poses to the eye (Pflugfelder et al., 2014).

Inhibitory properties of natural product such as honey on ocular microbiota and inflammatory effects was said to have occurred due to its low pH value, high osmolarity, hydrogen peroxide components such as methylglyoxal (Albietz and Lenton, 2006). In a recent study on New Zealand Manuka honey particularly *Leptospermum scoparium,* demonstrate a potential novel therapy in managing blepharitis. It achieves this with the methylglyoxal which is largely found in the sample with more affinity in resisting physiological breakdown by heat and enzymes than peroxides components in antimicrobials (Snow and Manley-Harris, 2004).

As the possibility of developing the condition increases with age prompting the growing number of cases seen in recent times, clinicians are forecasting increased number of cases in the coming years considering the high number of aging populations. Although the treatment of blepharitis

starts when symptoms are present, asymptomatic blepharitis may also need to be addressed before procedures are carried out in most patients. Lid hygiene has traditionally been referred as the first line of treatment however, limited success has been achieved with this measure alone (Lindstrom, 2009).

1.1.2. Epidemiology

Blepharitis is among the commonest misdiagnosed ocular infection in optometry practice globally (McCulley and Shine, 2000). However, anterior blepharitis is said to be more prevalence in fair-skinned young female aged between 30-50 years with rosacea (Putnam 2016). Other ocular conditions associated with blepharitis infection include Dry Eye Diseases (DED), dematitis, Serborrheic dermatitis and atopy (Putnam, 2016). According to Nelson et al., 2001, Meibomian Gland Disease (MGD) is the most associated ocular condition accounting to about 30% of cases that coexist in blepharitis infection. He found that MGD is common in aging population affecting males above 65 years and female between the ages of 45-65 years. However, a study by Nicolas et al., (2011) comprising 90 patients diagnosed with chronic blepharitis found that the mean age of patient with blepharitis to be within 50 years. This also collaborate the finding by Bienat et al., (2018) in the prevalence of blepharitis been higher in older individuals although he found no significant difference between male and female.

1.1.3. Classification of blepharitis by progression.

There are series of classification of blepharitis however, there is yet to be a single accepted model (Jackson, 2008). However, the existing classifications are primary or secondary blepharitis, anterior or posterior blepharitis.

Primary blepharitis results from staphylococcal toxins. This predominantly affects those infected with rosacea, hypersensitivity and seborrhea. Secondary blepharitis refers to bacterial infection processes or infestation by phthiriasis or *Demodex*. The primary and secondary causes exist with substantial overlap of signs and symptoms. According to Biernat et al., (2018), *Demodex* species are the most common mites living on human skin. There are two known species of *Demodex* identified in human namely, *Demondex folliculorum* and *Demodex brevis* which is often found in

the sebaceous glands and Meibomian glands while the former lives in the follicles. They are commonly situated on the face, forehead, eyelashes, and eyebrows and within the nose region (Cheng et al., 2015). Mite infection affects large human population especially those in aged above 70 years and could also be as high as 90% with its presence increasing with age (Biernat et al., 2018). Demodex infection is rarely found in children. This is due to low secretion of sebaceous glands in children which doesn't favor the development of *Demodex.* However, *Demodex* infection was first reported in children suffering from leukemia and in any form of immunodeficiency (Herron et al., 2005). Children without immunological diseases should also be considered in cases such as recurrent blepharitis and conjunctivitis where allergy is being considered in the etiological development and poor response to conventional treatment (Liang et al., 2010). There's increasing number of people expressing *Demodex* symptoms although the pathogenesis of the infection remains unclear in the light of numerous researches been carried out. Blepharitis is believed to be one of the infections caused by *Demodex* species which often have a recurrent course requiring long term treatment though often unsatisfactory (Biernat et al., 2018). Furthermore, primary blepharitis presents with complex etiology in the course of the disease while secondary blepharitis results from coexistence with other diseases. *Demodex* does carry other microorganisms whose antigen is capable of stimulating the immune towards synthesizing some proinflammatory cytokines; and some metabolites than can initiate allergic reactions with the hair follicles (Nicholls et al., 2017). *Demodex folliculorum* infection causes mechanical irritation of the epithelium of the hair follicles resulting to hyperplasia and hyperkeratization while *Demodex brevis* blocks Meibomian glands. In all these, no reliable hypothesis has been confirmed thus making the etiology of *demodex* infection a controversial factor in eye diseases (Biernat et al., 2018).

A. Acute and chronic

Further classification of blepharitis was done by Lindsley et al., (2012). They opined that the classification was relied on the duration of the diseased process. While they discovered that acute blepharitis could be of bacterial, viral or parasitic it's etiology and is commonly termed lid infection, acute ulcerative blepharitis are due to infections secondary to Staphyloccocal infection.

However, there has been evidence of polymicrobial infection in both acute and chronic blepharitis (Din and Patel, 2012). Most hospital-acquired infections, commonly recognized bacterial species in blepharitis, were found to be *S. epidermidis* followed by *S. aureus, Propionibacterium* etc (Din & Patel, 2012). Changes to the external eyelid due to inflammatory conditions such as blepharitis or meibomian gland obstruction is detected through the evertion of the lid and view through a magnifying lens as shown in the figure 1. In the image captured with keratograph 4 which was originally designed to produce information for corneal topography and contact lens fitting. (a) meibography of the right upper lid. There is no loss of meibomian glands. (b) The meibography of the left upper lid. About 50% of the meibomian glands are absent showing gland drop out in the location of resolving chalazion (nasal side). (c and d) Meibography images taken of the active chalazia in the right eye and left eye respectively. Chalazia in both eyelids appear as lumps on the palpebral conjunctiva of the eyelids with the chalazion on the right eye appearing larger than the chalazion on the left eye. There is disappearance of the meibomian glands in the area of the chalazion for both eyelids. Image e and f demonstrate the right and left upper lids post-surgery respectively. Up to 30% of the meibomian glands are absent in the area of the removed chalazion in the right upper lid. There appears to be no further loss of glands in the left upper lid following surgical removal of the chalazion.

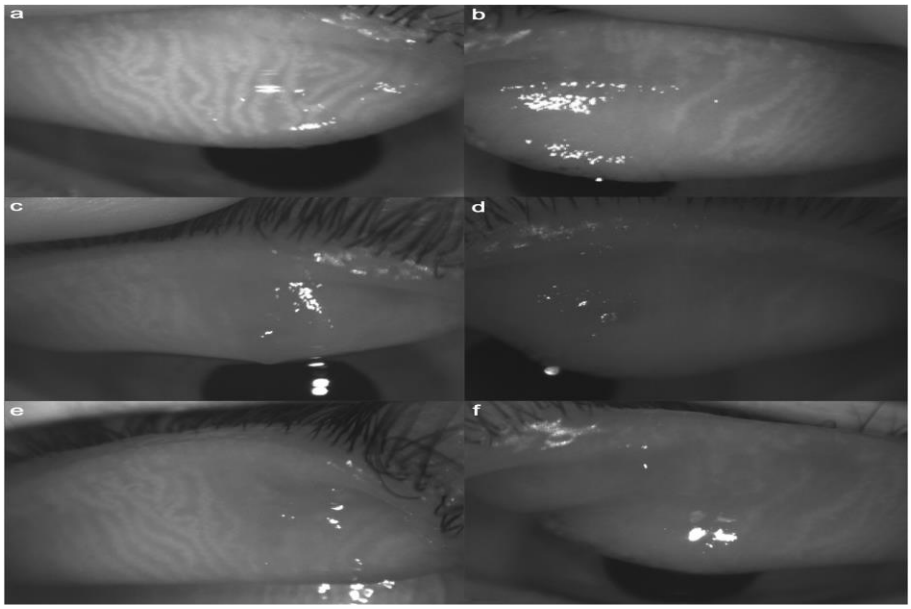


Fig. 1. Everted image of the eyelid in active meibomian gland infection: (a–f) The upper eyelids were everted and images of the meibomian glands were captured using Keratograph 4. Reproduced by Srinivasan et al., 2013.

1.1.4. Classification of blepharitis by location.

A. Anterior blepharitis.

Anterior blepharitis is known to be a frequently encountered eye infection encountered in Ophthalmology practice. Staphylococcal, *Demodex* and seborheic blepharitis constituted about 37-42% of incidence recorded in previous studies (De Paula et al., 2019). Anterior blepharitis primarily affects the bases of the eyelashes. The common causes include staphylococcal and seborheic blepharitis (Lindsley, 2012). The glands primarily affected in seborrheic blepharitis are the gland of Zeiss and the meibomian glands; in mild condition, the pilosebaceous glands situated within the lid margin are involved as show n in figure 2. However, meibomian gland dysfunction also manifest in seborrheic blepharitis due to the nearness between the meibomian and the sebaceous gland (Raskin, 1992). According to Donnenfeld et al., 2008, several conditions such as compromised immune system, age, changes in hormone, rosacea, allergy and dermatitis could trigger blepharitis. The link between the pathogenicity of blepharitis involving mites and bacteria is shown in the figure 2.

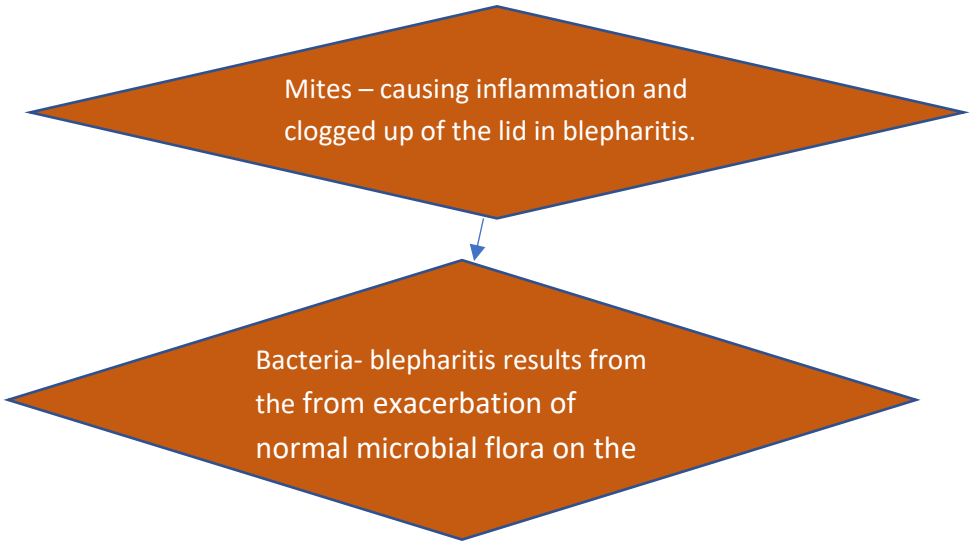


Fig. 2. Shows the link between mites and bacteria in the etiology of blepharitis.

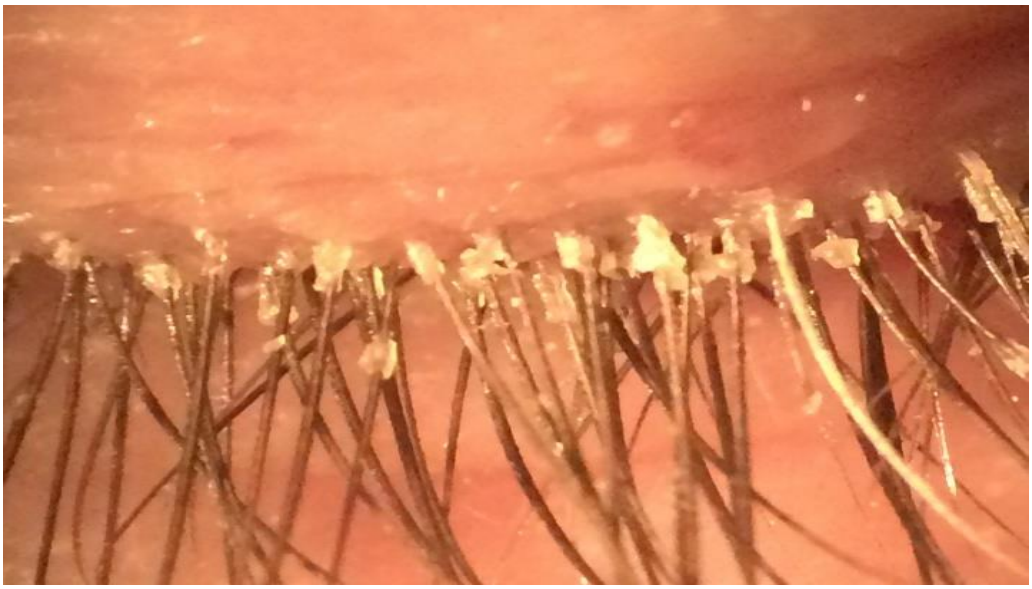


Fig. 3: Image of the upper lid margin showing crust on the base of the lashes in anterior blepharitis. Reproduced from Moyes, 2018.

B. Posterior blepharitis.

Posterior blepharitis is primarily caused by a dysfunctional meibomian gland arising from a metabolic disorder, allergic or infective conjunctivitis, some systemic conditions such as Rosacea, atopy and eczema (Nelson, 2011). While previous studies identify marginal blepharitis as involving both anterior and posterior blepharitis, angular blepharitis involves the canthal region of the lid although it may not affect the anterior or posterior part of the lid as shown in figure 3.

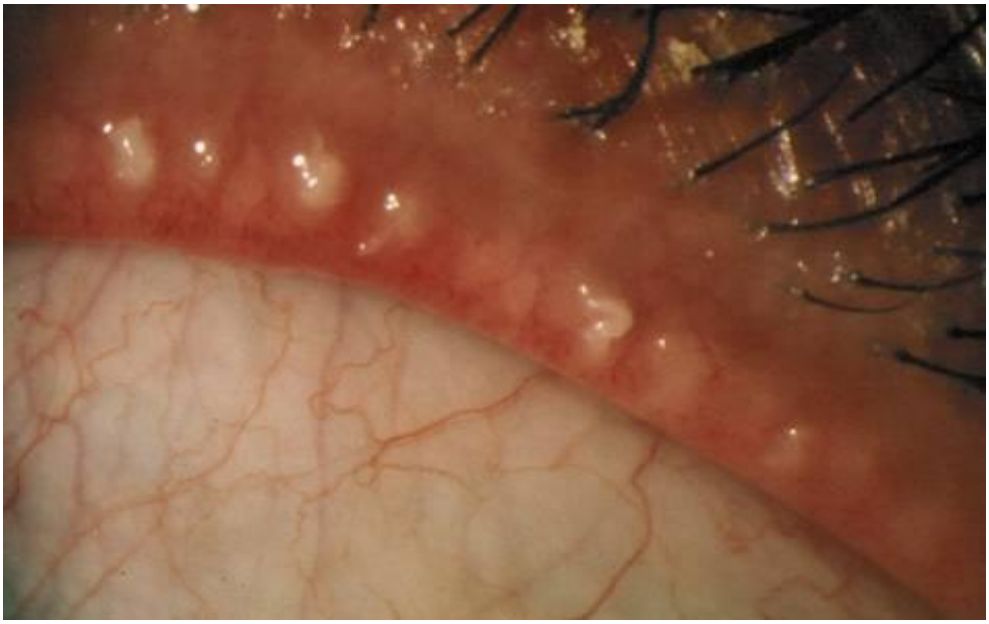


Fig: 4. An image of posterior blepharitis showing blocked meibomian gland and crust eye lashes. Reproduced from Moyes, 2018.

It mostly affects the eyelash margin and the meibomian gland which secrets the oily component posterior to the lid as shown in figure 4. This eventually cause the blockage of the glands leading to the retention of the sebum and increasing the risk of other complications of meibomian gland diseases such as meibomian cyst, chalazion, Internal hordeolum and secondary infections (Fredrick, 2018, Putnam, 2016).

The major two categories of meibomian gland diseases are the low delivery states and the high delivery states. The low delivery states comprise of the obstructive with cicatrical and non-cicatrical subdivisions (figure 4). Hyposecretory glands are glands secreting lesser meibum than it ought to due to disorder the meibomian gland in absence of a significant obstruction. In cicatrical MGD, the duct orifices are displaced posteriorly into the mucosa of the lid while this is not affected in non cicatrical form. Figure 5 also illustrates different stages of meibomian gland diseases and causes leading to other ocular surface diseases such as dry eye. In high delivery hypersensitivity MGD, copious amount of lipids are released along the lid margin that is more prominent when pressure is exerted on the tarsal conjunctiva during an eye examination (Nelson et al., 2011).

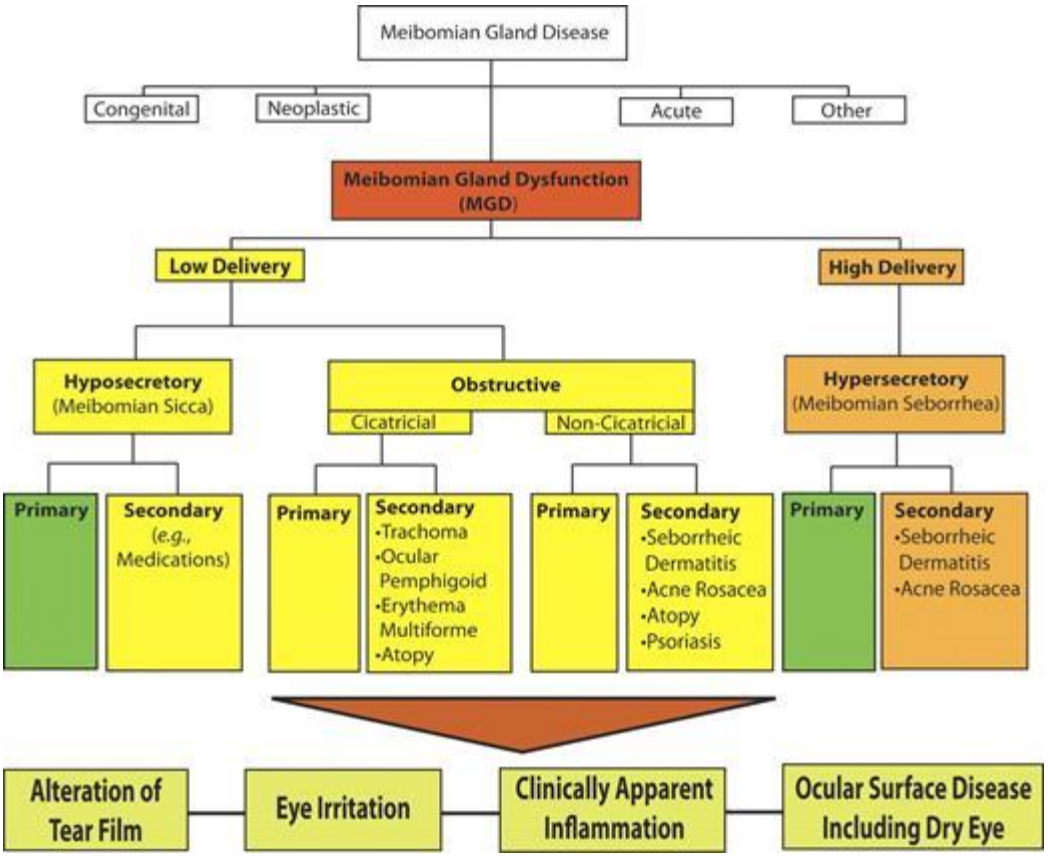


Fig. **5** Image showing subdivisions of MGD. Reproduced from **Nichols et al., 2011.**

1.2.0. Infection pathways.

While Scheinfeld and Berk, 2010 established that the chronic blepharitis often results to loss of eyelashes (madarosis) poliosis, corneal scarring and eyelid hypertrophy, staphylococcal species are commonly associated with infectious blepharitis and recurrent hordela (Probst, 2005). Bacteria cell growth occurs through duplication leading to production of offspring of equal size. However, cell multiplication involves series of complex steps which includes mass doubling of cells, initiating and terminating of a circle of chromosomal replication, disenabling and separating sister formed chromosomes also known as nucleoids, assembling of all the facilitating mechanism and a coordinated access into the host cell leading to synthesizing of the cell wall and eventual development of a full septum (Chien et al 2012).

