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Efficacy of 0.5% Levofloxacin against Aerobic-Anaerobic Bacterial Flora in Chronic-Blepharoconjunctivitis Patients: A Prospective Semi-Randomized Study

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1. Introduction

1.1. Role of conjunctival normal flora

The normal bacterial flora of the lids and conjunctiva have been thoroughly studied since the inception of bacteriology [11,64]. The conjunctival surface is colonized by a population of bacteria and fungi that, although fairly constant in number, undergo a continual cyclic repetitive change in species [11,64]. Before birth, the conjunctiva and eyelids are sterile if the amniotic sac is not ruptured. Bacterial flora are acquired during passage through the birth canal. After birth, healthy persons of all ages show approximately the same distribution of bacterial species. As is true of age, other variables such as right versus left eye, sex, or seasons of the year do not affect the bacterial population [11,64]. The source of bacteria populating the eye is the skin. This has been determined by culturing conjunctiva, lids, and face, nose and hand skin [7]. Previous studies from Allansmith et al.[3] suggested that the bacterial flora of the two eyes is similar. A specific bacteria was from two to ten times more likely to be cultured from one eye if present in the other eye [32].

Coagulase-negative *Staphylococc*us (CNS) (37%) are a major component of the normal bacterial flora of human eyelids and conjunctiva [4,67]. Less frequent bacteria are *Staphylococcus aureus* (17%*) and Corynebacterum sp* (1%) [4]. Studies of anaerobic bacteria in the eye have been performed less frequently than those involving cultures of aerobes. *Propionibacterium acnes* is by far the most common and should be considered as indigenous micro-flora [53]. Fungi are normal inhabitants of the eye, probably falling out of dust from the air onto the surfaces of the eye [53]. While a great number of species can be cultured from the eyelid margin and ocular surface, it is known by special studies that in 45% to 52% of subjects, most of these species are not considered pathogens [23,53]. However, following trauma or inmunosupression, normally benign fungi, even those never known previously to cause human disease, are capable of inducing corneal infection with subsequent intraocular extension and blindness [7,67].

1.1.1. Natural control mechanisms of indigenous flora of the eye

Probably the most important mechanism for control of the bacterial flora of the eye are the dust-filtering eyelashes, the eyelids, and the lacrimal apparatus [56]. The human blinks 10 to 15 times a minute. Each time the eyelids blink, they mechanically sweep tear film fluid, mucus, and suspended bacteria medially toward the lacrimal puncta [56]. The mechanical vacuuming effect of the eyelid muscles on the tear sac sucks the bacterial flora down the lacrimal duct to the nose [56]. Increased production of tears might aid in removal of microorganisms [7]. Competitive interactions exist among those bacterial species found on the eye. In particular, staphylococci and diphtheroids seem to have antibiotic properties directed against each other [7]. There is some evidence that these two genera tend to inhibit each other and that an overgrowth of one leads to suppression of the other [40]. Further, different strains or species of staphylococci may have the ability to interfere with the growth of each other. This may be a reflection of nature's ecological balance among species so that one bacterium is able to live in relative symbiosis with another without depleting nutritional resources [7,40]. The way how the bacteria, sometimes considered normal flora, become pathogenic is not clear, but one step is adherence of the microorganism to the host cell [7]. Adherence of bacteria by means of pili to depressions in epithelial cells is another mechanism by which disease pathogenesis might occur. Adherence occurs more to damaged epithelial cells than to non-damaged epithelium or denuded stroma [40].

The tear fluid is a potent inhibitor of bacterial growth on the eye. Lysozyme is a protein tear component that has a non-specific antibacterial effect that lyses bacterial cell walls [46]. Although this substance is found in higher concentration in tears than other body fluids, it is probably a minor component of the ocular protection. Also lysozyme may possibly have an adverse effect in that *S. aureus* that are resistant to lysozyme may be selectively increased in β-lactamase production and hence have increased antibiotic resistance [46]. Another protein component of the tears, lactoferrin may be of some importance in inhibiting the bacterial flora

of the eye by its iron-binding capacity [46,10]. An important tear component in resisting bacterial infection is immunoglobulin A [48,10]. As in other glandular tissues that secrete fluids or mucus on the internal or external surface of the body, the lacrimal gland has the ability to couple a secretory “T piece” to the immunoglobulin molecules circulating in the blood [48]. This secretory immunoglobulin A is a highly effective antimicrobial agent against some organisms and occurs in the normal tear fluid in a concentration of 50 mg to 60 mg/dl [48,10]. Since the external surface of the eye is joined from cell to cell with no gaps in between, the normal eye has an effective mechanical barrier composed of its normal cells. When this physical barrier is broken mechanically, infection and invasion by foreign agents are facilitated [27].

1.2. Chronic Blepharitis

Chronic blepharoconjunctivitis (CBC) is one of the most common ocular diseases of the eyelids and conjunctiva encountered in clinical practice, but surprisingly, it is not often recognized and can be associated with symptoms such as a burning sensation, irritation, tearing, photophobia, blurred vision, and red eyes [10,65]. Clinical examination typically reveals the presence of scurf, telangiectatic vascular changes of the eyelid margin, inspissated meibomian glands, conjunctival hyperemia, and rarely, punctuate keratopathy, cornea vascularization and ulceration [12,28,45]. Epidemiologic data indicates that blepharitis and conjunctivitis account for approximately 71% of cases presenting to general medical clinics with ocular inflammation [12,65]. The prevalence is higher in elderly patients. Attempts to classify this disorder are difficult because of the complex mechanisms underlying its pathogenesis. Clinical and laboratory investigations have clearly established bacteria and meibomian gland abnormalities as major etiologic determinants as well as changes in tear film dynamics and underlying dermatologic diseases [65]. The conjunctival flora in patients with CBC has been reported to be comprised of elevated amounts of bacteria compared to normal individuals. Bacterial lipase changes the secretion of the meibomian glands. Thus, cholesterol concentration increases through the cholesterol esterase enzymatic division that favors

bacterial growth and proliferation. This suggests that bacterial populations and their corresponding lipase/esterase activity may play an important role in promoting the development of blepharitis [17,24].

1.2.1. Clinical features and Classification

The eyelid is a complex structure anatomically and functionally. Anatomically, the eyelid margin is comprised of diverse elements, including skin, lashes, their associated pilosebaceous apparatus, the cartilage-like tarsus in which the meibomian glands are found and the conjunctival mucous membrane [56]. Functionally, the eyelid provides mechanical protection of the ocular surface, maintains a continuous tear film over the epithelial surface, and supplies essential components of the tear film, especially the lipid secretory product of the meibomian glands that forms the outer layer of the tear film [44,45,54].

Blepharitis is often low grade, chronic and asymptomatic. Common symptoms are mild ocular irritation with a frequent foreign body sensation, redness, crusting, itching and burning [34]. There is often a history of styes or chalazia of the eyelid [5]. The signs associated with blepharitis may be confined to the eyelid, such as erythema of the lid margin, collarettes (scales surrounding the base of the eyelashes), madarosis (loss of eyelashes), trichiasis (misdirection of eyelashes), notching of the lid margin, and overflow or plugging of the meibomian glands [5]. Frequently, there is an associated conjunctivitis with erythema and edema, but this is usually without discharge. Papillary hypertrophy of the tarsal conjunctiva is often noted. Superficial punctuate erosions of the inferior cornea epithelium, frequently concentrated at the inferior limbus, are common and are associated with more severe symptoms [5]. Because of the intimate relationship between the lids and ocular surface, chronic blepharitis may cause secondary changes in the conjunctiva and cornea [34,48,65,].

Blepharitis has been difficult to categorize because it consist of a varied collection of clinical entities, which manifest differently but sometimes present with overlapping signs and

symptoms. Over the years, several classification schemes for categorizing the different types of blepharitis have been proposed [45].