The pathophysiology of chronic blepharitis occurring in three sequential orders was described by Din and Patel (2012). The first stage involve infection by bacterial, secondly is the exotoxin

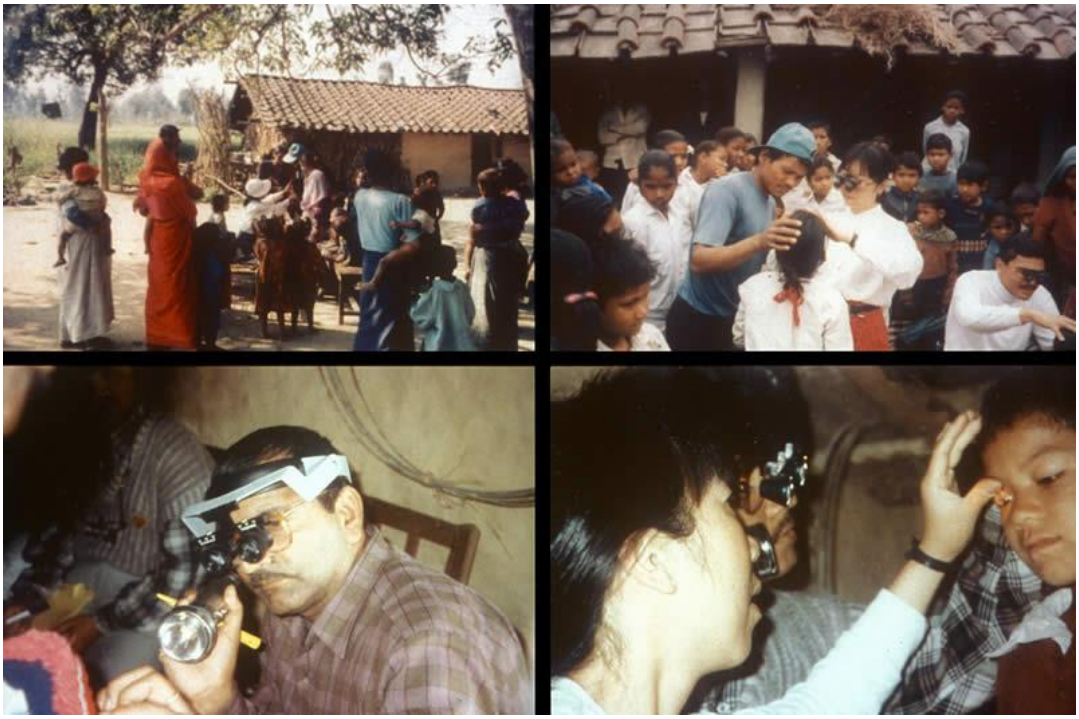
hypersensitivity and lastly, a mediated immune hypersensitivity response. According to Kim et al., (2011), blepharitis results to inflammation of the eyelid margin and ocular surface through increased cytokines level such as IL-17 which is responsible for an autoimmune response and telangiectasias within the lid margin. In bacterial infection, pro-inflammatory cytokines are released by the interaction of bacterial antigens and the elevated exotoxins essential to an inflammatory response (Din and Patel, 2012). Cytokines found in the tears plays major role in the series of pathological conditions involving the anterior ocular surface. There is obvious improvement in recent studies towards understanding the roles of cytokines on the ocular surface. Recent studies found that cytokines IL-1β, IL-7, IL-7, IL-12, IL-13, IL-17, and MIP-1β were found to be raised in blepharitis with IL-7, IL-12, and IL-17 significantly found in cases involving *demodex* blepharitis. Table 1 shows a summary of some of the cytokines found in blepharitis and their role.

Cytokines	function
IL-7	Mediates inflammatory response.
IL-12	Mediates inflammatory response.
IL-13	Mediates inflammatory response.
IL-17	Autoimmune inflammatory response.
IL-1β	Pro inflammatory response.
MIP-1β	Pro inflammatory response.

Table 1 Shows the list of cytokines associated in blepharitis and their function.

Comparing the effect of cytokines in cases of *demodex* caused blepharitis and blepharitis of different pathogenic origin on the tear break-up time (BUT) on ocular surface; it was found that the tear break up time decreased more than *demodex*-free blepharitis. This may be attributed to

the level of cytokines on the ocular surface, which destabilizes the ocular surface (Kim et al., 2011). The high level of IL-7 and IL-12 is thought to be as a result of inflamed pre-existing blepharitis in plugged meibomian glands. Allergic conjunctivitis with diffuse conjunctival erosion, inflammation and hyperemia is believed to be mediated by IL-17 (Maizels et al., 2009). Other autoimmune related conditions where IL-17 is found to be elevated include allergic contact dermatitis, allergic rhinitis, psoriasis, asthma and rheumatoid arthritis (Oboki et al., 2008). Potent angiogenic chemokine is said to play crucial role in the formation of new vessels on the conjunctiva causing hyperemia and telangiectasias on the eyelid in chronic blepharitis infection (Kim et al, 2011). According to the findings by Venturino et al., 2003) in identifying the prevalence of chronic blepharitis in a population of 1148 patients diagnosed with general eye diseases presenting with symptoms of discomfort and irritable eyes as can be detected in eye examinations as shown in figure 6. Anterior blepharitis constituted 12% of the symptoms. It was observed that anterior blepharitis was more common amongst the younger population with mean age of 4% among male and 80% of the female patients. The glans of Zeiss and meibomian glands are mildly affected in seborrheic blepharitis involving the pilosebaceous glands within the lid margin. Meibomian gland dysfunction as a characteristic of a sebaceous blepharirtis is due to the closeness between the meibomian gland and epidermal sebaceous glands (Raskin, 1992).



Fig; 6. Examination of ocular infection in rural community. Reproduced from Albert et al 2007.

1.2.1 Clinical features of pseudomonas aeruginosa.

According to Teweldemedin et al., 2017, *P. aeruginosa* is known to be one of the Gram-negative organisms responsible for anterior eye infection on patients diagnosed with blepharitis and infectious keratitis (corneal infection). *P aeruginosa* easily gets access into the ocular system through an injured surface (Hazeltt et al., 2004). Loss of cilia, corneal neovascularization and ulceration are also known as some of the complications of blepharitis infection. If not treated, it could delay visual recovery after surgery and can also cause more serious ocular infection such as endophthalmitis in deep surgical procedures such as penetrative keratoplasty (Holland, 2014). *P. aeruginosa* survives even in limited nutrients and different environmental conditions including hospital environments (Hazlett, 2004). Ulceration of the cornea progresses with the help of the enzymes released by the bacteria into various layers of the corneal including the epithelial, stromal and the inflammatory cells of the cornea. Also, pathogenicity response of the cornea to bacterial invasion occurs through proliferative response to released exoenzymes in corneal diseases (Hazlett et al., 2004), while the ensuing tissue destruction is majorly caused by the

release of exoproducts from the raised leukocytes and residing corneal cells (Lausch et al., 1996). However, non-ulcerative blepharitis is triggered by allergic reactions such as rosacea, seasonal or atopic dermatitis (Eberhardt and Rammohan, 2017). Ring infiltrates commonly lodges around the paracentral sides of the cornea and hypopyon, which is made of a dense coagulated inflammatory material within the anterior chamber of the eye. Other consequence of corneal perforation is the loss of corneal outer layer otherwise known as descemetocele (Wilhelmus, 1995) illustrated in figure 7.

In countering the progression of diseases, *P. aeruginosa* is eliminated through polymorphonuclear neutrophils (PMN) linked with lysosomal degranulation, phagocytosis and elimination of the bacteria into the phagolysosomal segment of the cell. Polymorphonuclear neutrophil in the cell engulf the invaded bacteria that are degranulated by stimulating the phagocytic respiratory build-up and attack through oxidative process. In continuation, toxic oxygenated metabolites are created, other oxidizing elements such as hydrogen peroxide, creation of complex molecules like superoxide anions (Hazlett, 2004).

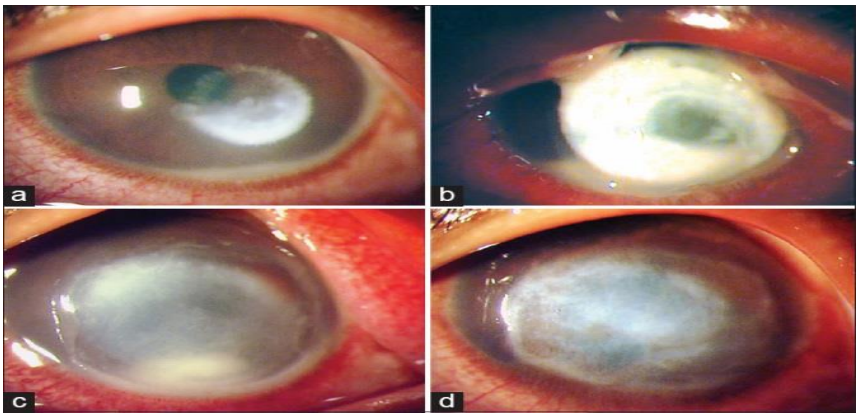


Fig 7: An image of corneal ulceration due *to P. aeruginosa* infection following a trauma. (a) 48 hours after onset showing cornea melting. (b) Image of the ulceration after 4 days the treatment commenced. (c) Image showing reduced infiltration and density preceding healing of the ulcer. (d) Appearance after 30 days of ulceration. Reproduced from Chatterjee and Agrawal, 2016.

Enzymes and free radicals derived from the toxic oxygen causes stromal extermination through collagen break, severance of stromal keratocytes and digesting glycoaminoglycans. Macrophages

accord to corneal edema and stromal necrosis in bacterial keratitis. Some biological elements are also thought to initiate the release similar active elements such as tumor necrosis factor TNF-a, IL-1, IL-6 with several cytokines material synergistically towards inflammatory activities (Hazlett et al., 2004). According to Strieter et al., 2004, the common features of most inflammatory processes starts from infiltrative leukocytes. The overall process of phagocytic processes during infection is championed by the timely response of cytokines IL-1, cell attaching molecules that support cell to cell extracellular matrix interplay and the expression of the molecules outside the cell surface or the extracellular matrix (Leonard, 1990). Chemotactic cytokines are responsible for cell mobilization along a gradient pathway as required by the chemoattractant (Ogita, 2008). The gathering and intrusion of inflammatory cells into the inflamed tissues is mainly controlled by inflammatory agents within the infected area. The most active inflammatory chemoattractant and polymorphonuclear neutrophils found in mouse are double cross-linked cysteines amino acid group of chemokines and the macrophage inflammatory protein (Driscoll, 1995). However, macrophage inflammatory proteins and chemokines varies within the ocular tissues such that both play different role and in differing magnitude (Kernack et al., 2000). Chemokines are known to facilitate angiogenesis and enhance the multiplication of epithelial cells (Cole et al., 2005).

1.2.2. Susceptibility affinity of *Pseudomonas* to antibiotics.

One of the conventional approaches in the management of bacterial eye infection by the clinician, is the use of broad-spectrum antibiotics prior to culture and identification of the pathogenic organisms (Chalita et al., 2004). Sensitivity tests were performed to identify the particular microbe responsible for the infection. This is a useful approach towards reducing antibiotics resistance by bacteria. However, there is increasing record of antibiotic insensitivity. Pathogenic organisms achieve this by horizontal gene transfer. These genes produce enzymes that destroy the antibiotics and change the cause of the applied drug to the target site (Tenover, 2006).

1.3.0. Some ocular conditions associated with blepharitis.

A. Blepharo-keratoconjunctivitis.

Blepharo-keratoconjunctivitis (BKC) is an inflammatory condition affecting the whole surface of the eyes with a common diverse prevalence in both adult and children (Rhee and Mah 2007).

Some causes of BKC include infection of the ocular system including blepharitis, allergic conditions and dermatological causes. Clinical manifestations of BKC include inflammations of the eyelids, conjunctiva and cornea. It is capable of causing crusting on the base of eye lashes due to the accumulation of fibrous tissues, misdirection of the eyelashes and maldrosis, lid notching, moderate follicular hypertrophy and conjunctival pappilae, marginal infiltrates or sterile marginal infiltrates. It can also present with anterior lid telangiectasia (Viswalingam et al., 2005). Blepharitis and BKC shares similar treatment approach however, adult patients respond better to treatment including lid hygiene and antibiotics than in children especially in poorly characterized and chronic cases. Prompt control of inflammations and other symptoms is vital towards preventing complications such as reduced vision and corneal thinning. Both topical and systemic antibiotics such as erythromycin and tetracycline are used for several weeks or months including lid hygiene (Cehajic-Kapetanovic and Kwartz, 2010. Daniel et al., 2017).

B. Chalazion.

The eyelid comprises of a multi- layered anatomical structure with the outermost layer of stratified epithelial layer, the tarsal plate made up of a loose connective tissue, a layer of loose connective tissue, the palpebral conjunctiva layer and the orbicularis oculi or the muscular layer. The meibomian gland is situated within the tarsal plate. It produces the oily layer of the tear film that prevents evaporation and dryness of the tear film. The glands opening is found along the lid margin through which the secreted lipids in the gland escape to nourish the ocular surface and in turn, prevents dryness or dry eyes (Srinivasan 2013).

Chalazion also known as a meibomian cyst results from granulomatous inflammatory reaction within either location of the lid (Srinivasan 2013). It's one of the complications of meibomian gland dysfunction or blepharitis. Blocked gland over time gets inflamed resulting in irritation, mild pain and dry eyes. Although chalazion may resolve without treatment Honda 2010, the fibrous sac containing the hard nodule may calcify and enlarge on to the tarsal conjunctiva forming a fleshy round or oval shaped mass (Srinivasan 2013).

Red eye.

Red eye is one of the commonest ocular diseases that presents to eye clinic departments. Eye care practitioners may face challenges in the cause of managing the condition (Watkinson and Seewoodhary, 2017). Blepharitis including other eye diseases like acute iritis, dry eye, conjunctivitis and close angle glaucoma are the commonest cause of iritis however, blepharitis induced red eye is not seen as a sight threatening condition (Watkinson, 2013), although the clinician should be capable to identify a benign red eye and a sight threatening condition. Red eye patients are classified into depending on the severity of case presented. These include those referred to primary eye care center needing the attention of optometrists and ophthalmic nurses and those requiring the attention of tertiary eye care practitioners mostly in hospital practice (Du Toit and Van Zyl, 2013). A thorough case history taken should unravel the impending risk and discomfort to the more serious ocular symptoms such as pain, sensitivity to light (photophobia), diminish vision, discharge and findings from ophthalmoscopy. Major area of focus on the eyes is the visual acuity, the pupillary reaction to light, shape and preauricular node palpation. Some characteristic features that differentiates a simple case of red eye to a more threatening cause (Du Toit and Van Zyl, 2013).

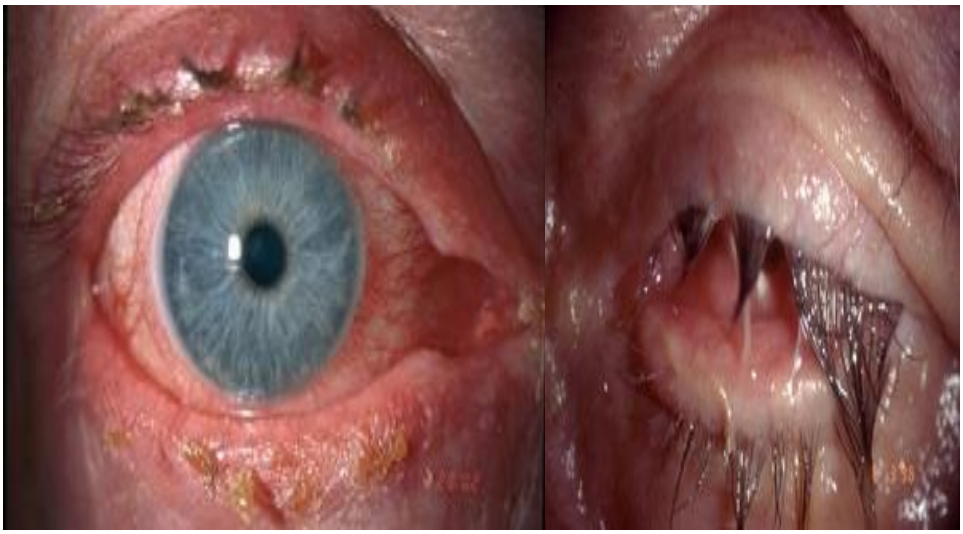
1.3.1. Bacterial conjunctivitis

According to the American Academy of Ophthalmology, bacterial conjunctivitis is one of the associated eye infections with blepharitis (Putnam, 2016). Some pathogenic organisms responsible for bacterial conjunctivitis include *S. aureus, Haemophilus influenza, Chlamydia and Gonococcus bacteria.* Common symptoms of bacteria conjunctivitis include mild pain and photophobia while purulent discharge, redness and lid sticking in the morning often seen as a differential feature of the diseases are the common signs observed on patients with bacteria conjunctivitis in figure 8. It's commonly managed with topical antibiotics however, infection due to *Chlamydia* and *Gonococcus* are best managed through systemic antibiotics due to its mode of contraction (Tarabishy and Jeng, 2008).

Most acute bacterial conjunctivitis result from infection of the genital organ caused by *Neisseria gonorrhea* though cases may be asymptomatic to the patient (Tarabishy and Jeng, 2008).

However, *chlymadia* conjunctivitis is the major organism responsible for neonatal conjunctivitis. Infants contract this infection on passing through the birth canal. Signs include profuse discharge; in complicated cases, patients may present with corneal perforation with consequent reduction in vision (Tarabishy and Jeng, 2008).

Other causative organisms of bacterial conjunctivitis include *Haemophilus influenza*. Studies by Buznach et al., 2005, found that the infection is easily contracted in large gathering and commonly associated with upper respiratory tract infection and conjunctivitis-otitis syndrome in about 70% presenting cases with ipsilateral ear inflammation (Bodor, 1992).



a **b**

Fig. 8. (a) Image of bacterial infective conjunctivitis and (b) Sticky eyelid in acute bacteria conjunctivitis. Reproduced from Høvding, 2008.

1.3.2. Presentation of blepharitis.

A. Anterior blepharitis.

Staphylococcal blepharitis is characterized by crusting, scaling, and erythema of the eyelid margin with collarets formation at the base of the cilia (American Academy of Ophthalmology, 2013). Eberhardt & Rammohan (2017) concluded that telangiectasia may exist at the external part of the eyelid in addition to madarosis, poliosis and trichiasis. The American Academy of

Ophthalmology (2013) also included mild to moderate conjunctival injection, eyelid ulceration in severe cases, hordeolum, eyelid scarring, corneal changes such as neovascularization, erosions, scarring, pannus, phlyctenules, thinning and infiltrates as part of the clinical signs.

Seborrheic blepharitis usually manifests with greasy lashes that result in matting across the anterior lid margins of both eyes, with less inflammation and telangiectasia than Staphylococcal blepharitis (Putnam, 2016). Clinical presentation ranges from benign dandruff to exfoliate erythroderma (Jackson, 2008).

Posterior blepharitis Meibomian gland dysfunction manifestation of the eyelids includes foamy discharge along the eyelid margin, crossing of prominent blood vessels at the mucocutenous junction, scalloping and hardening of the eyelid margin, expression of the meibomian secretions. This ranges from turbid fluid to hard chesses-like material, pouting, plugging of the meibomian orifices, chalazion and triachiasis (American Academy of Ophthalmology, 2013). Overall, the management of any type of blepharitis has encountered towards identifying the pathogenic agents and the presence of polymicrobial organisms that may be presence during infection (Ta et., 2003).

1.4.0. Molecular characterization of organism's and DNA.

Previous study on comparative assessment with cultured-based method showed the presence of multi-microbial on patients with and without blepharitis could vary in terms of the population of the bacteria isolated (Ta et al., 2003). Many common causes of blepharitis are not well defined, which makes it difficult to give a definite pathophysiological cause of the disease (Lee et al., 2002). This could be attributed to the limitations encountered in identifying the causative organisms, which is performed conventionally in the clinic through the culture method (Ta et al., 2003). One of the advantages of this conventional method of identification is that it is inexpensive thereby resolving the need for the expensive molecular technique (Patel, 2001). Certain limitation of conventional techniques indicates the need to apply molecular identification in clinical laboratory practice. For example, slow growing bacteria like *Mycobacterium* spp known for is slow-growing nature of about 6-8 weeks with another time frame of about 4 to 6 weeks to identify is a proof that conventional identification is time consuming and requires great expertise which may take

more than a year to develop (Patel, 2001). A major factor in the unreliability of culture samples is the possibility of multiple bacterial growth within the media and specific growth requirements of the cultured sample (Graham et al., 2007). However, previous studies have shown that applying the molecular identification method in the detection and characterization of the causative organisms demonstrated high level of reliability and as such, is often applied in clinical investigation of microorganisms (Schabereiter-Gurtner et al., 2001). As the aetiology of chronic blepharitis is not well understood, it therefore leads to failed treatment regimen in some cases. With the knowledge of role play by microbial infection in the pathogenesis of chronic blepharitis either as singular cause of the disease or as part of the disease process; therefore, identifying the causative organisms and antibacterial agents sensitive to the particular organisms is considered to be helpful in managing the condition (Karimian et al., 2011). Karimian suggested for studies on the relationship in severity of chronic blepharitis, presentation on the ocular surface and results from cultured sample. Some steps involved in the molecular method of detecting and characterization include the PCR technique, denatured gradient, gel electrophoresis and sequencing. Although previous studies were on molecular characterization of microbial communities in patients affected by blepharitis, this study will further elaborate our understanding in specifics organisms identified in established case of blepharitis and so enhance in the treatment and management of the condition. Molecular identification techniques are advantageous in recognizing some fastidious bacteria and also for those that cannot be well differentiated through the conventional techniques. In a bid to solve this problem, a method of culture-independent analysis relied on the 16S ribosomal RNA (16S rRNA) gene sequences was developed and used to investigate the microbial community in human body (Costello et al., 2009 and Fierer et al., 2008). Sequencing of the 16S rRNA gene is an accepted method of bacterial identification (Patel, 2001). It is currently used by taxonomists in measuring the similarity between DNA and isolates.