Thygeson [70]	1946	Divided blepharitis into 3 etiologic types based on distinct clinical characteristics	a. Staphylococcal b. Seborrheic c. Diplobacillary blepharitis.
McCulley et al.[45]	1982	More elaborate classification scheme subdivided into 6 groups, based on the characteristic of the eyelid, lashes, hair follicles, meibomian orifices, debris on the lid margin and corneal changes.	a. Staphylococcal blepharitis b. Seborrheic alone c. Seborrheic with staphylococcal d. Seborrheic with meibomian seborrhoea e. Seborrheic with secondary meibomitis f. Primary meibomitis
Huber-Spitzy et al.[28]	1991	Based on clinical features	a. Blepharitis sicca b. Blepharitis seborrheica c. Blepharitis Ulcerosa
Mathers et al.[42]	1991	Based on the degree of meibomian gland loss, tear film osmolarity, and the results of Schirmer testing.	a. Seborrheic meibomian gland dysfunction b. Obstructive meibomian gland dysfunction c. Obstructive with sicca d. Pure sicca

Wilhelmus [73]	1992	Based on an anatomic delineation of the lid margin according to the gray line. The gray line (muscle of Riolan) divides the lid into an anterior lamella (skin and muscle) (eyelashes follicles and associated glands of Zeiss are part) and posterior lamella (tarsus and conjunctiva) (the meibomian glands are part of this)	a. Anterior lid margin blepharitis b. Posterior lid margin blepharitis

According to all previously mentioned classification schemes, blepharitis can be classified anatomically in 2 principal groups.

a. Anterior lid margin

- Staphylococcal
- Seborrheic
- Mixed

b. Posterior lid margin

- Meibomian Seborrhoea
- Meibomianitis

Anterior lid margin: Staphylococcal blepharitis

Staphylococcal blepharitis is caused by a chronic inflammation of the lash basis with tiny intrafollicular abscesses. Dermal and epidermal ulceration and tissue destruction are the consequences. Staphylococcal blepharitis is frequently found in patients with atopic eczema and demonstrates a gender predilection, with 80 % of patients being female. In addition it tends to occur

in younger patients [45]. The mean age of patients with staphylococcal blepharitis is 42 years, whereas the mean age of patients with other forms of blepharitis is 51 years [35,45].

Clinical Features: The characteristic symptoms associated with staphylococcal blepharitis include burning, itching, photophobia, and an irritating sandy, gritty sensation, which is frequently worse upon awakening [27]. The principal clinical signs manifest primarily on the anterior lid margin, palpebral conjunctiva and the cornea. Distinctive lid margin features are hard, fibrinous crusting scales on the anterior lid margin that surround individual cilia as collarette. Dilated blood vessels (rosettes) produce hyperemia of the lid margins [27]. Chronic inflammation leads to irregularity or notching and thickening of the lid margin (tylosis) thinning or loss of lashes (madarosis), white lashes (poliosis), and misdirected lashes (trichiasis) [34]. Kanski [28] previously showed that acute staphylococcal infections may produce external hordeola from acute purulent occlusion of the glands of Zeiss or internal hordeola from acute inflammation of the meibomian glands. Secondary changes through hyper sensitivity to staphylococcal toxins include the following features:

- Low papillary conjunctivitis,
- Punctate epithelial keratitis, which commonly affects the inferior one-third of the cornea
- Marginal keratitis (catarral ulcers)
- Phlyctenular keratitits and a peripheral wedge-shaped pannus may develop in the area of previous ulceration,
- Associated instability of the tear film will be observed in 50% of the cases

Anterior lid margin: seborrheic blepharitis

The seborrheic blepharitis is a disease of the Zeiss- Moll glands and is frequently associated with seborrheic dermatitis which may involve the scalp, eyebrows, nasolabial folds, retroauricular areas, and sternum. The two principal forms of seborrheic blepharitis are: the greasy type, with which the scales eruptions are greasy, and the dry type (Pityriasis capitis or dandruff) [34]. It has

been postulated that excessive amounts of neutral lipids in patients with seborrhoea are broken down by *Corynebacterium acnes* into bacterial lipase and irritating fatty acids. The seborrheic blepharitis can appear isolated or with staphylococci or interconnected with posterior blepharitis. The symptoms of purely seborrheic blepharitis are similar to staphylococcal blepharitis, but less heavily, with seldom exacerbation. Approximately 30 % of the patients have associated tear film instability. Clinical Features: hyperaemic and greasy anterior lid margins with sticking together of lashes. The scales are soft and located anywhere on the lid margin and lashes [35].

Posterior lid margin: blepharitis

Posterior blepharitis is manifested as a meibomian dysfunction and can be associated with anterior blepharitis or it may occur in isolation. Complications are chalazion formations, which may be recurrent, as well as tear film instability in about 30% of patients. This is probably the result of an imbalance between the aqueous and lipid components of the tear film, allowing increased evaporation and dryness. Others complications are papillary conjunctivitis and inferior corneal epithelial erosions. The two main types are meibomian seborrhoea and meibomianitis [35].

Meibomian seborrhoea is characterized by excessive meibomian gland secretions. It is easily missed because symptoms may be severe but clinical signs of blepharitis are mild. The meibomian gland orifices are often capped by small oil globules. Pressure on the tarsus results in expression of copious amounts of meibomian oil. The tear film is oily and foamy and in severe cases froth accumulates on the lid margins or inner canthi (meibomian foam) [35].

Meibomianitis: this is characterized by inflammation and obstruction of the meibomian glands [46]. The posterior lid margin shows hyperaemia, telangiectasia and obstruction of meibomian gland orifices. Long-standing cases are characterized by cystic dilatation of meibomian ducts, with thickening and notching of the lid margin. Expressed meibomian gland secretions in long-standing cases may be turbid or inspissated, appearing as toothpaste-like plaque. In every severe cases no secretions can be expressed [35].

1.2.2. Pathogenesis

Skin Diseases

Frequently, a skin illness underlies the chronic blepharitis. One third of these patients suffer from seborrheic dermatitis, which is characterized through the hyper keratinisation of the skin, especially in sebaceous glandular rich areas. An overproduction of sebum, however, should not be present. Seborrheic dermatitis was found in 100% of the patients with seborrheic blepharitis and secondary meibomitis and in 82% of the patients with seborrheic blepharitis [45].

In more than one third of the patients with chronic blepharitis, rosacea is present. Practically, all rosacea patients suffer from chronic blepharitis with meibom gland involvement and 20% of blepharitis precedes a skin manifestation. Chalazions develop often in patients with rosacea. Ten percent of patients with chronic blepharitis suffer from atopic dermatitis. An ectodermal dysplasia, in which the meibom glands are missing, can also lead to chronic blepharitis [27].

An abnormal keratinisation of the meibom gland excretory duct, which occurs frequently in the above mentioned skin diseases, seems to be important for the pathogenesis of chronic blepharitis. This is especially true for the form of blepharitis with blockage of the meibom gland excretory duct without substantial bacterial participation. Histologically, an increase in keratinocytes is present in the openings and excretory ducts of the meibom glands [5].

Bacterial Infections

Bacteria have been implicated to play a significant role in the pathogenesis of chronic blepharitis [4,24,52]. Studies showed, that this is the case in 2 subtypes, namely staphylococcal blepharitis and combined seborrheic/staphylococcal blepharitis. In this case, coagulase-negative staphylococci, *Propionibacterium acnes* or *Pityrosporum ovale* were detected in eyelid margin cultures that had a bacterial lipase which altered meibomian gland secretion. *Staphylococcus aureus* and *Propionibacterium acnes* build wax esterase and triglyceride lipase, in addition *Staphylococcus aureus* builds a cholesterol esterase [17,45]. The bacteria mentioned are also found in normal eyelid

margins, however they are possibly present in a greater number in chronic blepharitis and special strains could be responsible for the inflammatory eyelid illness [19].