1.4.1. The 16S rRNA gene.

A vital phylogenetic characteristic of the 16S rRNA gene is that it is pivotal in clinical identification. Another important component of this gene in clinical laboratory is that it is found in all bacteria thereby enhancing the characterization of bacteria universally (Patel, 2001). The role of 16S rRNA

gene has been consistent over the years; however, a change in the sequence of a gene could result from a random change than a selection of changes that would alter the molecular function of the rRNA gene. The 16S rRNA is referred as an ''ultimate molecular chronometer'' largely due to the relevant information about the organism it carries in a molecular confinement. Furthermore, an increase in the number of functional domains with the rRNA gene reduces the effect of selected changes bears on phylogenetic relationships in clinical identification (Woese, 1987). However, 16S rRNA gene sequencing techniques lack some measures in total sequencing differences between bacteria. DNA-DNA reassociation essay is considered to be more reliable in measuring the diversity between bacteria strains (Reischl et al., 1998).

Although there must be a high level of relativity between the species to achieve such result within the rRNA gene. Although phylogenetic information is carried throughout the gene, the vital heterogenic information is seen in the first 500 bases of the 5' end of the strand. (Tang et al., 1998 and Rogall et al., 1990).

So, it is believed that the identification of the isolate can still be achieved by sequencing the first 500 bases however, a full gene sequencing provides more precise information of the isolates especially for a novel isolate (Patel, 2001). Furthermore, the cost of acquiring laboratory instrument and reagents needed for a holistic sequencing of gene.

1.5.0. Outline of research areas and key question.

There is heightened interest towards proper identification of organisms that would eventually reduce the overuse of antibiotics. Also, the rising report of bacterial resistance particularly those caused by the multidrug resistant strain emerging as a global health challenge and required urgent attention; also, of consideration is the recent report by the WHO about the limited availability of antibiotics in the near future (Sin-Yeang et al., 2016).

1.5.1. Aim:

Objective 1: Isolation of sample from patients suffering chronic blepharitis and gaining ethical approval for collecting and analysing patients' data and medical information.

Objective 2: Identifying organisms responsible for blepharitis with a view to enhancing its management.

Objective 3: Molecular identification of 20 clinical strains.

Objective 4: Determination of the strains' antibiotic resistance (micro-broth dilution and antibiotic susceptibility testing).

Chapter 2

2.1.0. Material and method.

A. Recruitment of patients in the rural areas.

Some of the initial work completed include sensitization and outreach programs in the rural areas in Abia State with no functional eye care service. Hand bills were shared to the people living in these areas to come out for a visual screening on a stated date. Some places visited for publicity include town center and churches. Participants included children, adults and old adults. Participants information such as age and occupation were recorded with their consent and ethical approval secured through the hospital management. Visual screening was done in the rural clinic while suspected cases of blepharitis and other dictated ocular conditions were advised to visit the specialist and teaching hospital.

B. Visual acuity measurement.

Visual Acuity is the most common means of assessing visual function and its measures the ability of the patient to resolve fine details at a given distance. Chronic blepharitis can obscure visual acuity especially when patient's cornea are affected through Staphylococcal infection (Chirinos- Saldana, 2013). The visual acuity of the subjects were tested using Snellen's chart developed by Herman Snellen in 1860. Both literate chat and illiterate chart as seen in figure 9, were used and measurement done at various distance of 6 meters and 3 meters (Masden et al., 2014)

C. Procedure for Visual acuity measurement.

The chart was positioned in a room with bright illumination of about 80 dc/m² luminance. VA of the patients were taken at 6 meters distance from the chart with an unobstructed view. Three principle VA method taken were; Unaided VA often referred as vision, habitual VA or VA with prescription and lastly, optimal visual acuity where a patient wears his best corrective lenses. Vision were taken conventionally with the right eye (OD) first and then on the left eye (OS) and finally with both eyes (OU) at 6 meters. Same step was taken in measuring the near acuity of the patients at the reading distance of 25 cm.

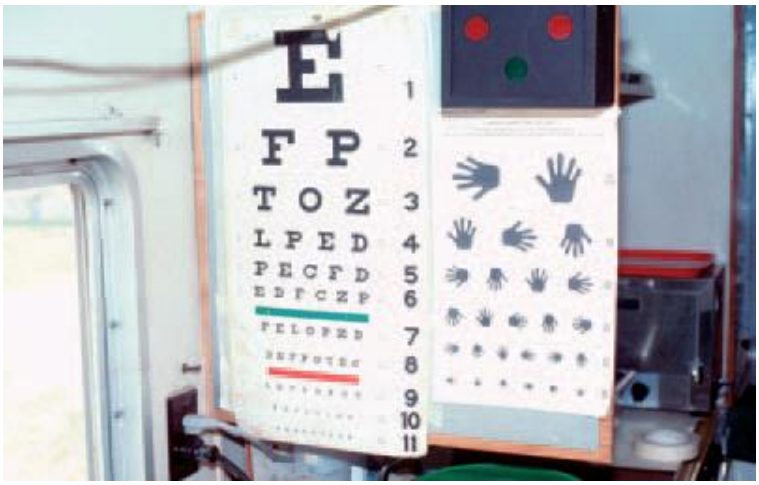


Fig 9: Snellen's visual acuity chart. Reproduced from Sue, 2007.

D. External and internal examination of the patients with slit lamp.

The second stage of examination carried on the patients was to examine the external adnexia (structures in front of the eye) and internal ocular examination with the help of slight lamp biomicroscopy in figure 10. Patients were instructed to position as recommended for slit lamp examination. Diffuse illumination system with a wide beam and magnification of 16D (16 magnification) was used in the examination of the adnexia such as the lashes and eyelid. The tear film was specifically assessed with optic illumination with a magnification of 25D. Assessment of the anterior chambers to check for cells and flares was performed with a conical section illumination at 1mm beam. Fluorescein dye was used in the examination of the corneal integrity and structure. Three scans were performed on the four quadrants of the anterior eye starting from the central to the medial, temporal, superior and the inferior of the ocular structures examined. Volks lens 78D was used with parallelepiped illumination in examining the posterior medias of lens and the vitreous humour. These were done to completely evaluate the health of the front and back of the eyes of the participants.

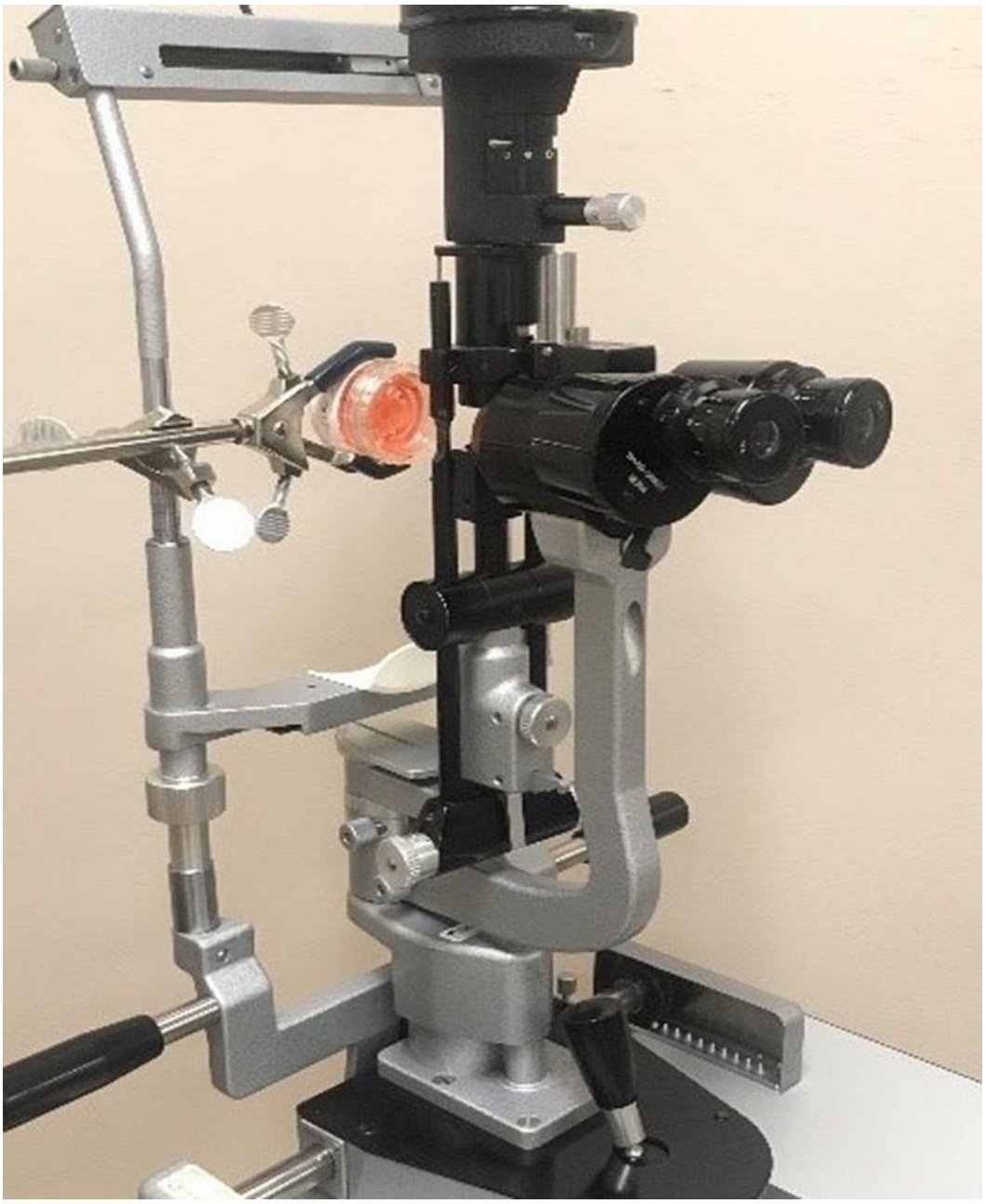


Fig. 10. Slit lamp biomicroscopy; an ophthalmic instrument used in the external and internal examination of the eye. Reproduced from Clover, 2020.

E. Examination of patients and isolation of sample.

The first stage of this study was conducted in Aba, Abia State in Southern Nigeria. Aba is popularly known by the industrial prowess of the residents of about two million people out of three and half million population of Abia State; the state is also among the oil producing state in Nigeria known as the Niger Delta region, which is the major source of revenue of Nigeria. Furthermore, most rural dwellers engage in subsistence farming with a limited number of citizens involved in commercial farming. Subjects were gathered from rural and urban areas of Aba. The Chief Medical Director of Abia State University teaching hospital Nigeria granted consent of the study and all ethical approval was successfully submitted. Patients were initially screened out at the GP department before eye examination. Demographic data of the patients such as place of residence (rural or urban), use of contact lenses, previous ocular condition and clinical presentation were among the data collected in the clinic. Clinical strains were isolated from samples taken aseptically from the eyelid margins of patients who presented to the ophthalmic clinics with blepharitis infection. Collection of samples was performed using a sterile swap. The swaps were rolled in the upper and lower eyelid margin from the lateral to the medial canthus of the eyes. The swaps were immediately inoculated on a blood agar plates and incubated in 37^0C overnight. Following incubation pure strains were isolated by performing the streak plate technique. Investigations of these patients were carried out with the use of slit lamp bio-microscopy after recording their habitual visual acuity. The isolates were initially stored in -80^0 C before been shipped LJMU microbiology laboratory having gotten approval for the transportation of the UN3373 biological substances. On arrival to LJMU the strains were stored at -80^0 C in cryopreservation vials according to the manufacturer's instructions (MASTGroup Ltd).

2.1.1. Cultivation and culture.

Nutrient agar (Oxoid) was prepared according to manufacturing instructions prior to autoclaving at 121^0C for 15 minutes. 50ml of the nutrient agar were added in petri dish and allowed to cool. Samples were incubated overnight at 37^0C.

2.1.2. Scanning electron microscope (SEM).

Samples were grown in shakes flasks containing 50ml of sterile nutrient broth and incubated in shaking incubator for 24 hours at 37ºC. A sized amount of 1ml in each of the media was pipetted into a 1.5ml eppendorf tube and centrifuged using the eppendorf centrifuge 5418R at 13,000 rmp at 25^0C for 1 minute. The media were aspirated and the subsequent supernatant discarded. Mobilized samples of bacteria samples were stored overnight in 1ml of 2.5% gluteraldehyde solution and kept in fridge of 2^0C temperature. Samples were washed with 1ml of distilled water and vortexed. They were later centrifuged at 13,000 rmp at 25ºC for 1 minute. The media was aspirated and discarded. This procedure was repeated three times to thoroughly washed of the gluteraldehyde. Samples were left in room temperature for 2 hours to dry. They were later loaded in stubs and photographed.

2.1.3. Susceptibility testing of samples with antibiotics.

Antibiotic susceptibility by the organisms was carried out using a range of broad spectrum antibiotics. Initial step involved inoculating the microorganisms in 250 ml flasks containing 50 ml of nutrient broth prior to incubation at 37ºC shaking incubator for 24 hours. Later, 100µl was pipetted into a petri dish plate containing sterile nutrient agar. Inoculated plates were kept in a safety cabinet for 10 minutes to dry before introducing antibiotics with the use of the plunger. The antibiotics used were gentamycin, penicillin, fusidic acid and erythromycin on Gram negative bacteria while ciprofloxacin, gentamycin, cefotaxime and ampicillin on Gram positive bacteria. The antibiotics were chosen as the are commonly prescribed for blepharitis. This process was performed under sterile conditions to ensure that the samples were not contaminated. Susceptibility measurements were performed according to the European Committee on antimicrobial susceptibility testing (EUCAST).

2.1.4. Molecular characteristics.

A. Bacterial DNA extraction protocol.

Extractions of genomic DNA were performed using the E. Z. N. A. Bacterial Kit (Omega Bio-Tek). Samples were cultured in 250ml LB media to about 20 hours. 3ml of the sample was centrifuged at 4000x g for 10minutes at room temperature using the eppendorf centrifuge 5418R. The media

was aspirated and discarded; 100µl of TE buffer was added to suspend the pellet. 10µl Lysozyme and incubated at 37ºC for 10 minutes to enhance the complete digestion of the bacteria cell wall for efficient lysis. 100µl TL Buffer and 20µl Proteinase K solution, vortex to mix thoroughly and incubated at 55ºC in a shaking water bath for one hour for bacterial lysis. 5µl RNase A was added and tube was inverted to mix thoroughly and then incubated for 5 minutes. The mixture was centrifuged at 10,000 x g for 2 minutes to pellet any indigested material in the sample. The subsequent supernatant was later transferred to a new 1.5ml microcentrifuge tube while leaving the pellet undisturbed. After that, 220µL BL buffer was added and vortex to mix. It was later incubated in AccuBlock™ digital dry bath for 10 minutes for DNA recovery process. 220µl 100% Ethanol was added and vortex for 20 seconds at a maximum speed to ensure thorough mixture. Sample was later transferred to into Hiband® DNA mini column placed in a 2mll tube before 500µl HBC buffer was added (HBC was diluted with isopropanol before been used). HBC buffer removes contaminants thereby improving high-quality genomic DNA. Sample was later centrifuged at 10,000 x g for 1 minutes and the filtrate discarded. 700µl DNA wash buffer diluted with 100% ethanol was used to wash the sample and centrifuged for 1 minute. This process of washing with the DNA wash buffer was repeated to ensure thorough washing of the sample. To ensure that there was no trace of ethanol in the sample, the Hiband DNA mini column containing the sample was centrifuged at a speed of 10,000 x g for further 2 minutes. To elute the DNA sample, 50µl Elution buffer heated to 65ºC in AccuBlock™ digital dry bath was added and allowed to sit for 5 minutes in room temperature before been centrifuged at 10,000 x g for 1 minute release to release the DNA product. This last stage of the extraction method was repeated, and product stored at -20ºC.

B. DNA amplification and electrophoresis.

Amplifications of the 16S rRNA sequence was performed with a Perkin-Elmer Cetus GeneAmp Thermal Cycler, 9600, using 35 cycles of 95^0 C for 1 minute, 52^0 C for 1 minute and 72^0C for 2 minutes. The reaction mixture contained 45 µl of 1.1 x PCR MasterMix (1.5 mM $MgCl_2$) [ABgene], 2 µl of DMSO, 1 µl of genomic DNA (50 ng), 1 µl of sterile dH_2O and 0.5 µl of each primer by Fisher™ (forward and reverse). The bacterial primers for the PCR amplifications are (5' to 3'): GTT

TGA TCM TGG CTC AG (forward) and CCG TCA ATT CMT TGG AGT TT (reverse). These primers were chosen because they are generic and conservative.

C. Agarose electrophoresis.

The amplified products along with Hyperladder II biomarkers (Bioline) were visualized on a 2% agarose gel prior to purification with the QIAquickR PCR purification kit (Qiagen). 2 % agarose was heated for 2 minutes in 10ml invitrogen UltraPure™ TAE buffer to dissolve the mixture while been shake for every 20 seconds during the heating. This was to enable proper melting of the agarose and also to avoid bubbles. 7µl of the sample and 3µl of novel juice were mixed using pipette and then loaded in the gel. Also, 6µl of Thermo scientific™ DNA ladder was loaded to enable interpretation of the gel result. Gel electrophoresis was carried out on BIO-RAD machine at 60 Voltage for 55 minutes. Gel picture were taken with the BIO-RAD ChemiDoc™ MP imaging system. DNA concentration measurement was done using the Thermo Fisher Scientific NanoDrop 2000/2000c spectrophotometer.

D. DNA sequencing (Sanger sequencing with capillary electrophoresis, ABI).

The PCR products were sent to Source Bioscience (Nottingham) according to their requirements. Sanger sequencing leads to the formation of extended length of the dideoxynucleotides at the 3' end. This product was the separated through capillary electrophoresis and the produced molecules were injected by electrical current in a capillary made with a gel polymer and sent to Source Bioscience (Nottingham). The sequence obtained was individually checked for errors and manually aligned. Pairwise sequence comparison and retrieval of homologous sequences was conducted using the NCBI BLAST database (available at: http://www.ncbi.nih.gov/). The process undertaken from the isolation of samples to identification is summarized in figure 11.

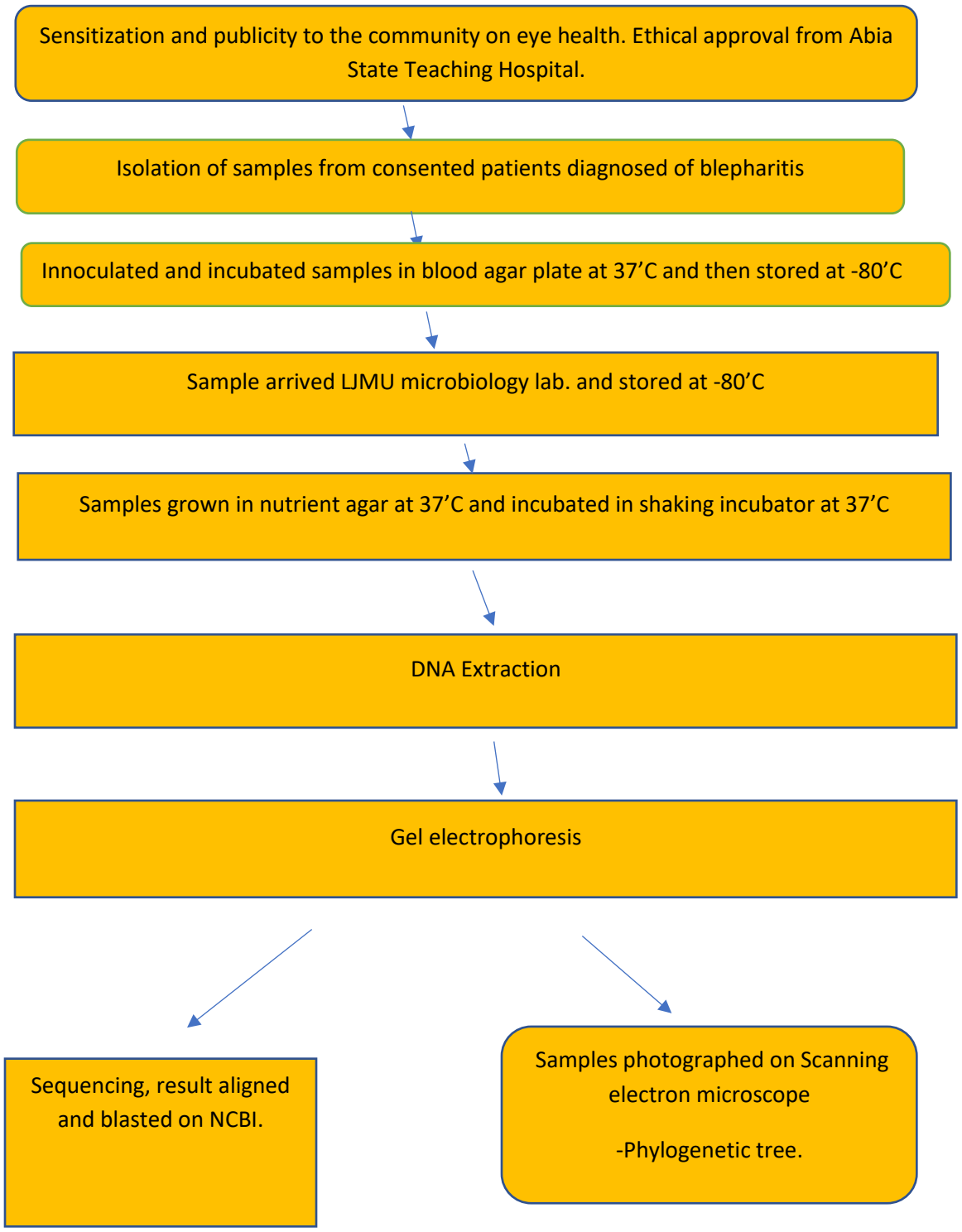


Fig. 11. A flow chat of the process taken from the isolation of samples to identification.

Chapter 3

3.1.0. Results and Discussion.

To further elaborate on the outlined approach in our study, the PCR products from the isolates were sent to Source Bioscience Nottingham™ for sequencing. Initially, all the 20 samples extracted were sent for the sequencing however, 8 of the samples were poorly differentiated as a result of what was suspected to be caused by their fastidious nature although previous study had recorded a successful sequencing of fastidious bacteria (Patel, 2001). According to the finding by Patel, sequencing helps to identify unusual and slow-growing bacteria. There is evidence of this finding in our study through the identification of coagulase-negative *Staphylococcus*, which has not been reported as much in convention identification techniques. The DNA bands were visualised using agarose gel electrophoresis; the image was captured (Figure 12). Before samples were sent for sequencing, DNA concentration measurement was performed using the Thermo Fisher Scientific NanoDrop 2000/2000c spectrophotometer samples were sequenced within 48hours of delivery to the sequencing center. This is advantageous as it offers a rapid and quick identification of a variety of species. However, the identification of coagulase negative bacteria within the study population is a novel development in the pathogenesis of blepharitis. One benefit of this method of identifying bacteria is that it's accepted universally and so can be applied in unravelling the causative organisms in various forms of anterior eye infection.

Agarose gel electrophoresis.

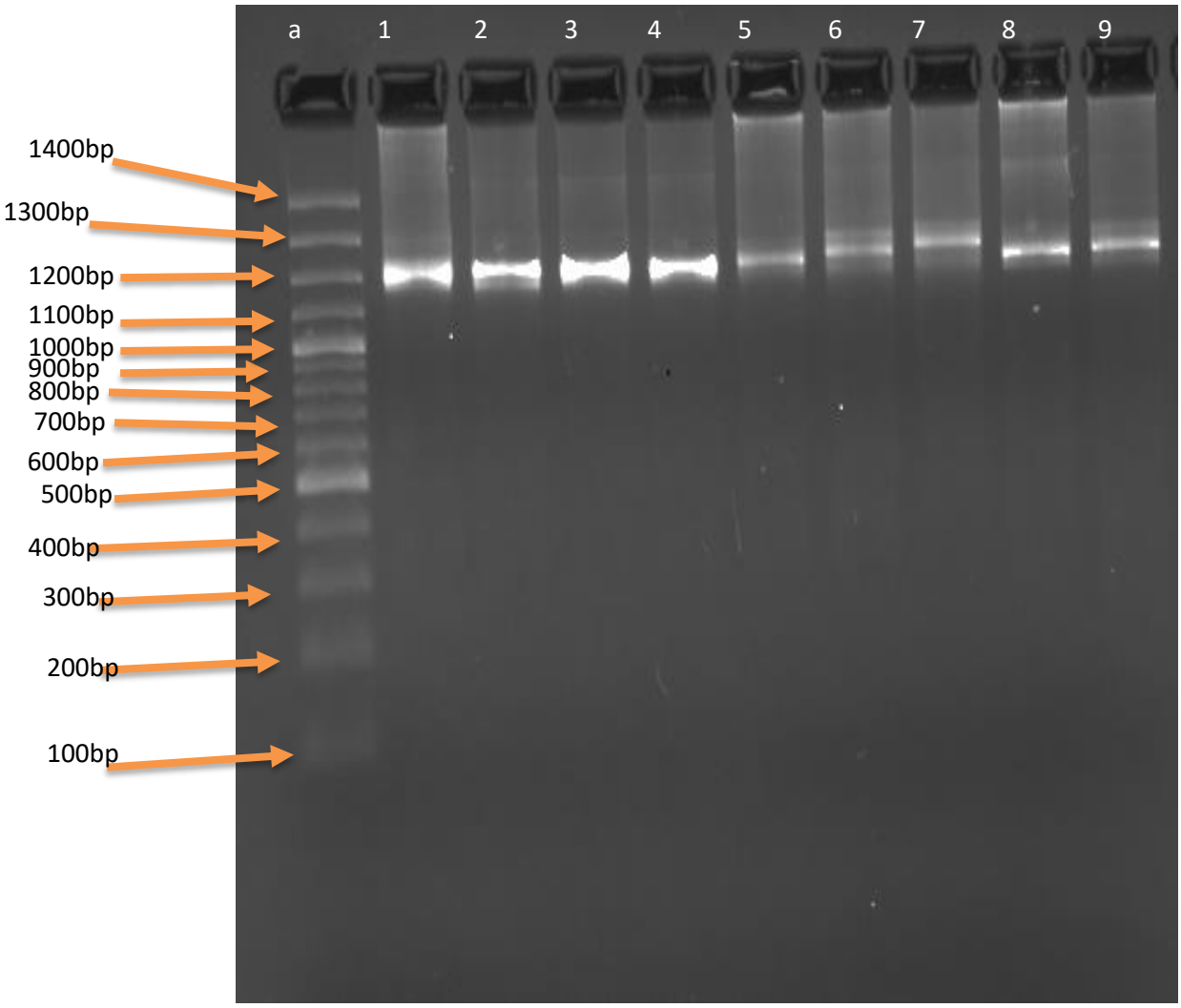


Figure 12: Agarose gel electrophoresis. The clearer PCR products as was displayed in the gel picture could be due to normal DNA concentration obtained with the nano-drop spectrophotometer. Samples number as indicated in the gel and weights were as follow; 1 (30ng), 2 (26ng), 3 (33ng), 4 (28ng/µl), 5 (48ng), 6 (60ng), 8 (45ng), 9 (56ng).

3.1.2. Phylogenetic studies of the identified genes.

The study probed further to characterize the organisms according to their phylogenetic relationship using the MEGA 7 software application. It was aimed at providing more information on how closely the species are related to one another.

The phylogenetic tree shows how close different species of the organism relates to one another using the Neighbor-Joining method conducted with MEGA7 software as shown in figure 13. The accurate percentage level of similarity among the species of the genes in shows the conservative nature of the 16S rRNA gene. It could also be said to have contributed in their similar characteristic features in blepharitis.

Among the aim of the study was to molecularly identify isolated organisms from the patients and explore options towards finding the relationship between species of organisms. Initial step followed was by analyzing the sequenced 16S rRNA gene to expound the taxonomic positions of the isolated agents.

The aim of phylogenetic study in disease control is generally geared towards determining the source of the responsible organisms within a population; the factors enabling the spread of the disease with the population and relationship among the identified organisms (Hall and Barlow 2006). Although the pathogenicity of most infections can be ascertained through the behavioral, chronicity and or noninfectious characteristics of the disease within a population however, it is not uncommon to miss the source of the pathogenic organisms through this method. There are several other factors that negate the tracking of some infectious diseases. This include a mixed up those infected by the disease with turnover of infected individuals, some clinical laboratories lacks some necessary facility to identify causative agents and reporting cases as a result of various cases that present with similar signs and symptoms; and for the fact that most affected individuals readily report to clinic on time for treatment. It is believed that the analogy of the nucleotide through some forms such as the phylogenetic tree will contribute immensely in the understanding of the relatedness among selected organisms.

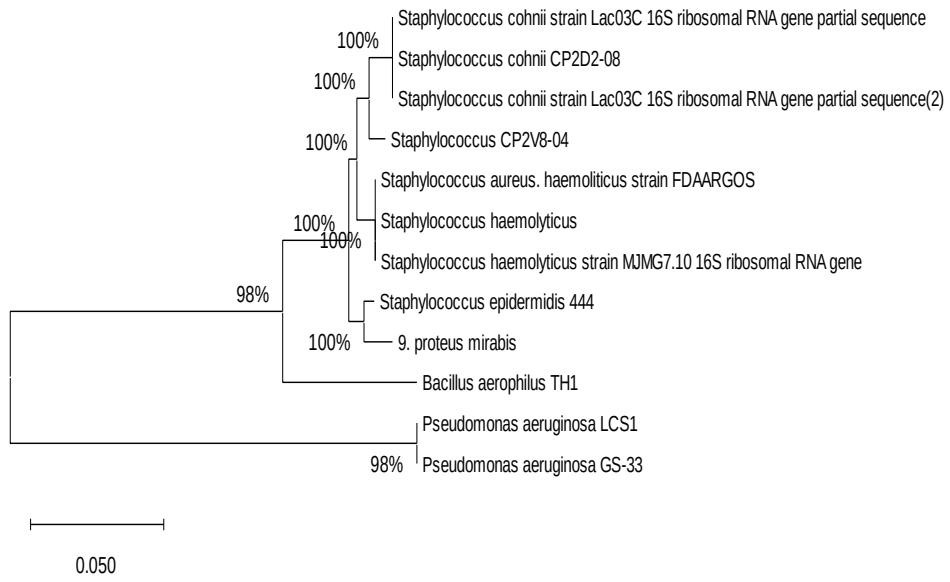


Figure 13. Evolutionary relationships of organism.

NO of samples	Samples	Values of DNA homology (percentage)
S1.	*S. heamolyticus*	1468/1468 (100%)
S2.	*S. haemolyticus*	335/335 (100%)
S3.	*S. conhii*	1361/1361 (100%)
S4.	*S. conhii*	799/799 (100%)
S5.	*S. Saprophyticus*	912/914 (99%)

S6.	*S. saprophyticus*	750/750 (100%)
S7.	*S. aureus*	1400/1401(99%)
S8.	*S. aureus*	900/900 (100%)
S9.	*S. epidermidis*	778/778 (100%)
S10.	*B. aerophilus*	1399/1400 (99%)
S11.	*P. mirabis*	1435/1435 (100%)
S12.	*P. aeruginosa*	908/909 (99%)
S13.	*P. aeruginosa*	496/497 (99%)

Table 2. Showing the homology of the DNA to sample identity

3.1.3. Features of some isolated organisms.

Staphylococcus saprophyticus is a coagulase-negative *Staphyloccocus* found mainly in the urinary tract. It's considered to be among the major causative pathogens of urinary tract infection in younger women contributing about 40% of infection among this group with mean age of about 26 years according to study by Eriksso and Giske et al., 2013. This organism was isolated from a patient in the rural area where poor hygiene is more prevalent which is associated in causing blepharitis.

Staphylococcus heamolyticus strain belongs to the coagulase-negative *Staphylococci* frequently isolated and possess the ability to produce biofilm enhancing its emergence as one of the nosocomial pathogens. It's ability to form biofilm could be one of the features contributing in blepharitis.

Staphylococcus cohnii is a novobiocin-resistant, coagulase-negative strain of Staphylococcal strain colonizing human skin. Typically known to be a methicillin resistant and often times, associated with plasmids resistance to many antibiotics which is a contributory factor in the cause of blepharitis. Although, *S. cohnii* is not usually pathogenic (Vinh and Nichol et al., 2006).

Staphylococcus epidermidis is among the commonest bacterial organisms constituting about 90% of the total aerobes. It's localized below the skin surface in hair follicles, sweat and sebaceous glands. It's thought that changes in puberty often favors the multiplication of this species, which is associated with blepharitis and acnes. It can also be found in the urethra of both sexes though in relative amount. Commonly associated with urinary tract infection with high incidence seen in hospital patients. Most hospital acquired infection commonly recognized bacterial species in blepharitis infection was found to be *S. epidermidis* followed by *S. aureus, Propionibacterium* etc (Din & Patel, 2012). Other study of infection blepharitis found multiplicity of organisms involving S*. epidermidis* and *S. aureus* in isolated sample indicating polymicrobial and multifactorial etiology in the cause of the disease (Ficker et al., 1991).

Proteus mirabilis is one of the most common Gram-negative pathogens commonly encountered in variety of community including hospital environment and in wound infection including urinary infection. It's known to be highly resistance to antibiotics with increasing report of resistance to extended-spectrum cephalosporins as a result of its ability to produce extended- spectrum β-lactamases (ESBLs) that has been a concern*.* Also spread by poor hygiene, *P. mirabilis* is among the common cause of nosocomial infections with ESBL strain often seen to be resistant to most antibacterial agents resulting to difficulty in treatment of the diseases (Hu et al., 2012).

Staphylococcus aureus is a known human commensal and responsible for a diverse acute and chronic infection in human. Chronic infection of *S. aureus* such as blepharitis persist and in severe cases causes morbidity to the patients which result from the resistance to treatment agents and recalcitrant biofilm formed on the affected area (Boles et al., 2010). *S. aureus* and coagulase negative *S. aureus* were the most organisms found in isolated samples from the lid margin, conjunctiva and cornea in a study done in Aba Udo 2009. The high pathogenicity of S*. aureus* is attributed to affinity to multiply and spread within the host tissue. It achieves this through the production of extracellular materials such as coagulase that limits the ingestion of phagocytic cells through the production of fibrin on its surface Udo 2009. Alpha toxin produced by these pathogens lyses the erythrocytes and damage platelets (Schubert al., 2011). Although the significant role of biofilms in the disease cause is been highlighted in several studies, there is no

significant understanding on the molecular mechanisms that initiates the formation of biofilm (Boles et al., 2010). According to the recent studies, the extracellular matrix of *S. aureus* is made up of several elements including DNA, protein and polysaccharide which is also known as the polysaccharide intercellular adhesion or PIA) Boles et al 2010. These factors are thought to contribute to its resistant to antibiotics.

Pseudomonas aeruginosa. Several studies affirm that the most common Gram negative bacterial identified in isolated cases of ocular infection are *P. aeruginosa (*Schiferaw et al., 2012). Although the most common infection attributed to *P. aeruginosa* are corneal infection such as keratitis and other corneal ulcers, it often results to lid scarring which is a characteristic of blepharitis infection (Teweldemedhin et al., 2017). Another study carried out in Ethiopia also supported this finding with Blepharitis constituting 23.8% of case found to be caused by *P.* aeruginosa coming behind keratitis with 50% of diagnosed cases and blepharoconjuctivitis 16.7% (Tesfaye et al., 2013).

Ocular infection caused by these organisms majorly result from poor hygiene and a longstanding infection. They can also be contracted through unsterilized hospital equipment (Madhukumar and Ramesh, 2012). There are several factors contributing to the virulence of the pathogens which enhance the risk of infection (Mazin et al., 2016). Poor hygiene, immune-compromised patients, reduced resistance of the body to infective agents. Other factors include social economic status with most blepharitis patients in this identified as farmers living in rural areas, nutrition, physiology and age Vandenbroucke-Grauls et al., 2002). The most vulnerable part of the eyes commonly infected by pathogens is the lid, cornea and conjunctiva. Blepharitis as one of the major infections of the lid presents with scaling occurring on the base of the lashes and crust; telengiectasis and hyperaemia. However, the infection can progress deeper into the tissue and glands such as the glands of Zeis and Moll leading to meibomian gland disease and other conditions like chalazion and internal hordeolum (Mazin et al., 2016, Sethuraman., 2009).

3.1.4. Scanned images of isolates from the INCA x-act electron microscope.

Further investigation was performed using Scanning Electron Microscopy (SEM) in order to obtain the morphological representation of the sample. This is an important diagnostic tool in microbiology towards identifying the responsible organisms in an infection and also allow for more specific test such as primers and antibiotics to properly identifying the isolated agent. It's also considered a useful technique in a novel study of pathogens where there's no prior knowledge. Another reason behind the decision to perform the SEM was to enable confirm the presence of some negative coagulase staphylococcal in the sample which has not been recorded in so large amount in a similar study on blepharitis. Additionally, having recorded more resistance to selected antibiotics under the biogram test, other measures of identification like SEM were considered to confirm the morphological structure of the sample (figure 14-22). Result showed a well mobilized and clear bacteria colony.

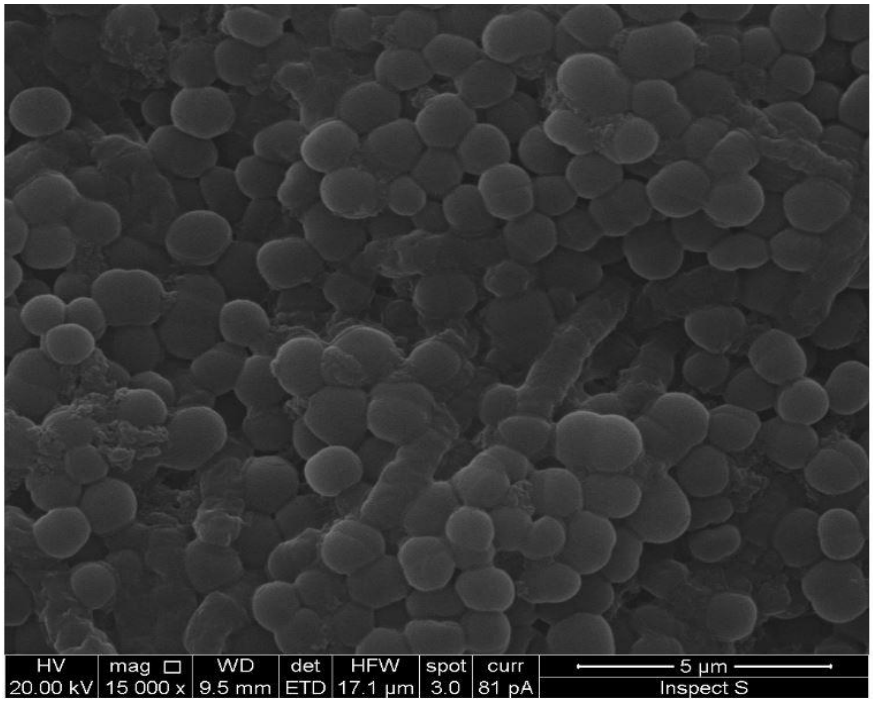


Fig 14. Scanning electron microscopy demonstrating clear image of *S. haemolyticus*. Magnification: X15 000.