Bacterial lipases change the secretion of the meibomian glands, whose cholesterol content increases through enzymatic scission of cholesteryl ester, which in turn benefits bacterial growth and propagation, especially from *Staphylococcus aureus*. The bacterial colonization of the eye lid margins and their lipase/ester activity support the development of chronic blepharitis. The presence of cholesteryl ester (and its decomposition product cholesterol) is clearly necessary for the development of a dysfunction of the meibom glands [61].

Bacterial lipoposaccharide trigger the formation of cytokine- like tumor necrosis factor-α (TNF- α), through phagocyte white blood cells or through normal cells such as keratinocytes. Cytokines lead to a rise in reactive oxygen species (ROS), which favors chronic blepharitis and keratoconjunctivitis through a subsequent accumulation of nitrogen oxide (NO) and 4-hydroxy nonenal (HNE) [36].

Chronic blepharitis is possibly a consequence of a cell mediated hypersensitivity reaction, an exotoxin, or from changes of the meibom-gland-fat through the location specific bacterial flora and their respective lipase, and rarely from an immediate infection [5].

Others lid infections

Parasitic: *Demodex folliculorum*. Is a microscopic, obligate, hair follicle mite that is the most common permanent ectoparasite of humans [57]. *Demodex folliculorum* as a further pathogenic factor of chronic blepharitis remains in discussion. Studies have demonstrated that the incidence of the parasite was very high in patients with chronic blepharitis compared with normal subjects (52%) [15,71].

Treatment of demodicosis of the eyelids as a general rule lasts a few months. The use of yellow mercurial ointment, sulphur ointment, camphorated oil, crotamiton, choline esterase inhibitors, sulfacetamide, steroids, antibiotics, as well as antimycotic drugs offers some

improvement. A good response has been observed after oral application of ivermectin along with topical application of cream permethrin. However, the best results were obtained after 2% metronidazole gel or ointment treatment [14].

Fungal: Candida infections of the eyelid are uncommon and are usually associated with candidal infections elsewhere. Normally, the infection occurs in immunosuppressed patients or those taking glucocorticoids. Small ulcers, vesicles, or pustules can develop at the bases of the eyelashes. The infection responds to topical nystatin dermatologic cream or topical amphotericin B [30]. A study by Huber et al.[29] suggested when Candida species happen to coincide with severe inflammation in atopic patients a blepharitis of the ulcerative type will develop or deteriorate thereby implying that these microorganisms may play an important role in the development or deterioration of this severe chronic inflammation.

Ringworm (tinea faciale) is a dermatophyte that can affect the eyelid primarily or spread to the eyelid from other parts of the face. The early lesions begin as flattened, reddish papules that spread peripherally while the central area heals. The fully developed lesion has a ring-like appearance, with a reddish, scaly, sharply defined border and a central pinkish scaly area. The lesions usually respond to topical salicylic acid (1%) and precipitated sulphur (3%) in hydrophilic ointment twice daily for 3 to 5 days [30].

Viral: *Molluscum contagiosum* infections are characterized by elevated, round, waxy, pearly-white, noninflammatory lesions with umbilicated centers. The lesions can be single or multiple and, when located on or near the lid margin, usually cause a chronic follicular conjunctivitis, superior pannus, and superior epithelial keratitis simulating trachoma. Removal or expression of the nodule, allowing permeation of blood into its substance, is curative [30].

Herpes simplex virus can infect the lid either as primary or recurrent infection. The infection is characterized by vesicles on an erythematous base that usually progress to ulcers. Cleansing of the eyelid with cool saline solution is helpful [30].

Herpes zoster virus involvement of the ophthalmic branch of the fifth cranial nerve often affects the upper eyelid, whereas involvement of the maxillary branch often affects the lower eyelid. The lesions have a dermatomal distribution and are vesicular, and later ulcerative. Treatment with systemic acyclovir is indicated [30].