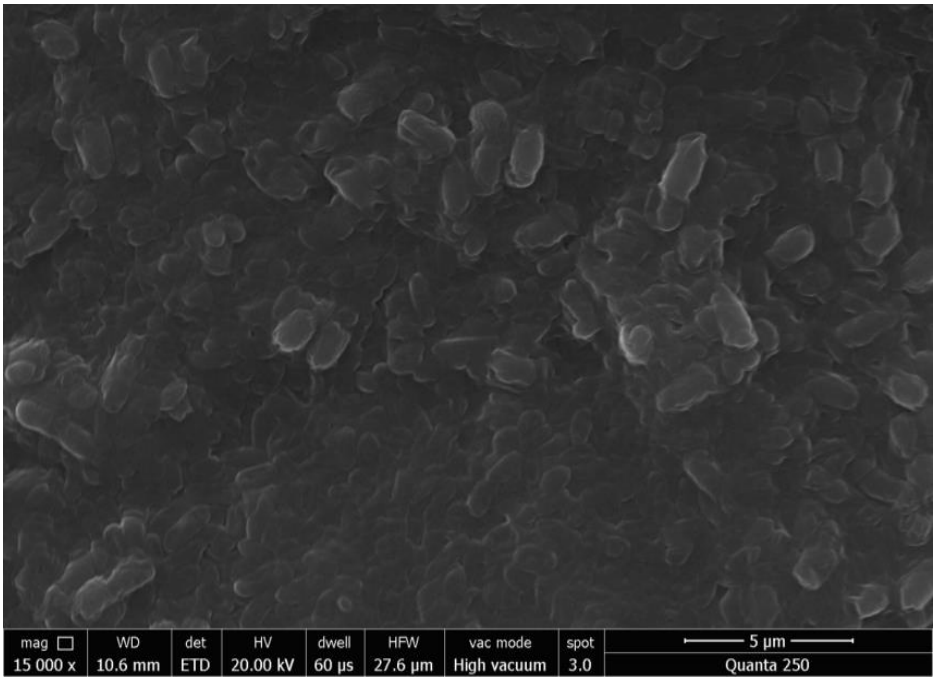


Fig. 15. Scanning electron microscopy demonstrating clear image of *S. conhnii* . X*15 000.*

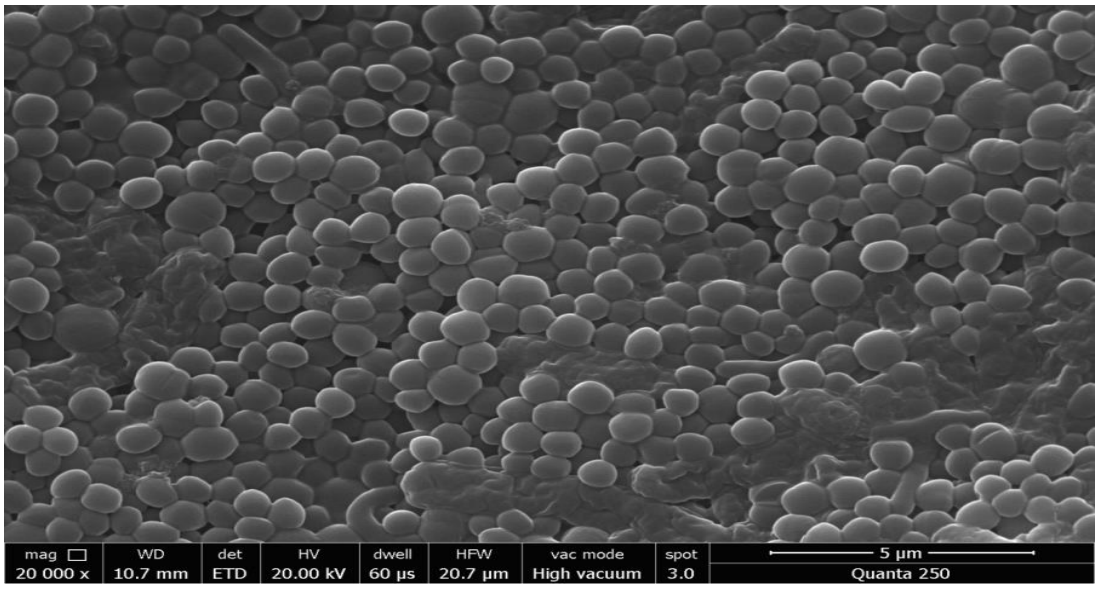


Fig. 16. Scanning electron microscopy demonstrating clear image of *S. conhni*. X *10 000.*

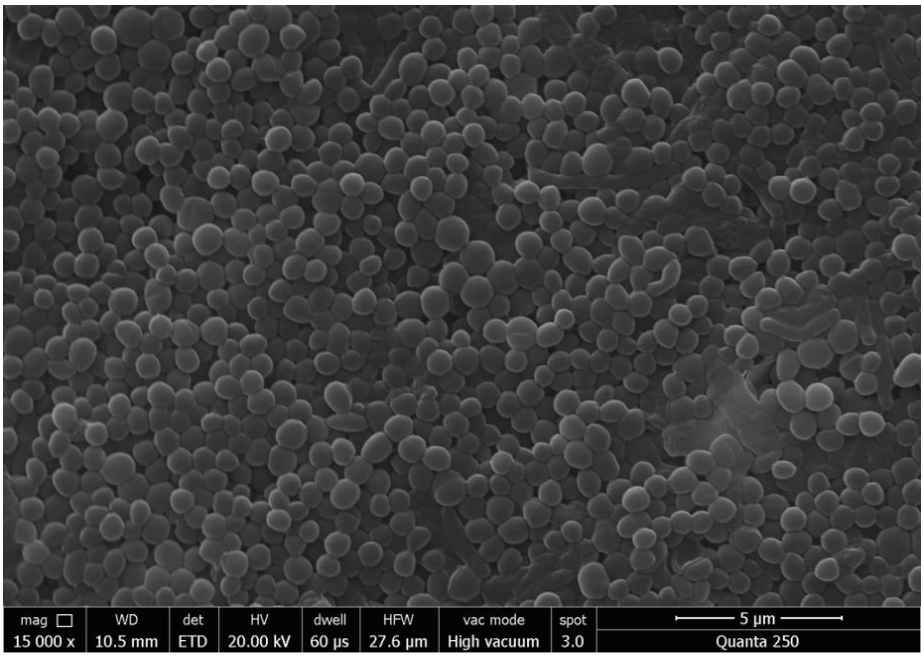


Fig.17. Scanning electron microscopy demonstrating clear image of *S. saprophyticus* X*15 000.*

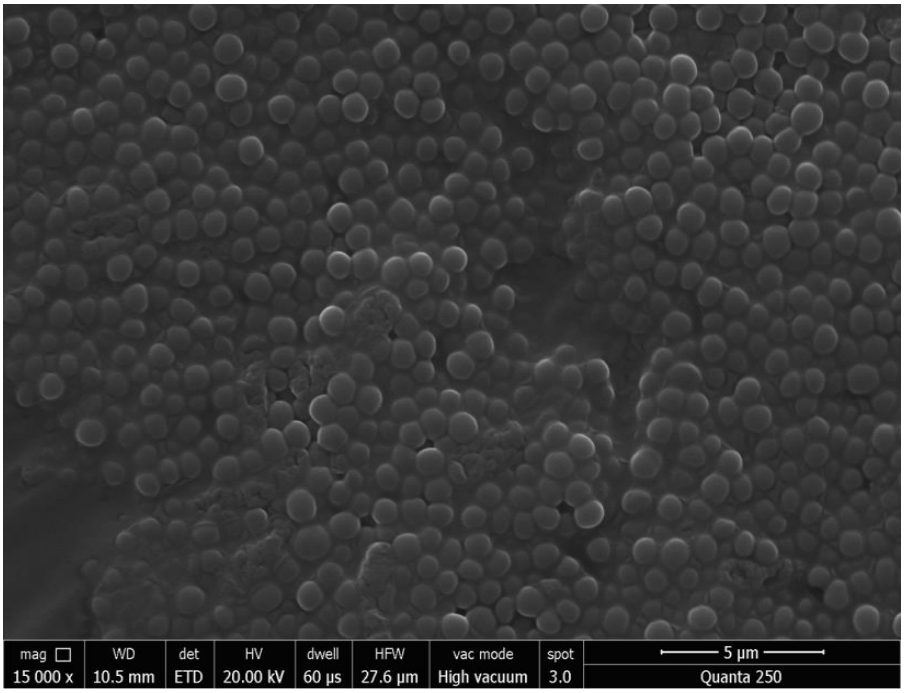


Fig. 18. Scanning electron microscopy demonstrating clear image of *S. saprophyticus*. X*15 000.*

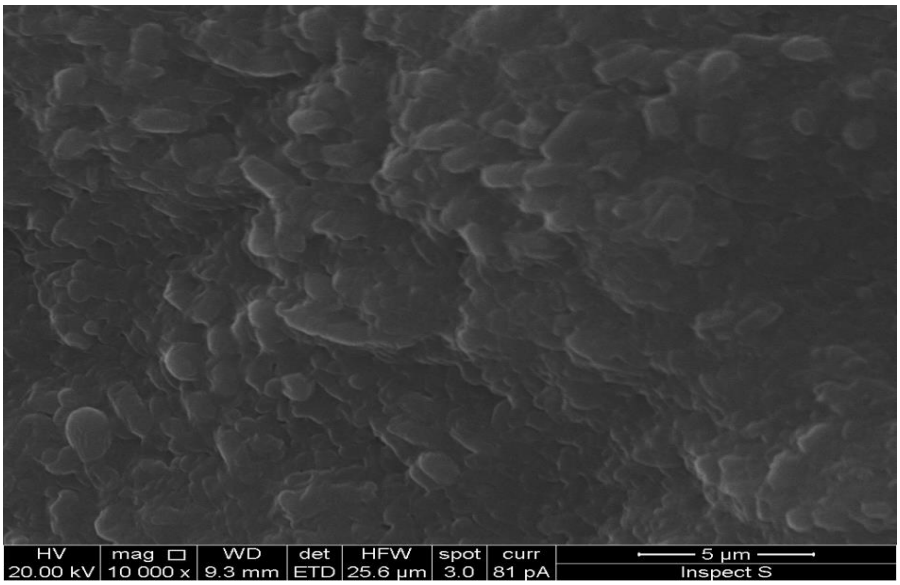


Fig. 19. Scanning electron microscopy demonstrating clear image of *S. epidermidis*. X*10 000.*

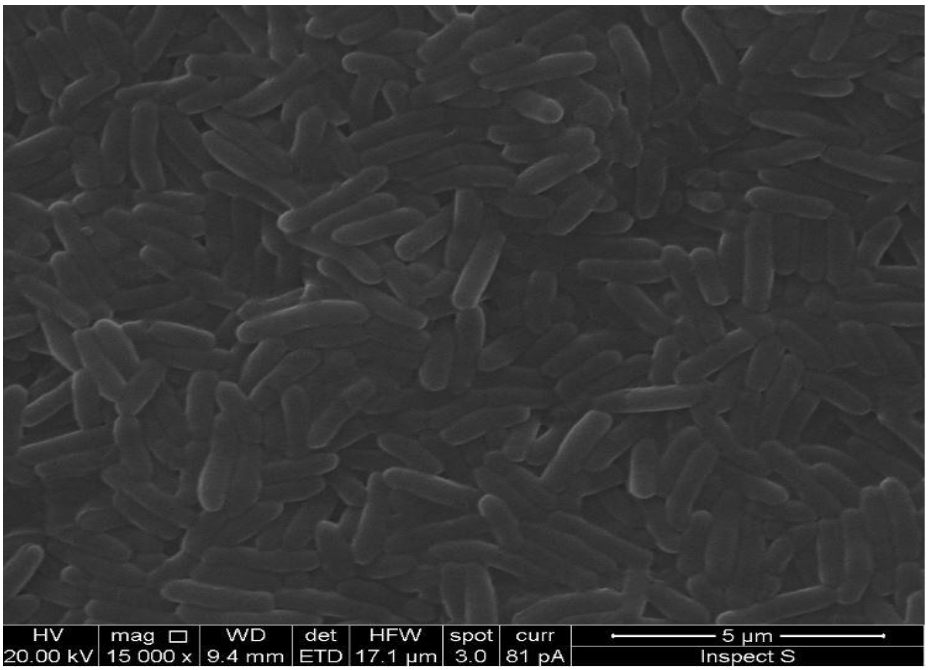


Fig. 20. Scanning electron microscopy demonstrating clear image of *B. aerophillus* . X*15 000.*

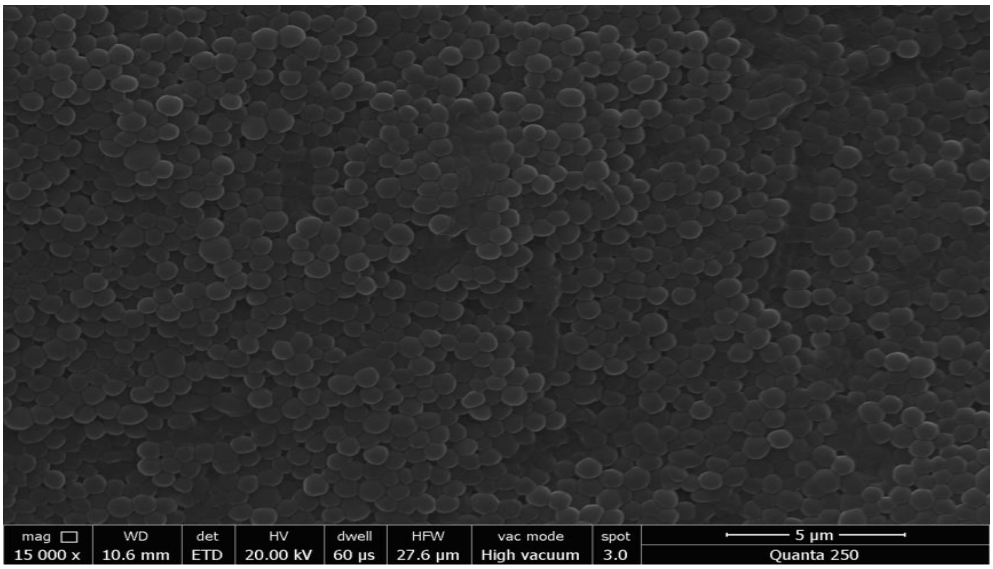


Fig. 21. Scanning electron microscopy demonstrating clear image of *S. aureus*. X*15 000.*

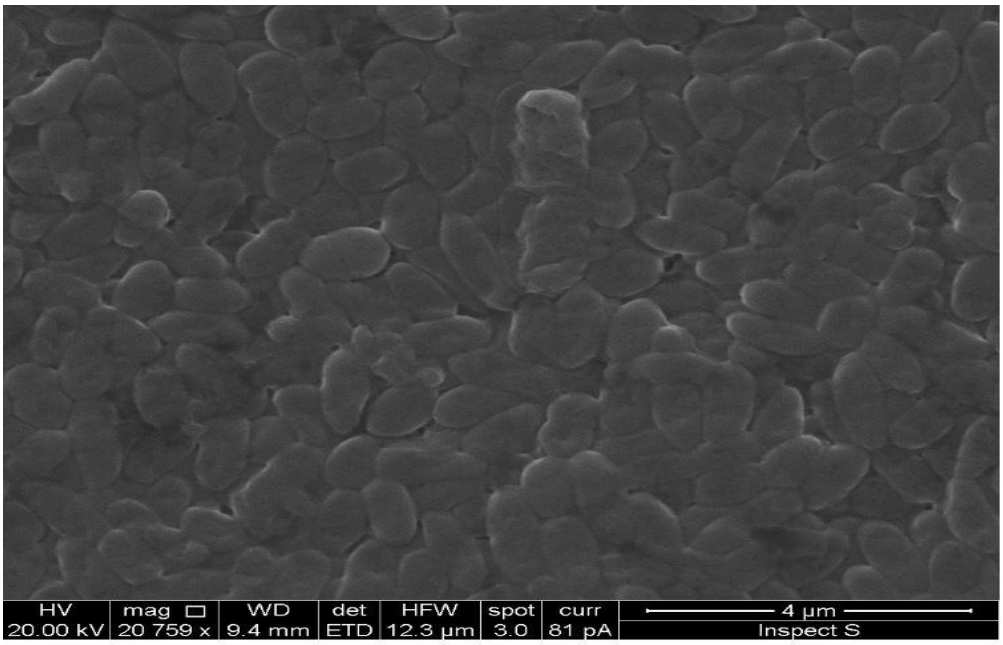


Fig. 22. Scanning electron microscopy demonstrating clear image of *P. aeruginosa*. X20 759.

3.1.5 Susceptible test with antibiotics.

The susceptibility of microbial organisms is one of the many techniques used in phenotypic study of organisms. The strains were tested with the selected antibiotics and kept for 24 hours at a temperature of 37®C.

Overall, most of the samples showed resistance to the antibiotics used. However, a lesser number of the sample were sensitivity to both the Gram positive and Gram negative antibiotics used. The zone diameter break points were measured with a ruler to determined susceptibility or resistance according to EUCAST's break values (figure 23).

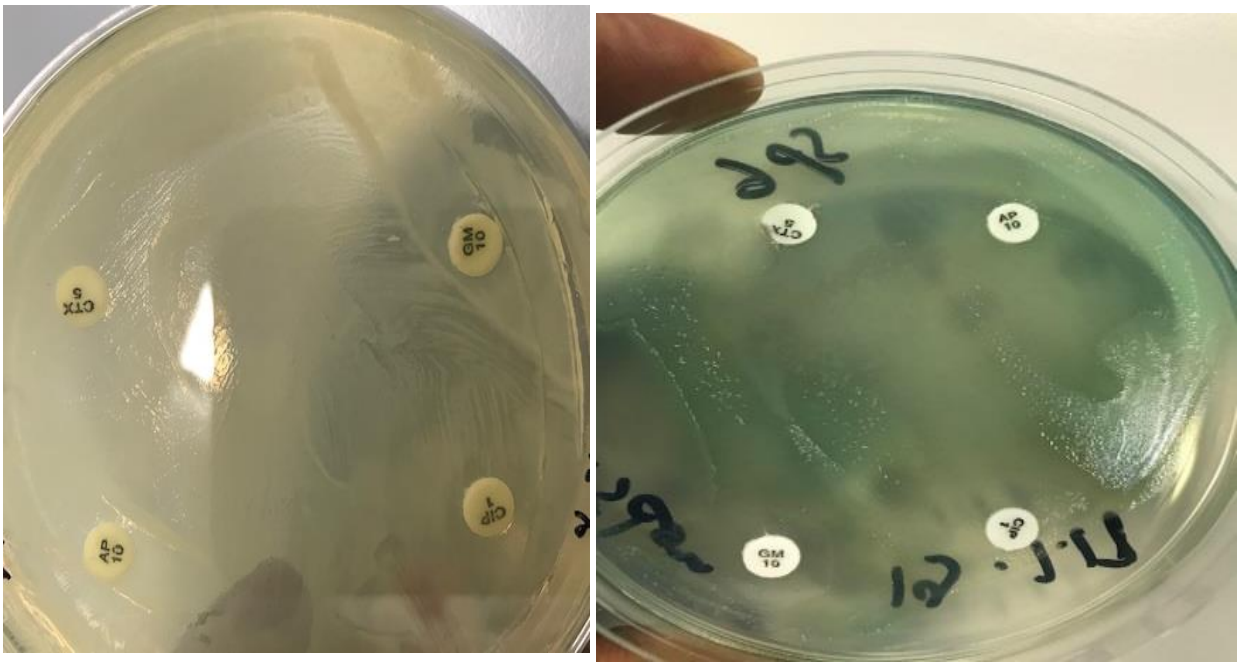


Figure 23. Image showing the zone of inhibition of bacteria growth by selected antibiotic.

Gentamycin (10µg), penicillin (1µg), fusidic acid (10µg), and erythromycin (5µg) were used to test on Gram positive samples while ciprofloxacin (1µg), gentamycin (10µg), cefotaxime (5µg) and ampicillin (10µg) all from Mast Group™ pharmaceutical were used to perform sensitivity on Gram negative samples. Interestingly, the results revealed that among all the Gram positive bacteria tested, only *S. epidermidis* showed sensitivity to gentamycin while *S. epidermidis* was sensitive to penicillin (table 1). By this result, the sensitivity of *S. epidermidis* isolated from blepharitis patients

to gentamycin is a development that would enhance the treatment of blepharitis. In a similar study by Okesola and Salako 2010, it was found that *S. aureus* recorded high susceptibility rate with erythromycin in the treatment of conjunctivitis although blepharitis as one of the anterior eye infection with conjunctivitis was not among the list of case studied. However, this study support the idea that gentamycin can be useful as a treatment option in anterior eye infection including blepharitis. While *P. aeruginosa* was sensitive to only ciprofloxacin*, P. mirabilis* was resistant to all the antibiotics in which it was tested upon (table 3).

Group A/No	Strain	Gentamycin(10µg) ZDB ≥ 18	Result	Penicillin (1µg)ZDB ≥ 26	Result	Fusidic Acid (10µg)ZDB ≥ 24	Result	Erythromycin (5µg) ZDB ≥21	Result
S1	*S. haemolyticus*	14mm	I	0	I	0	I	11mm	I
S2	*S.* haemolyticus	15mm	I	3mm	I	18mm	I	10mm	I
S3	*S. cohnii*	17mm	I	10mm	I	10mm	I	10mm	I
S4	*S. cohnii*	14mm	I	9mm	I	11	I	12mm	I
S5	*S. saprophyticus*	12mm	I	9mm	I	6mm	I	13mm	I
S6	*S. saprophyticus*	10mm	I	12mm	I	3mm	I	13mm	I
S7	*S. aureus*	7mm	I	3mm	I	8mm	I	15mm	I
S8	*S. aureus*	8mm	I	3mm	I	22mm	I	16mm	I
S9	*S. epidermis*	18mm	S	26mm	S	3mm	I	13mm	I
S10	*B. aerophilus*	10mm	I	2mm	I	5mm	I	6mm	I

Group B/No	Strain	Ciprofloxacin (1µg). ZDB ≥12	Result	Gentamycin (10µG). ZDB ≥4	Result	Cefotaxime (5µg). ZDB ≥19	Result	Ampicillin (10µg). ZDB ≥ 14	Result
S11	*P. mirabilis*	8mm	I	4mm	S	3mm	I	3mm	I
S12	*P. aeruginosa*	27mm	S	3mm	I	3mm	I	3mm	I
S13	*P. aeruginosa*	27mm	S	3mm	I	3mm	I	3mm	I

Abbreviation: ZDB- Zone diameter breakpoint. R- Resistance. I- Insensitive.

Table 3. Susceptibility test with the antibiotics.

3.1.6 Socio demographic characteristic of the patients.

Table 3 shows the age and medical condition of the patients that presented with blepharitis. Most patients were within 30 years and overall average of 46 years. However, 58.3% of the population are above 50 years confirming the findings by Lindstrom 2009 who observed that blepharitis was more prevalence in aging population. This finding was also supported by Nicholas et al., who reported that 50 years was found to be the mean age of patients diagnosed with blepharitis. This collaborated the idea that blepharitis is prevalence is high among the middle-aged adults. Moreover, the higher number of females found to have blepharitis in this study was supported by McCulley et al., 1982, McCulley and Dougherty, 1985). Furthermore, 10 out of the total number (12) of the subjects were found to be suffering different ailment that compromised the immune system which collaborated the findings by Tobisnick, 2003. The study also find that the incidence of blepharitis is more on females (table 4). This study affirms that blepharitis is common among females.

Organisms	Gender	Age	Occupation	Medical condition
S. aureus	Male	30	Trader	Malaria
S. aureus	Female	56	Farmer	Typhoid
S. epidermidis	Female	19	Farmer	Fever
S. haemolyticus	Male	55	Security	Diabetes
S. haemolyticus	Female	45	Secretary	Urinary tract infection
S. cohnii	Male	60	Farmer	Fever
S. saprophyticus	Female	58	Trader	Urinary tract infection
S. saprophyticus	Female	22	Student	No abnormality detected
P. mirabis	Male	60	Retired	Urinary tract infection
B. aerophilus	Female	60	Farmer	No abnormality detected
P. aeruginosa	Male	33	Student	Typhoid
P. aeruginosa	Female	60	Housewife	Typhoid

Table 4. Isolates from blepharitis patients including their gender, occupation and associated systemic condition

3.1.7. Social demography of study population.

The pie chart in Figure 24 shows the proportional value of the isolated organisms with coagulase negative staphylococcus seen as the largest in number of isolated organisms. A total of 20 patients were clinically diagnosed to have blepharitis. However, only 12 of the samples were able to be isolated and identified while 8 samples were fastidious to grow. The age and gender distribution of the subjects (Figure 23). Furthermore, the presence of *S. aureus, S. epidermidis*, and *P. auruginosa* confirms the findings by Lee et al., 2012, Watters et al., 2017 of bacteria responsible for the blepharitis infection. Moreover, study carried out by Okesola and Salako, 2010, in Ibadan Nigeria, found that *Staphylococcus* and coagulase-negative staphylococci were the most bacteria species isolated from patients diagnosed of bacteria conjunctivitis. However, and most remarkable, coagulase negative S*taphylococcus* was found to be the most bacteria species identified in the study. This result confirms the increasing case of ocular infections due to coagulase negative staphylococcus bacteria (Chirinos-Saldana, 2003).

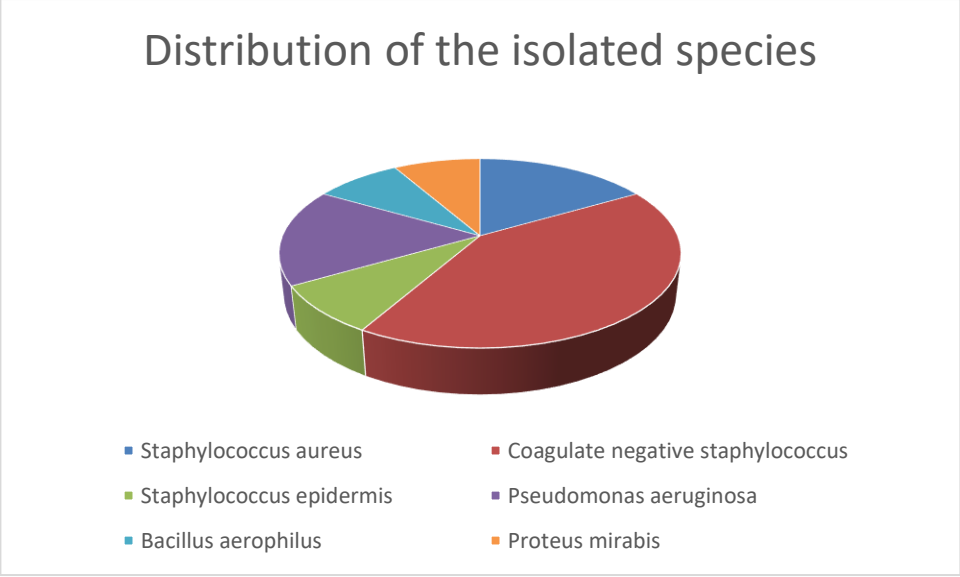


Fig. 24. Pie chart showing all the isolated bacteria species.

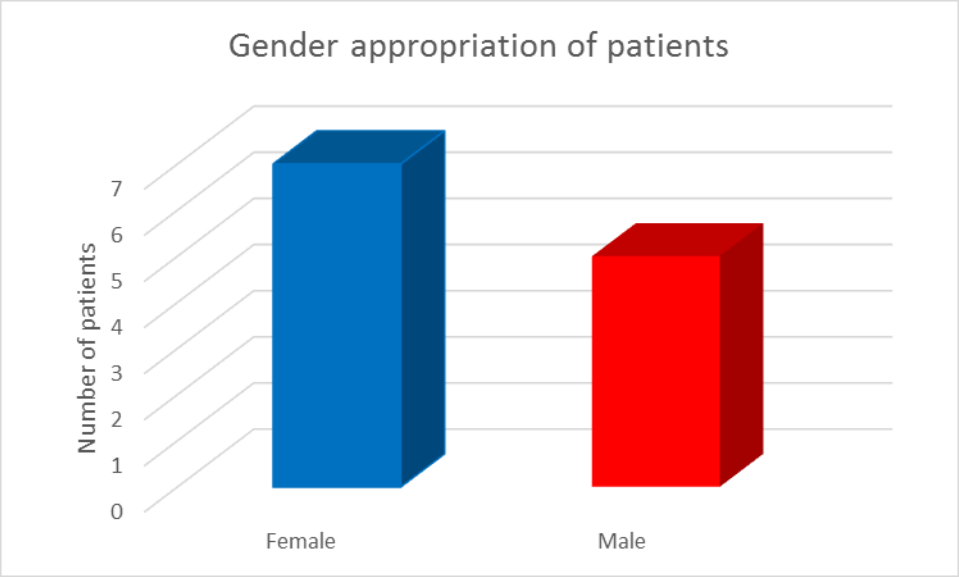


Fig. 25. Bar graph showing gender distribution of the patients.

The graph in figure 25 shows the gender distribution of the patients. The number of female patients is seven (7) constituting 58.3% of the study populations as against 41.7% of four (4) male patients recorded. This finding collaborated with the research of Putnam 2016, which recorded high prevalence of anterior blepharitis on female. However, a similar study in South West Ethiopia by Teweldemedin et al., 2017, recorded higher number of male to females in his study on a broad range of ocular infection and antimicrobial susceptibility although his findings did not indicate how many number of females that were tasted; so this evidence cannot be used to contradict the findings in this study. A higher number of subjected needs to be analyzed to draw a more accurate conclusion.

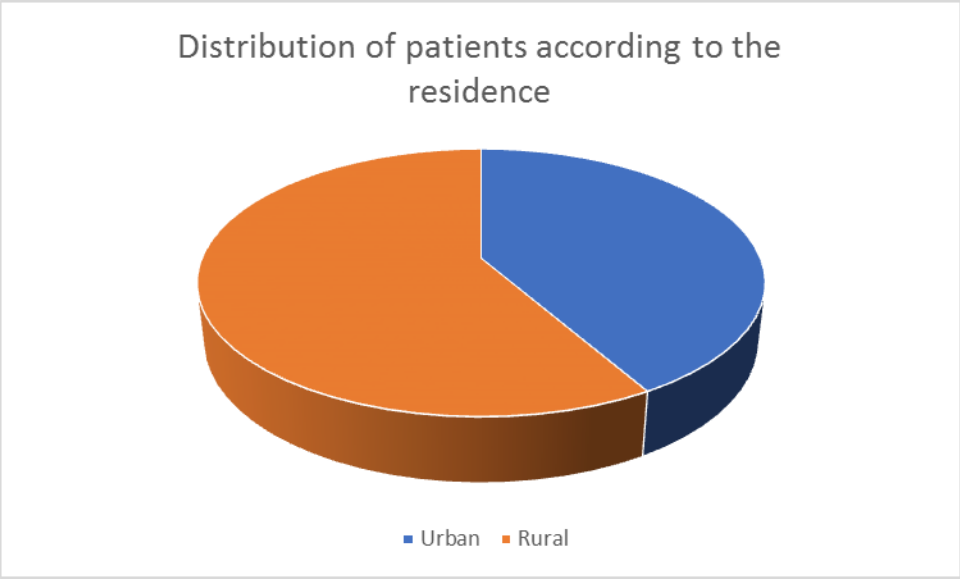


Fig. 26. Pie chart showing the distribution of residential areas of the patients from which organisms were isolated.

The residence of the patients was taken into consideration in this study. Figure 26 shows that most of the patients in the study were found in the urban area although, previous study suggested that most blepharitis cases were found in the rural community. However, Teweldemedin et al., in their study of external ocular infection recorded that the number of patients from the urban area was higher than the number recorded in the rural area although blepharitis been one of the infections of the ocular adnexia was not the main focus of the study. In this study, we tasted equal number of male and female for the purpose knowing the prevalence among the genders and analysis. However, there is no yet an evidence to suggest why female are more affected than the male.

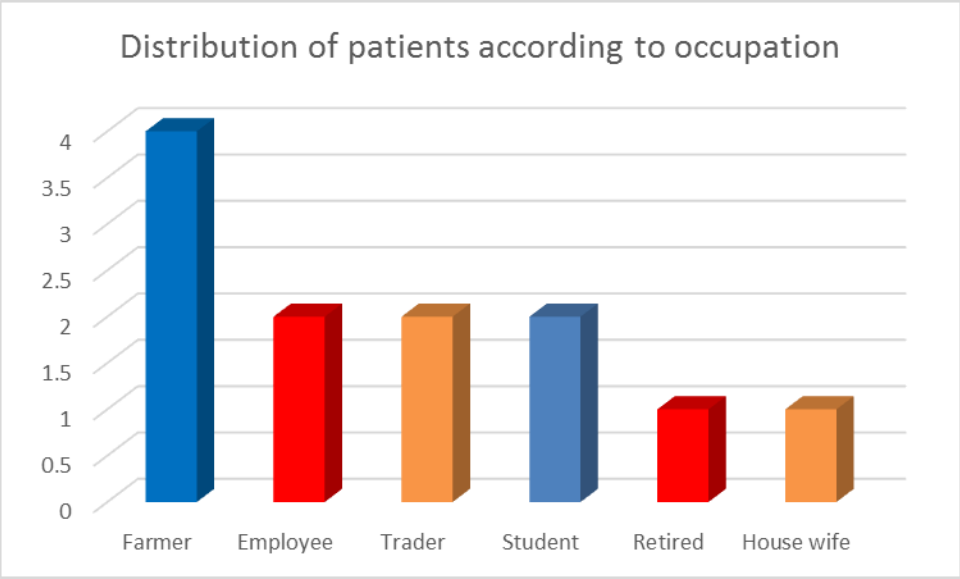


Figure 27. Shows the occupation of patients in view.

Chapter 4

A. CONCLUSION.

Blepharitis is known as one of the anterior ocular infection often encountered in clinical practice. It has been noted that clinical presentation does not provide enough diagnostic evidence in management thereby prompting the need for further microbial analysis. More so, the relapse in the treatment of blepharitis is not limited to any of the causative agent. Many common causes of blepharitis are not well defined, which makes is difficult to give a definite pathophysiology in the cause of the disease (Lee et al., 2002). This could be attributed to the limitations encountered in identifying the causative organisms, which is performed conventionally in the clinic through the culture approach (Ta et al., 2003). This finding was supported in a study done by Polage et al., 2005 and Tuttle et al., 2011, which revealed that the microorganisms are difficult to culture, although, most clinical bacterial isolates can be identified in an ample time and accurately through this technique while a discrete identification of the isolates can be attained within 24 hours.

These lapses influenced the need for further investigation towards enhancing the treatment of blepharitis with advanced technique that would enable identifying the causative organism. We believe that a successful application of advanced clinical investigation on the pathogenicity of this condition would in turn enhance it' treatment and possible cure of blepharitis. To start this process, we conducted sight test and anterior eye examinations on patients with suspected case of blepharitis in Abia State University Teaching Hospital, Aba, Nigeria. In all, 20 patients were diagnosed with blepharitis and samples collected aseptically from the eyelid margins of patients that presented at the ophthalmic clinic with blepharitis and isolated and stored in -80®C before been shipped to the microbiology laboratory of LJMU with approval for the transfer of UN3373 biological sample. The isolated samples identified through the conventional culture identification method showed that most of the organism are staphylococcal species including *S. aureus* and *coagulase negative staphylococcus*; *P. aeruginosa, P. mirabilis* and *B. aerophilus* specie although not all the samples were identified. However, the results suggested that there are potentially new discovery of more strain of staphylococcal bacteria that are responsible in causing blepharitis. This collaborated with the study by Lee et al., 2002, who noted the

eyelash and tear samples are polymicrobial. In order to confirm our result from the cultured sample, we extracted the DNA of the samples, amplified the result and sequenced the products. We obtain a more clarified result, which reveals different strain of bacteria species as been responsible in the aetiology of blepharitis. Molecular identification method is a vital and more reliable tool in the investigation of microorganisms. This finding was supported in a study done by Polage et al., 2005 and Tuttle et al., 2011, which revealed that the microorganisms are difficult to culture, although, most clinical bacterial isolates can be identified in an ample time and accurately through this technique, while a discrete identification of the isolates can be attained within 24 hours. Also, this study proves that molecular identification technique is a choice method in identifying bacteria. Furthermore, we investigated the relationship between the organisms by expounding the taxonomic positions of different species in a phylogenic tree. To advance the aim of this study, we introduced a novel technique of using the SEM to produce a phenotypic profile of the agents which has not been employed in the previous studies of blepharitis. According to our finding, coagulase negative staphylococcal bacteria species were the major strain identified followed by S. *aureus* and *P. aeruginosa*. However, these wasn't in total agreement of the previous study on blepharitis suggesting that more study and a larger sample size need to be analyzed to support these findings.

Although there is no available data on global prevalence of blepharitis, a study in the US demonstrated that about 37-47% of patients seen in ophthalmology and optometry clinics present with history of blepharitis (Lemp and Nicholas, 2009). Proper identification of causative organisms with the help of more sophisticated and enhanced technique of molecular identification is believed to proffer better outcome in clinical practice. It also provides more robust alternative of identifying multiple organisms in a single technique (Patel, 2002). Currently, erythromycin with its antimicrobial and anti-inflammatory efficacy stands as the drug of choice for infectious blepharitis in patients who are sensitive to bacitracin, but resistance rate of about 50% remains a big problem (Lemp and Nicholas 2009). Bacteria biofilms is said to be at forefront of sustained infection and damages caused by inflammatory processes (Lynch and Robertson 2008). The resistance to the newest formulation of treatment with azithromycin has been reported, which results from the sub therapeutic concentration of antibiotics over extended period of time (Verdman and Colby, 2011). A research from the Moorefield eye hospital revealed that chronic blepharitis is the major risk factor for bleb-related infection following

surgery, mainly caused by bacteria able to form biofilms (Rai et al., 2016). Recent trial with antibiotics has shown improvement in the control of blepharitis however; more clinical trials are needed to identify more effective therapies (Pflugfelder et al., 2014). Several studies have suggested the use of quorum sensing inhibitors through which the bacteria use to synchronize the expression of the specific genes to be seen as a possible drug target in tackling antibiotic resistance (Jakobsen et al., 2012).

It is an untapped new channel of delivery of antibiotics with great possibility of developing unique micro-selective antibacterial agents that limit the occurrence of bacterial resistance (Wencewicz et al., 2013). Although this method does not lead to discovery of new bacterial scaffolds, neither validating new bacterial target known to be extremely difficult to identify. Finding a useful biological pathway through which bacterial walls can be penetrated remains a biggest challenge (Braun and Endrib, 2007). An ideal membrane transport pathway is suggested to be specific to bacterial cell walls to rid the toxicity of bacteria, which enables its virulence against eukaryotic cells.

B. FURTHER WORK

In a rarefaction pyrosequence analysis to identify the 16S rRNA gene in collected samples from blepharitis patients, showed that eyelash and tear samples are highly polymicrobial suggesting further study on identifying causative agents through sequencing could be useful in obtaining more information on the cause of disease and management. Also, a larger number of samples need to be analysed.

REFERENCE

Adegboye, M.B., Zakari, S., Ahmed, B.A. and Olufemi, G.H. 2018. Knowledge, awareness and practice of infection control by health care workers in the intensive care units of a tertiary hospital in Nigeria. *African health sciences*, 18 (1), 72-78.

Albietz, J.M. and Lenton, L.M., 2006. Effect of antibacterial honey on the ocular flora in tear deficiency and meibomian gland disease. *Cornea*, *25*(9), 1012-1019.

Alkatan, H.M., Al-Mohizea, A. and Alsuhaibani, A. 2017. A case of localized amyloidosis of the eyelid misdiagnosed as recurrent chalazion. *Saudi journal of ophthalmology*, 31 (3), 180-182.

American Academy of Ophthalmology. 2013. Preferred Practice Pattern: Blepharitis. Available at: http://one.aao.org/preferred-practice-pattern/blepharitisppp. [Accessed on 20 November 2019].

Amsalu, A., Abebe, T., Mihret, A., Delelegne, D. and Tadesse, E. 2015. Potential bacterial pathogens of external ocular infections and their antibiotic susceptibility pattern at Hawassa University teaching and referral Hospital, Southern Ethiopia. *African Journal of Microbiology Research*, 9 (14), 1012-1019.

Bertino Jr, J.S. 2009. Impact of antibiotic resistance in the management of ocular infections: The role of current and future antibiotics. *Clinical ophthalmology (Auckland, NZ)*, 3, 507.

Biernat, M.M., Rusiecka-Ziółkowska, J., Piątkowska, E., Helemejko, I., Biernat, P. and Gościniak, G. 2018. Occurrence of Demodex species in patients with blepharitis and in healthy individuals: a 10-year observational study. *Japanese journal of ophthalmology*, 62 (6), 628-633.

Bodor, N., Loftsson, T., & WU, W.M., 1992. Metabolism, Distribution and Transdermal Permeation of a Soft Corticosteroid, Loteprednol Etabonate. *Pharmaceutical Research*. 9. 1275-1278.

Boles, B.R., Thoendel, M., Roth, A.J. and Horswill, A.R., 2010. Identification of genes involved in polysaccharide-independent *Staphylococcus aureus* biofilm formation. *PloS one*, *5*(4).

Braun, V. and Endriß, F. 2007. Energy-coupled outer membrane transport proteins and regulatory proteins. *BioMetals*, 20(3-4), 219-231.

Bruce Jackson, W. 2016. Blepharitis. current strategies for diagnosis and management. *Canadian Journal of Ophthalmology,* 43**,** 170-179.

Cehajic-Kapetanovic, J. and Kwartz, J. 2010. Augmentin duo™ in the treatment of childhood blepharokeratoconjunctivitis. *Journal of pediatric ophthalmology and strabismus*, 47 (6), 356-360.

Chabaud, M., Lubberts, E., Joosten, L., van den Berg, W. and Miossec, P. 2001. IL-17 derived from juxta-articular bone and synovium contributes to joint degradation in rheumatoid arthritis. *Arthritis Research & Therapy*, 3 (3), 168.

Chalita, M.R., Höfling-Lima, A.L., Paranhos, A., Schor, P. and Belfort, R. 2004. Shifting trends in in vitro antibiotic susceptibilities for common ocular isolates during a period of 15 years' *American Journal of Ophthalmology*, 137(1), 43–51.