Other changes of the meibom-gland secretion

Apart from the above mentioned bacterial changes in the composition of cholesteryl ester, other pathological changes of the secretion of the meibom glands have been described in chronic blepharitis:

Polar fats with a coat thickness of 1-3 molecules act as surfactants between the aqueous phase and the non-polar fat phase of the lachrymal coat. The polar lipids and the fatty acids that derive from them are highly unsaturated in meibomitis and they are different not only from the control group without blepharitis, but also from those with other blepharitis subtypes [60].

Meibom gland secretion from patients with chronic blepharitis contains twice the amount of secreted phospholipases A2 (sPLA2) in comparison to healthy patients. This is significant because the sPLA2-activity induces the release of the pro-inflammatory arachidonic acid, an unsaturated fatty acid which acts as a precursor of prostaglandin E2 (PgE2) and leukotriene B4. PgE2, leukotriene B4 and arachidonic acid activate the inflammation-promoting TNF-α. The inflammation-promoting fat aldehyde HNE is produced in the presence of ROS from unsaturated fatty acids like linoleic acid. The loss of polar lipids, including the “transition”-triglycerides can destabilize the tear film by the non-polar lipid layer only restraining the water evaporation insufficiently or the influence on the boundary layer and its triglycerides between the polar and non-polar layer [47].

Other studies discovered that simple unsaturated fatty acids from wax/sterol ester are elevated in patients with chronic blepharitis, in comparison to healthy patients. In contrast to this, a decrease of simply unsaturated non-polar oil acids in chronic blepharitis, especially in meibomitis, was found with an increase in the melting point and secretal hardening along with normal surface temperature of the eye with a following obstruction of the meibom gland excretory duct. Under normal conditions, the melting point of the secretion from the meibom glands lies between 10°C to 32°C [18,47].

The accumulation of irritating substances like fatty acids, the destabilisation of the fat layer through an increase of polar substances and a suitable environment for bacteria promote chronic blepharitis [19].

Impression-cytology of the conjunctiva in patients with chronic blepharitis showed defects in the epithelial cells with tears in the intercellular connections, lost of becher´s cell, and subsequently, a defect in the mucin secretion. These changes demonstrate the result as well as the cause of a chronic inflammatory reaction of the eye surface [8].

In 75% of patients with blepharitis meibom the glands that are reduced in number compares to the average, which is true in only 20% of healthy people. This can be detected through a meibography with an infrared video camera or through an infrared photograph [42].

Dysfunction of the meibomian glands with inflammation and obstruction has been suggested to be an important factor in the pathogenesis of chronic blepharitis. However, few objective tests are available to examine the meibomian glands directly. Studies by Messmer et al.[49] suggest that in vivo confocal microscopy allowed the examination of the tear film, the tarsal conjunctiva, the lid margin including the lash follicles and the meibomian glands. In patients with meibomian gland disease pathological changes could be visualised and documented objectively. The presence of an inflammatory infiltrate permitted us to differentiate between meibomitis and meibomian gland dysfunction. Changes of the lash follicles do not seem to play an important role in blepharitis. Thus,

in vivo confocal microscopy represents an objective technique in the classification and follow-up of patients with blepharitis.

Accompanying illnesses of the chronic blepharitis

It occurs with such frequency that CBC often coexists with keratoconjunctivitis sicca (KCS); there can be significant overlap of the symptoms and signs of KCS and CBC, and possibilities exist that both conditions may be contributing to the overall clinical picture, although a casual association is unproven There is a complex and dynamic interaction between eyelid surface abnormalities, the host's immune system, and natural immunity involving the eyelids and tear film [10].

Infiltrating lid tumours should be suspected in patients with apparently asymmetrical or unilateral chronic blepharitis, particularly when associated with madarosis [22].