Chatterjee, S. and Agrawal, D. 2016. Multi-drug resistant *Pseudomonas aeruginosa* keratitis and its effective treatment with topical Colistimethate, *Indian Journal of Ophthalmology*, 64(2), 153.

Cheng, A.M., Sheha, H. and Tseng, S.C. 2015. Recent advances on ocular *Demodex* infestation. *Current opinion in ophthalmology*, 26 (4), 295-300.

Chien, A.-C., Hill, N.S. and Levin, P.A. 2012. 'Cell size control in bacteria', *Current Biology*, 22(9), 340–349.

Chirinos-Saldaña, P., de Lucio, V.M.B., Hernandez-Camarena, J.C., Navas, A., Ramirez-Miranda, A., Vizuet-Garcia, L., Ortiz-Casas, M., Lopez-Espinosa, N., Gaona-Juarez, C. and Bautista-Hernandez, L.A. 2013. Clinical and microbiological profile of infectious keratitis in children. *BMC ophthalmology*, 13 (1), 54.

Clover, J. 2020. Cornea: *Slit-Lamp Biomicroscopy*.

Cole, N., Hume, E., Khan, S., Madigan, M., Husband, A.J., Garthwaite, L. and Willcox, M., 2005. Contribution of the cornea to cytokine levels in the whole eye induced during the early phase of Pseudomonas aeruginosa challenge. *Immunology and cell biology*, *83*(3), 301-306.

Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, et al. 2009. Bacterial community variation in human Amsalu, A., Abebe, T., Mihret, A., Delelegne, D. and Tadesse, E. (2015) Potential bacterial pathogens of external ocular infections and their antibiotic susceptibility pattern at Hawassa University teaching and referral Hospital, Southern Ethiopia. *African Journal of Microbiology Research*, 9 (14), 1012-1019.

Daniel, M.C., O'Gallagher, M., Hingorani, M., Larkin, D.F., Tuft, S. and Dahlmann-Noor, A. 2017. Medical management of blepharokeratoconjunctivitis in children: a Delphi consensus. *Journal of pediatric ophthalmology and strabismus*, 54 (3), 156-162.

De Paula, A., Oliva, G., Barraquer, R.I. and de la Paz, M.F. 2019. Prevalence and antibiotic susceptibility of bacteria isolated in patients affected with blepharitis in a tertiary eye centre in Spain. *European journal of ophthalmology*, 11, (2), 211-235.

Deutch, C. 2017. Limited effectiveness of over-the-counter plant preparations used for the treatment of urinary tract infections as inhibitors of the urease activity from *Staphylococcus saprophyticus*. *Journal of applied microbiology*, 122 (5), 1380-1388.

Din, N., & Patel, N.N., 2012. Blepharitis – a review of diagnosis and management. *Int J Ophthalmol Prac*. 3(4), 150–155.

Donnenfeld, e. d., mah, f. s. and macdonald, m. 2008. New considerations in the treatment of anterior and posterior blepharitis. *refract eye care,* 12**,** 3-14.

Dougherty, J.M., McCulley, J.P., Silvany, R.E. and Meyer, D.R. 1991. The role of tetracycline in chronic blepharitis. *Investigative Ophthalmology Vision Science*, 32 (11), 2970-2975.

Driscoll, K.E., Hassenbein, D.G., Howard, B.W., Isfort, R.J., Cody, D., Tindal, M.H., Suchanek, M. and Carter, J.M., 1995. Cloning, expression, and functional characterization of rat MIP-2: a neutrophil chemoattractant and epithelial cell mitogen. *Journal of Leukocyte Biology*, *58*(3), 359-364.

Duncan, K., & Jeng, B.H., 2015. Medical Management of Blepharitis. *Current Opin Ophthalmol.* 26, 289-294.

Du Toit, N. and Van Zyl, L. 2013. The red eye. *South African Family Practice*, 55 (1), 33-40.

Eberhardt, M., & Rammohan, G., 2017. Blepharitis. Stat Pearls Publishing: Jan 2018. [Online] Available at: https://www.ncbi.nih.gov/bwks/NBK4593051. [Accessed on 20, 2019].

Endimiani, A., Luzzaro, F., Brigante, G., Perilli, M., Lombardi, G., Amicosante, G., Rossolini, G.M. and Toniolo, A. 2005. *Proteus mirabilis* bloodstream infections: risk factors and treatment outcome related to the expression of extended-spectrum β-lactamases. *Antimicrobial agents and chemotherapy*, 49 (7), 2598-2605.

Eriksson, A., Giske, C.G. and Ternhag, A. 2013. The relative importance of *Staphylococcus saprophyticus* as a urinary tract pathogen: distribution of bacteria among urinary samples analysed during 1 year at a major Swedish laboratory. *Apmis*, 121 (1), 72-78.

Ficker, L., Ramakrishnan, M., Seal, D. and Wright, P., 1991. Role of cell-mediated immunity to staphylococci in blepharitis. *American journal of ophthalmology*, *111*(4), 473-479.

Fierer, N., Hamady, M., Lauber, C.L. and Knight, R. 2008. The influence of sex, handedness, and washing on the diversity of hand surface bacteria. *Proceedings of the National Academy of Sciences*, 105 (46), 17994-17999.

Fredrick, D. R. 2018. Conjunctivitis Beyond the Neonatal Period. *In:* Long, S. S., Prober, C. G. & Fischer, M. (eds.) *Principles and Practice of Pediatric Infectious Diseases (Fifth Edition).* Elsevier.

Freitas, L.L., Soriano, E., Muccioli C., Hofling-Lima, A.L., & Belfort, R., 2007. Efficacy and tolerability of a combined moxifloxacin/dexamethasone formulation for topical prophylaxis and reduction of inflammation in phacoemulsification: a comparative, double masked clinical trial. *Current Medical Research and Opinion*. 23(12), 3123–3130.

Forton, F., Germaux, M.-A., Brasseur, T., De Liever, A., Laporte, M., Mathys, C., Sass, U., Stene, J.-J., Thibaut, S. and Tytgat, M. 2005. Demodicosis and rosacea: epidemiology and significance in daily dermatologic practice. *Journal of the American Academy of Dermatology*, 52 (1), 74-87.

Graham, J.E., Moore, J.E., Jiru, X., Moore, J.E., Goodall, E.A., Dooley, J.S., Hayes, V.E., Dartt, D.A., Downes, C.S. and Moore, T.C. 2007. Ocular pathogen or commensal: a PCR-based study of surface bacterial flora in normal and dry eyes. *Investigative ophthalmology & visual science*, 48 (12), 5616-5623.

Geerling, G., Tauber, J., Baudouin, C., Goto, E., Matsumoto, Y., O'Brien, T., Rolando, M., Tsubota, K. and Nichols, K.K., 2011. The international workshop on meibomian gland dysfunction: report of the subcommittee on management and treatment of meibomian gland dysfunction. *Investigative ophthalmology & visual science*, *52*(4), 2050-2064.

Hall, B. G. and M. Barlow 2006. Phylogenetic analysis as a tool in molecular epidemiology of infectious diseases. *Annals of epidemiology* 16(3), 157-169.

Hazlett, L.D. 2004. Corneal response to Pseudomonas aeruginosa infection, Progress in Retinal and Eye Research, 23(1), 1–30.

Herron, M.D., O'Reilly, M.A. and Vanderhooft, S.L. 2005. Refractory Demodex folliculitis in five children with acute lymphoblastic leukemia. *Pediatric dermatology*, 22 (5), 407-411.

Holland, E.J., McDonald, M.B., Parekh, J.G. and Sheppard, J.D., 2014. Antibiotic resistance in acute postoperative endophthalmitis. *Ophthalmology*, *121*(11), 1-59.

Hsu, C.-K., Hsu, M.M.L. and Lee, J.Y.-Y. 2009. Demodicosis: A clinicopathological study. *Journal of the American Academy of Dermatology*, 60 (3), 453-462.

Hu, Y.-y., Cai, J.-c., Zhang, R., Zhou, H.-w., Sun, Q. and Chen, G.-x. 2012. Emergence of *Proteus mirabilis* harbouring blaKPC-2 and qnrD in a Chinese hospital. *Antimicrobial agents and chemotherapy*, 56 (5), 2278-2282.

Jackson, W.B. 2008. Blepharitis: current strategies for diagnosis and management. *Canadian journal of ophthalmology*, 43 (2), 170-179.

Kabataş, N., Doğan, A.Ş., Kabataş, E.U., Acar, M., Biçer, T. and Gürdal, C. 2017. The effect of *Demodex* infestation on blepharitis and the ocular symptoms. *Eye & Contact Lens: Science & Clinical Practice*, 43 (1), 64-67.

Karimian, F., Zarei-Ghanavati, S., Baradaran-Rafii, A., Jadidi, K. and Lotfi-Kian, A. 2011. Microbiological evaluation of chronic blepharitis among Iranian veterans exposed to mustard gas: a case-controlled study. *Cornea*, 30 (6), 620-623.

Kernacki, K.A., Barrett, R.P., Hobden, J.A. and Hazlett, L.D. 2000. Macrophage inflammatory protein-2 is a mediator of Polymorphonuclear Neutrophil influx in ocular bacterial infection. *The Journal of Immunology*, 164(2), 1037–1045.

Kheirkhah, A., Casas, V., Li, W., Raju, V.K. and Tseng, S.C. 2007. Corneal manifestations of ocular demodex infestation. *American journal of ophthalmology*, 143 (5), 743-749.

Kim, J.T., Lee, S.H., Chun, Y.S. and Kim, J.C., 2011. Tear cytokines and chemokines in patients with Demodex blepharitis. *Cytokine*, *53*(1), 94-99.

Koo, Kim, H., K, T.H., Kim, K.W., Wee, S.W., Chun, Y.S. and Kim, J.C., 2012. Ocular Surface Discomfort and Demodex: Effect of Tea Tree Oil Eyelid Scrub in Demodex Blepharitis*. Journal of Korean Medical Science.*27(12), 1574-1579.

Kumar S., Stecher G., and Tamura K. 2015. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution.*

Lausch, R.N., Chen, S.-H., Tumpey, T.M., SU, Y.-H. and Oakes, J.E. 1996. Early cytokine synthesis in the excised mouse cornea. *Journal of Interferon & Cytokine Research*, 16(1), 35–40.

Lee, S.H., Oh, D.H., Jung, J.Y., Kim, J.C. and Jeon, C.O. 2012. Comparative ocular microbial communities in humans with and without blepharitis. *Investigative ophthalmology & visual science*, 53 (9), 5585-5593.

Lemp, M. and Nicholas, K. 2009. Blepharitis in the United States 2009. A Survey-based Perspective on Prevalence and Treatment. *The Ocular Surface*, 7(2), S1-S14.

Leonard, B.E. 1990. 'Book reviews: Basic Neurochemistry: Molecular, cellular and medical aspects. *Journal of Psychopharmacology*, 4(1), 49–49.

Liang, L., Safran, S., Gao, Y., Sheha, H., Raju, V. and Tseng, S.C. 2010. Ocular demodicosis as a potential cause of pediatric blepharoconjunctivitis. *Cornea*, 29 (12), 1386-1391.

Lin, J., Morrison, D. and Oriade, D. 2005. The discovery of a multi-resistant *Staphylococcus haemolyticus* clone in the hospital and community environment in south western Nigeria. *Ostomy/wound management*, 51 (1), 67-70.

Lindsley, K., Matsumura, S., Hatef, E. and Akpek, E.K. 2012. Interventions for chronic blepharitis. *Cochrane Database of Systematic Reviews* (5).

Lindstrom, R. 2009. The Effects of Blepharitis on Ocular Surgery. *The Ocular Surface*, 7(2), S19-S20.

Lynch, A. Robertson, G. 2008. Bacterial and Fungal Biofilm Infections. *Annual Review of Medicine*, 59(4), 415-428.

Madhukumar, S. and Ramesh, G. 2012. Study about awareness and practices about health care waste management among hospital staff in a medical college hospital, Bangalore. *Iranian Journal of Basic Medical Sciences*, 3 (1), 7-11.

Maizels, R.M., Pearce, E.J., Artis, D., Yazdanbakhsh, M. and Wynn, T.A. 2009. Regulation of pathogenesis and immunity in helminth infections. *Journal of Experimental Medicine*, 206 (10), 2059-2066.

Marsden, J., Stevens, S. and Ebri, A., 2014. How to measure distance visual acuity. *Community eye health*, *27,* (85), p.16.

McDonald, M.B., M.D. 2009, "Blepharitis: the importance of proper diagnostics and treatment", *Ocular Surgery News,* vol. 27, no. 21, 58-59.

Miller, D., 2017. Update on the epidemiology and antibiotic resistance of ocular infections. *Middle East African journal of ophthalmology*, 24 (1), 30.

Mohager, M.O., Kaddam, L.A. and Mohager, S.O. 2016. External ocular bacterial infections among Sudanese children at Khartoum State, Sudan. *African Journal of Microbiology Research*, 10 (40), 1694-1702.

Mohamed Mostafa, E., Abdellah, M.M., Elhawary, A.M. and Mounir, A. 2019. Noncontact Meibography in Patients with Keratoconus. *Journal of Ophthalmology*, 2019.

Mostovoy, D., Vinker, S., Mimouni, M., Goldich, Y., Levartovsky, S. and Kaiserman, I. 2018. The association of keratoconus with blepharitis. *Clinical and Experimental Optometry*, 101 (3), 339-344.

Moyes Eye Center, 2018. [Photograph]. At Northland: 5151 NW 88TH Street Kanas City. [Online Photograph] [Viewed on 20th November 2019] Available at http://moyeseye.com/blepharitis.

Mcculley, J. P. & Shine, W. E. 2000. Changing concepts in the diagnosis and management of blepharitis. *Cornea,* 19**,** 650-658.

Nelson, J.D., Shimazaki, J., Benitez-del-castillo, J.M., Craig, J.P., Mcculley, J.P., Den, S. and Foulks, G.N., 2011. The international workshop on meibomian gland dysfunction: report of the definition and classification subcommittee*. Investigative Ophthalmology & Visual Science.* 52(4), 1930.

Nicholls, S.G., Oakley, C.L., Tan, A. and Vote, B.J. 2017. Demodex species in human ocular disease: new clinicopathological aspects. *International ophthalmology*, 37 (1), 303-312.

Numasaki, M., Watanabe, M., Suzuki, T., Takahashi, H., Nakamura, A., McAllister, F., Hishinuma, T., Goto, J., Lotze, M.T. and Kolls, J.K. 2005. IL-17 enhances the net angiogenic activity and in vivo growth of human non-small cell lung cancer in SCID mice through promoting CXCR-2-dependent angiogenesis. *The Journal of Immunology*, 175 (9), 6177-6189.

Oboki, K., Ohno, T., Saito, H. and Nakae, S. 2008. Th17 and allergy. *Allergology International*, 57 (2), 121-134.

Oechsler, R.A., Feilmeier, M.R., Ledee, D.R., Miller, D., Diaz, M.R., Fini, M.E., Fell, J.W. and AlfonAdegboye, M.B., Zakari, S., Ahmed, B.A. and Olufemi, G.H. 2018. Knowledge, awareness and practice of infection control by health care workers in the intensive care units of a tertiary hospital in Nigeria. *African health sciences*, 18 (1), 72-78.

Ogita, H., Ikeda, W. and Takai, Y. 2008. Roles of cell adhesion molecules nectin and nectin-like molecule-5 in the regulation of cell movement and proliferation, *Journal of Microscopy*, 231(3), 455–465.

Okesola, A. and Salako, A. 2010. Microbiological profile of bacterial conjunctivitis in Ibadan, Nigeria. *Annals of Ibadan postgraduate medicine*, 8 (1), 20-24.

Patel, J .b., 2001. 16s rrna gene sequencing for bacterial pathogen identification in the clinical laboratory. *Molecular diagnosis*, 6 (4), 313-321.

Payne, D., Gwynn, M., Holmes, D. and Pompliano, D. 2006. Drugs for bad bugs: confronting the challenges of antibacterial discovery. *Nature Reviews Drug Discovery*, 6(1), 29-40.

Pflugfelder, S.C., Karpecki, P.M. and Perez, V.L., 2014. Treatment of blepharitis: recent clinical trials. *The ocular surface*, *12*(4), 273-284.

Popoola, O., Agu, G., Oyeyipo, F. and Thomas, B. 2019. Biochemical and bacteriological profiles of asymptomatic bacteriuria among school children in Ago-Iwoye, Nigeria. *African Journal of Clinical and Experimental Microbiology*, 20 (4), 299-305.

Probst, L.E., 2005. Bacterial Eyelid Infections. In Krachmer, J.H., Mannis, M.J., and Holland, E.J. *Cornea 2nd Edition. Philadelphia. Elsevier Mosby*. 495-500.

Putnam, C. M. 2016. Diagnosis and management of blepharitis: an optometrist's perspective. *Clinical Optometry,* 8**,** 71.

Qiu, T.Y., Yeo, S. and Tong, L. 2018. Satisfaction and convenience of using terpenoid-impregnated eyelid wipes and teaching method in people without blepharitis. *Clinical ophthalmology (Auckland, NZ)*, 12, 91.

Raskin, E.M., Speaker, M.G., and Laibson, P.R., 1992. Blepharitis. *Infectious Disease clinics of North America.* 6(4), 777-787.

Reischl, U., Emler, S., Horak, Z., Kaustova, J., Kroppenstedt, R.M., Lehn, N. and Naumann, L. 1998. Mycobacterium bohemicum sp. nov., a new slow-growing scotochromogenic mycobacterium. *International Journal of Systematic and Evolutionary Microbiology*, 48 (4), 1349-1355.

Rhee, S.S., & Mah, F.S., 2007. Comparison of Tobramycin 0.3%/Dexamethasone 0.1% and Tobramycin 0.3%/Loteprednol 0.5% in The Management of Blepharo-keratoconjunctivitis. *Advances in Therapy*. 24(1), 60-67.

Saitou N. and Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*. (4), 406-425.

Salem, D.A.-B., El-shazly, A., Nabih, N., El-Bayoumy, Y. and Saleh, S. 2013. Evaluation of the efficacy of oral ivermectin in comparison with ivermectin–metronidazole combined therapy in the treatment of

ocular and skin lesions of Demodex folliculorum. *International Journal of Infectious Diseases*, 17 (5), 343-347.

Schabereiter-Gurtner, C., Pinar, G., Lubitz, W. and Rölleke, S. 2001. An advanced molecular strategy to identify bacterial communities on art objects. *Journal of Microbiological Methods*, 45 (2), 77-87.

Scheinfeld, N., & Berk, T., 2010. A review of the diagnosis and treatment of rosacea. *Postgrad Med*. 122 (1), 139–14.

Shiferaw, B., Gelaw, B., Assefa, A., Assefa, Y. and Addis, Z., 2015. Bacterial isolates and their antimicrobial susceptibility pattern among patients with external ocular infections at Borumeda hospital, Northeast Ethiopia. *BMC ophthalmology*, *15* (1), 103.

Schubert, S., Schwertz, H., Weyrich, A.S., Franks, Z.G., Lindemann, S., Otto, M., Behr, H., Loppnow, H., Schlitt, A., Russ, M. and Presek, P., 2011. *Staphylococcus aureus* α-toxin triggers the synthesis of B-cell lymphoma 3 by human platelets. *Toxins*, *3* (2), 120-133.

Sethuraman, U. and Kamat, D. 2009. The red eye: evaluation and management. *Clinical pediatrics*, 48 (6), 588-600.

Sin-Yeang T. Kitson L, Syed A. Alan and Suat-Cheng P. 2016. Antibacterial Action of Curcumin against *Staphylococcus aureus*. A Brief Review. http:dx.doi.org/10.1155/2016/285304.

Snow, M.J. and Manley-Harris, M., 2004. On the nature of non-peroxide antibacterial activity in New Zealand manuka honey. *Food Chemistry*, *84* (1), 45-147.

So, E.C. 2009. Utility of molecular sequence analysis of the it's rRNA region for identification of Fusarium spp. from ocular sources. *Investigative ophthalmology & visual science*, 50 (5), 2230-2236.

Srinivasan, S., Menzies, K.L., Sorbara, L. and Jones, L.W. 2013. Imaging meibomian glands on a patient with chalazia in the upper and lower lids: A case report. *Contact Lens and Anterior Eye*, 36 (4), 199-203.

Stern, M., Schaumburg, C., Dana, R., Calonge, M., Niederkorn, J. and Pflugfelder, S. 2010. Autoimmunityat the ocular surface: pathogenesis and regulation. *Mucosal immunology*, 3 (5), 425-442.

Strieter, R.M., Belperio, J.A., Phillips, R.J. and Keane, M.P., 2004, June. CXC chemokines in angiogenesis of cancer. In *Seminars in cancer biology* 14, (3), 195-200.

Ta, C.N., Shine, W.E., McCulley, J.P., Pandya, A., Trattler, W. and Norbury, J.W. 2003. Effects of minocycline on the ocular flora of patients with acne rosacea or seborrheic blepharitis. *Cornea*, 22 (6), 545-548.

Tamura K., Nei M., and Kumar S. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences*, 1 (101),11030-11035.

Tang, Y.-W., Ellis, N.M., Hopkins, M.K., Smith, D.H., Dodge, D.E. and Persing, D.H. 1998. Comparison of phenotypic and genotypic techniques for identification of unusual aerobic pathogenic gram-negative bacilli. *Journal of clinical microbiology*, 36 (12), 3674-3679.

Tarabishy, A.B. and jeng, B.H. 2008. 'Bacterial conjunctivitis: A review for internists, *Cleveland Clinic Journal of Medicine*, 75 (7), 507–512.

Tenover, F.C. 2006. Mechanisms of Antimicrobial resistance in bacteria, *The American Journal of Medicine,* 119 (6), S3–S10.

Tesfaye, T., Beyene, G., Gelaw, Y., Bekele, S. and Saravanan, M. 2013. Bacterial profile and antimicrobial susceptibility pattern of external ocular infections in Jimma University specialized hospital, Southwest Ethiopia. *American Journal of Infectious Diseases and Microbiology*, 1 (1), 13-20.

Teunissen, M.B., Bos, J.D., Koomen, C.W., de Waal Malefyt, R. and Wierenga, E.A. 1998 Interleukin-17 and interferon-γ synergize in the enhancement of proinflammatory cytokine production by human keratinocytes. *Journal of Investigative Dermatology*, 111 (4), 645-649.

Teweldemedhin, M., Gebreyesus, H., Atsbaha, A.H., Asgedom, S.W. and Saravanan, M. 2017. Bacterial profile of ocular infections: a systematic review. *BMC ophthalmology*, 17 (1), 212.

Teweldemedhin, M., Saravanan, M., Gebreyesus, A. and Gebreegziabiher, D. 2017. Ocular bacterial infections at Quiha Ophthalmic Hospital, Northern Ethiopia: an evaluation according to the risk factors and the antimicrobial susceptibility of bacterial isolates. *BMC infectious diseases*, 17 (1), 207.

Thygeson, P. 1946. Etiology and treatment of blepharitis: a study in military personnel. *Archives of Ophthalmology*, 36 (4), 445-477.

Tobinick, E.L., 2002. *Cytokine antagonists for the treatment of localized disorders*. U.S. Patent 6, 419, 944.

Tully, G., Kortsik, C., Höhn, H., Zehbe, I., Hitzler, W., Neukirch, C., Freitag, K., Kayser, K. and Maeurer, M.J. 2005. Highly focused T cell responses in latent human pulmonary Mycobacterium tuberculosis infection. *The Journal of Immunology*, 174 (4), 2174-2184.

Tuttle, M.S., Mostow, E., Mukherjee, P., Hu, F.Z., Melton-Kreft, R., Ehrlich, G.D., Dowd, S.E. and Ghannoum, M.A. 2011. Characterization of bacterial communities in venous insufficiency wounds by use of conventional culture and molecular diagnostic methods. *Journal of clinical microbiology*, 49 (11), 3812-3819.

Ubani, U.A. 2009. Bacteriology of external ocular infections in Aba, South Eastern Nigeria. *Clinical and Experimental Optometry*, 92 (6), 482-489.

Vandenbroucke-Grauls, C., Frénay, H., Van Klingeren, B., Savelkoul, T. and Verhoef, J. 2002. *Prescot, LM; H*ARLEY*, JP; Klein, DA Microbiology . 819-820*.

Veldman, P. and Colby, K. 2011. Current Evidence for Topical Azithromycin 1% Ophthalmic Solution in the Treatment of Blepharitis and Blepharitis-Associated Ocular Dryness. *International Ophthalmology Clinics,* 51 (4), 43-52.

Venturino, G., Bricola, G., Bagnis, A. and Traverso, C. 2003. Chronic blepharitis: treatment patterns and prevalence. *Investigative Ophthalmology & Visual Science,* 44**,** 774-774.

Vinh, D.C., Nichol, K.A., Rand, F. and Karlowsky, J.A. 2006. Not so pretty in pink: *Staphylococcus cohnii* masquerading as methicillin-resistant *Staphylococcus aureus* on chromogenic media. *Journal of clinical microbiology*, 44 (12), 4623-4624.

Viswalingam, M., Rauz, S., Morlet, N. and Dart, J. 2005. Blepharokeratoconjunctivitis in children: diagnosis and treatment. *British Journal of Ophthalmology*, 89 (4), 400-403.

Watkinson, S. 2013. Assessment and management of patients with acute red eye. *Nursing older people*, 25 (5).

Watkinson, S. and Seewoodhary, M. 2017. Assessment, care and management of patients with red eye. *Nursing Standard Journal*, 32 (15), 43-50.

Watters, G.A., Turnbull, P.R., Swift, S., Petty, A. and Craig, J.P. 2017. Ocular surface microbiome in meibomian gland dysfunction. *Clinical & experimental ophthalmology*, 45 (2), 105-111.

Wencewicz, T., Long, T., Möllmann, U. and Miller, M. 2013. Trihydroxamate Siderophore–Fluoroquinolone Conjugates Are Selective Sideromycin Antibiotics that Target *Staphylococcus aureus*. *Bioconjugate Chemistry*, 24 (3), 473-486.

Woese, C.R. 1987. Bacterial evolution. *Microbiological reviews*, 51 (2), 221.

Yanoff, M. 2014. *Ophthalmic Diagnosis and Treatment*. JP Medical Ltd.

APPENDIX

Genomic sequencing of DNA and result.

The sequences were aligned with bioedit software and the results as follows:

Sample 1: >CP033814.1 *Staphylococcus haemolyticus* strain FDAARGOS_517 chromosome, complete genome 455nt.

Staphylococcus haemolyticus.

TCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAACAGACAAGGAGCTTGCTCCTTTGACGTT
AGCGGCGGACGGGTGAGTAACACGTGGGTAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAAT
ACCGGATAATATTTCGAACCGCATGGTTCGATAGTGAAAGATGGTTTTGCTATCACTTATAGATGGACCCGCGCCG
TATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCGACGATACGTAGCCGGGAATCTTCCGCAATGGGCGAAAG
CCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACATACGT
GTAAGTAACTATGCACGTCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAAT
ACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGACCTGAGAGGGTGATCGGCCACACTGGAA
CTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGCGTAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCAC
GGCTCAACCGTGGAGGGTCATTGGAAACTGGAAAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGC
GGTGAAATGCGCAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTG
CGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGG
GGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACT
C

Sample 2: >CP033814.1 *Staphylococcus haemolyticus* strain FDAARGOS_517 chromosome, complete genome 837nt.

Staphylococcus haemolyticus.

TGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAACAGACAAGGAGCTTGCTCCTTTGACGTTAGCGGC
GGACGGGTGAGTAACACGTGGGTAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGA
TAATATTTCGAACCGCATGGTTCGATAGTGAAAGATGGTTTTGCTATCACTTATAGATGGACCCGCGCCGTATTAG
CTAGTTGGTAAGGTAACGGCTTACCAAGGCGACGATACGTAGCCGGGAATCTTCCGCAATGGGCGAAAGCCTGAC
GGAGCAACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACATACGTGTAAGT
AACTATGCACGTCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG
GTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGA
CACGGTCCAGACTCCTACGGGAGGCAGCAGTAGCGTAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCACGGCTCA
ACCGTGGAGGGTCATTGGAAACTGGAAAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAA
ATGCGCAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAG
CGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTC
CGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACT

Sample 3: >MH591774.1 *Staphylococcus cohnii* strain F 16S ribosomal RNA gene, partial sequence 1361nt.

Staphylococcus cohnii.

TAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTACCTATAAGACTGGAATAACTCCGGGAAACCGGGGCTAA
TGCCGGATAACATTTAGAACCGCATGGTTCTAAAGTGAAAGATGGTTTTGCTATCACTTATAGATGGACCCGCGCC
GTATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATACGTAGCCGACCTGAGAGGGTGATCGGCCACA
CTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGA
CGGAGCAACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACAAATGTGTAAG
TAACTGTGCACGTCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTA
GGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCA
CGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAG

CGGTGAAATGCGCAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGT
GCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAG
GGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAAC
TCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTAC
CAAATCTTGACATCCTTTGACAACTCTAGAGATAGAGCCTTCCCCTTCGGGGGACAAAGTGACAGGTGGTGCATG
GTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCAGCA
TTAAGTTGGGCACTCTAAGTTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCC
CTTATGATTTGGGCTACACACGTGCTACAATGGACAATACAAAGGGCAGCTAAACCGCGAGGTCATGCAAATCCC
ATAAAGTTGTTCTCAGTTCGGATTGTAGTCTGCAACTCGACTACATGAAGCTGGAATCGCTAGTAATCGTAGATCA
GCATGCTACGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGC
CGGTGGAGTAACC

Sample 4: >MF773752.1 *Staphylococcus cohnii* strain PYG49 16S ribosomal RNA gene, partial sequence 799nt.

Staphylococcus cohnii.

AATACATGCAAGTCGAGCGAACAGATAAGGAGCTTGCTCCTTTGACGTTAGCGGCGGACGGGTGAGTAACACGT
GGGTAACCTACCTATAAGACTGGAATAACTCCGGGAAACCGGGGCTAATGCCGGATAACATTTAGAACCGCATGG
TTCTAAAGTGAAAGATGGTTTTGCTATCACTTATAGATGGACCCGCGCCGTATTAGCTAGTTGGTAAGGTAACGGC
TTACCAAGGCAACGATACGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCC
TACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATGAA
GGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACAAATGTGTAAGTAACTGTGCACGTCTTGACGGTACCTA
ATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTG
GGCGTAAAGCGCGCGTAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGA
AACTGGGAAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAG
GAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAGGA
TTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGT

Sample 5: >LR134089.1 *Staphylococcus saprophyticus* subsp. saprophyticus strain NCTC7666 genome 912nt.

Staphylococcus saprophyticus.

GTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAACAGATAAGGAGCTTG
CTCCTTTGACGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTACCTATAAGACTGGGATAACTTCGGGAA
ACCGGAGCTAATACCGGATAACATTTGGAACCGCATGGTTCTAAAGTGAAAGATGGTTTTGCTATCACTTATAGAT
GGACCCGCGCCGTATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCGACGATACGTAGCCGACCTGAGAGGG
TGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGG
GCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATGAAGGGTTTCGGCTCGTAAAACTCTGTTATTAGGGAAGA
ACAAACGTGTAAGTAACTGTGCACGTCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCC
GCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTCTTAAGTCTG
ATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCAGAAGAGGAAAGTGG
AATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAA
CTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAG
GCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTAGACCGC
AAGGTTGAAACTCAAAG

Sample 6: >LR134089.1 *Staphylococcus saprophyticus* subsp. saprophyticus strain NCTC7666 genome 750nt.

Staphylococcus saprophyticus.

ACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAACAGATAAGGAGCTTGCTCCTTTGACGTTAGCGGCGGA
CGGGTGAGTAACACGTGGGTAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATAAC
ATTTGGAACCGCATGGTTCTAAAGTGAAAGATGGTTTTGCTATCACTTATAGATGGACCCGCGCCGTATTAGCTAG
TTGGTAAGGTAACGGCTTACCAAGGCGACGATACGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGA
GACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACG
CCGCGTGAGTGATGAAGGGTTTCGGCTCGTAAAACTCTGTTATTAGGGAAGAACAAACGTGTAAGTAACTGTGCA
CGTCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGC
GTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACC
GTGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATG

CGCAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCGT
GGG

Sample 7: >CP028165.1 *Staphylococcus aureus* strain CFSAN064037 chromosome, complete genome 1400nt.

Staphylococcus aureus.

TCGAGCGAACGGACGAGAAGCTTGCTTCTCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGATAACCTACCT
ATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATAATATTTTGAACCGCATGGTTCAAAAGTGAAA
GACGGTCTTGCTGTCACTTATAGATGGATCCGCGCTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAAC
GATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAG
CAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTCTTCGGATCG
TAAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAACTGTGCACATCTTGACGGTACCTAATCAGAAAGCCAC
GGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGC
GCGTAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAAAACTT
GAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGAACACCAGTGGC
GAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGT
AGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGC
ACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCA
TGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAATCTTGACATCCTTTGACAACTCTAGAGATAGAGCCTT
CCCCTTCGGGGGGACAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTAGATGTTGGGTTAAGTCCC
GCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGTTGACTGCCGGTGACAAACCGG
AGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGATTTGGGCTACACACGTGCTACAATGGACAATACA
AAGGGCAGCGAAACCGCGAGGTCAAGCAAATCCCATAAAGTTGTTCTCAGTTCGGATTGTAGTCTGCAACTCGAC
TACATGAAGCTGGAATCGCTAGTAATCGTAGATCAGCATGCTACGGTGAATACGTTCCCGGGTCTTGTACACACCG
CCCGTCACACCACGAGAGTTTGTAACACCCGAAGCCGGTGGAGTAACCTTT

Sample 8: >CP029474.1 *Staphylococcus aureus* strain USA 100 isolate 30-47 chromosome, complete genome 788nt.

Staphylococcus aureus.

GAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACC
CCCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGATCCCCACGCTTTCGCA
CATCAGCGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTGCGCATTTCACCGCTAC
ACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTTTTCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTT
CACATCAGACTTAAAAAACCGCCTACGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTAC
CGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGATTAGGTACCGTCAAGATGTGCACAGTTACTTACACATAT
GTTCTTCCCTAATAACAGAGTTTTACGATCCGAAGACCTTCATCACTCACGCGGCGTTGCTCCGTCAGGCTTTCGCC
CATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCCGATCACCC
TCTCAGGTCGGCTATGCATCGTTGCCTTGGTAAGCCGTTACCTTACCAACTAGCTAATGCAGCGCGGATCCATCTAT
AAGTGACAGCAAGACCGTCTTTCACTTTTGAACCATGCGGTTCAAAATATTATCCGGTATTAGCTCCGGTTTCCCGA
AGTTATCCCAGTCTTATAGGTAGGTTATCCACGTGTTACTCACCCGTCCGCCGCTAACATCAGAGAAGCAAGCTTCT
CGTCCGTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATCCTGAGCT

Sample 9: >LR134536.1 *Staphylococcus epidermidis* strain NCTC13924 genome 1399nt.

Staphylococcus epidermidis.

ATCTAATCCTGTTTGATCCCCACGCTTTCGCACATCAGCGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTG
TTCCTCCATATCTCTGCGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTTTTCCAGTTTCC
AATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAAAAACCGCCTACGCGCGCTTTACGCCCAATA
ATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGATTAGGTA
CCGTCAAGACGTGCATAGTTACTTACACATTTGTTCTTCCCTAATAACAGAGTTTTACGATCCGAAGACCTTCATCA
CTCACGCGGCGTTGCTCCGTCAGGCTTTCGCCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGA
CCGTGTCTCAGTTCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTAAGCCGTTACCTTA
CCAACTAGCTAATGCGGCGCGGATCCATCTATAAGTGACAGCAAAACCGTCTTTCACTATTGAACCATGCGGTTCA
ATATATTATCCGGTATTAGCTCCGGTTTCCCGAAGTTATCCCAGTCTTATAGGTAGGTTATCCACGTGTTACTCACCC
GTCCGCCGCTAACGTCAGAGGAGCAAGCTCCTCGTCTGTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGT
TCATCCTGAG

Sample 10: >MG384826.1 *Bacillus aerophilus* strain AA4 16S ribosomal RNA gene, partial sequence 1434nt.

Bacillus aerophilus.

AGTCGAGCGGACAGAAGGGAGCTTGCTCCCGGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCC
TGTAAGACTGGGATAACTCCGGGAAACCGGAGCTAATACCGGATAGTTCCTTGAACCGCATGGTTCAAGGATGAA
AGACGGTTTCGGCTGTCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGC
GACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGG
CAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGA
TCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCAAGAGTAACTGCTTGCACCTTGACGGTACCTAACCAGAAAG
CCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAG
GGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAA
ACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGT
GGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCC
TGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTA
AGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGA
GCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGC
TTTCCCTTCGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGCGTGAGATGTTGGGTTAAGTCCC
GCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCG
GAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAAC
AAAGGGCTGCGAGACCGCAAGGTTTAGCCAATCCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGAC
TGCGTGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACC
GCCCGTCACACCACGAGAGTTTGCAACACCCGAAGTCGGTGAGGTAACCTTTA

Sample 11: >MH091057.1 *Proteus mirabilis* strain UMAGOD06 16S ribosomal RNA gene, partial sequence 1434nt.

Proteus mirabilis.

AGCGCGGCCTAACACATGCAAGTCGAGCGGTAACAGGAGAAAGCTTGCTTTCTTGCTGACGAGCGGCGGACGGG
TGAGTAATGTATGGGGATCTGCCCGATAGAGGGGGATAACTACTGGAAACGGTGGCTAATACCGCATAATGTCTA
CGGACCAAAGCAGGGGCTCTTCGGACCTTGCACTATCGGATGAACCCATATGGGATTAGCTAGTAGGTGGGGTAA
AGGCTCACCTAGGCGACGATCTCTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCAG

ACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGA
AGAAGGCCTTAGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGTGATAAGGTTAATACCCTTATCAATTGACGTT
ACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAA
TTACTGGGCGTAAAGCGCACGCAGGCGGTCAATTAAGTCAGATGTGAAAGCCCCGAGCTTAACTTGGGAATTGCA
TCTGAAACTGGTTGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATGT
GGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAA
CAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGTCGATTTAGAGGTTGTGGTCTTGAACCGTGGCTTCTG
GAGCTAACGCGTTAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCC
GCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGAATCCAGCGAATCCT
TTAGAGATAGAGGAGTGCCTTCGGGAACGCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATG
TTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCACGTAATGGTGGGAACTCAAAGGAGACT
GCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTG
CTACAATGGCAGATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGAACTCATAAAGTCTGTCGTAGTCCGGATT
GGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGTTCCC
GGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGG
GCGCTTACCACTTGT

Sample 12: >KP893390.1 *Pseudomonas aeruginosa* strain GS-33 16S ribosomal RNA gene, partial sequence 909nt.

Pseudomonas aeruginosa.

TGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGATGAAGGGAGCTTGC
TCCTGGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACG
GGCGCTAATACCGCATACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGT
CGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCA
CACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCT
GATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAA
GTTAATACCTTGCTGTTTTGACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACG
AAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTTGGATGTGAAATC
CCCGGGCTCAACCTGGGAACTGCATCCAAAACTACTGAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGT

AGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAG
GTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTT
GGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAA
ACTCAAATGAATTGAC.

Sample 13: >*Pseudomonas aeruginosa* LCS1 499nt.

TGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAA
AGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCAACAGAATAAGCACCGG
CTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGC
GTAGGTGGTTCAGCAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTACTGAGCTAGA
GTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAA
GGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAG
TCCACGCCGTAAACGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGG

ABIA STATE UNIVERSITY TEACHING HOSPITAL

P.M.B. 7004 ABA

Chairman Board of Management
DR. KINGSLEY N. ENWEREMADU
M.B.B.S. FCBE&T, FAGP, FICA, DMPN

Director of Administration/Secretary to the Board
MR. MODESTUS NWAOBASI
Bsc (Sociology)

Chief Medical Director
PROF. CHUKS KAMANU
MBBCh (Cal), FWACS, F.I.C.S.
Dipl Reproductive Medicine and Embryology (Germany)
+2347030857540

Chairman Medical Advisory Committee
ASSOC. PROF. ASSUMPTA CHAPP_JUMBO
MBBS (NIG), FWACPaed

Website: www.absuthng.org
E-mail: info@absuthng.org, ict@absuthng.org
cmd@absuthng.org.

Our Ref: _____

Your Ref: ABSUTH/MAC/117/VOL.I/15

Date: 8/02/2018.

Dr. Wisdom Iwumune
School of Pharmacy and Biomedical Sciences
Liverpool Johnmore University

RE: ETHICAL APPROVAL OF RESEARCH PROJECT ON BLEPHARITIS

Ethical approval has been given to you for the conduct of the above research project on consenting individuals at the Abia state University Teaching Hospital, Aba, Abia State, Nigeria.

It is believed that findings from this study will be communicated to the Ethical and Research Committee of ABSUTH.

Any challenges encountered in the course of the research at the hospital should also be communicated to us.

Sincerely,

ABIA STATE UNIVERSITY TEACHING HOSPITAL

Dr. Chigbu Bright
FWACS,FICS
Chairman Ethics & Research Committee.

ABIA STATE UNIVERSITY TEACHING HOSPITAL
EXCELLENCE IN KNOWLEDGE AND SERVICE