1.2.3 Treatment

The most important point for the treating physician and the patient to understand relative to therapy is that we are dealing with a chronic disease for which we have no cure and that therapy will be directed initially at bringing the disease under control with intensive therapy and then establishing the minimal amount of chronic long-term therapy that will maintain control of the disease process [35].

Treatment of anterior chronic blepharitis

Lid hygiene with warm compress for some minutes on the lids to the softening of crusts at the lash basis:

Removing crusts and toxic products involves scrubbing the lid margins daily with a commercially available lid scrub, a cotton bud dipped in a 25% solution of baby shampoo or a week

solution of sodium bicarbonate. It is also useful to scrub the eyelids with diluted shampoo when washing the hair. Gradually, lid hygiene can be performed less frequently as the condition is brought under control but must not be stopped or blepharitis will recur [35].

Antibiotic ointment such as sodium fusidate (Fucidin), bacitracin or chloramphenicol is used to treat acute folliculitis but is of limited value in long-standing cases. Following lid hygiene, the ointment should be rubbed onto the anterior lid margin with a cotton bud or clean finger [35].

Oral azithromycin 500 mg daily for 3 days may be helpful to control ulcerative lid margin disease.

Weak topical steroids such as fluorometholone 0, 1% q.i.d. for one week are useful in patients with severe papillary conjunctivitis, marginal keratitis and phlyktänulose, although repeated courses may be required. Tear substitutes are required for associated tear film instability and dry eye [35].

Treatment of posterior chronic blepharitis

Lid hygiene with warm compress and lid massage to melt solidified sebum and mechanical expression of the meibomian glands are used to reduce the amount of irritating lipids within the glands [68].

Systemic tetracyclines are the mainstay of treatment but should not be used in children under the age of 12 years or in pregnant or breast-feeding women because they are deposited into growing bone and teeth and may cause staining of teeth and dental hypoplasia. One of the following preparations may be used: oxytetracyclin 250mg b.d for 6-12 week, doxycyclin 100mg b.d for 1 week and then daily for 6-12 weeks, minocyclin 100 mg daily for 6-12 weeks, erythromycin 250 mg daily or b.d. Local measures like antibiotics, steroids and tear substitutes for dry eye [35].

Treatment for CBC includes warm compresses, eyelid hygiene, topical antibiotics to reduce bacterial load, oral tetracycline analogues, and sometimes topical corticosteroids [33,62,68].

Local Antibiotics

Antibiotics should only be applied with staphylococcal, mixed staphylococcal-seborrheic and seborrheic blepharitis as well as in case of disturbance of the Meibomian glands due to Rosacea. Treatment should be guided by identification of the pathogenic organism and the corresponding antibiogram. The most popular antibiotics in these cases are aminoglycosides or quinolones. Due to its modulating effect on Meibomian glands in combination with its antibiotic activity, oral tetracycline is one the main pillars of the treatment of the chronic blepharitis caused by rosacea. Locally applied metronidazol also is effective with rosacea-associated blepharitis [5]. Duration of antibiotic treatment (application 2-3 times daily after lid-edge hygiene) should not exceed more than 14 days if possible. Locally applied Salicylic acid can have some additional effect by inhibiting the Prostaglandin synthesis and its lightly antibiotic effect [34].

Studies by Ta *et al*. suggest that minocycline effectively decreased eyelid bacterial flora in patients with acne rosacea or blepharitis. One of the mechanisms of newer generation tetracycline analogues may be a decrease or elimination of bacterial flora from the eyelids [68].

Others studies recommend that a 1-day treatment with topical 0.5% levofloxacin is as effective as a 3-day application in healthy patients without CBC [69].

Local cortisone therapy

Especially corneal s and episodes of heavy inflammation make cortisone application absolutely necessary. However, the patient must be informed about possible side-effects and the necessity of keeping this therapy for a short time only. Aim of all therapies, must be to avoid the steroid phase or to keep it rare and short. Especially peripheral corneal infiltrates in the initial stage can easily be intercepted with non-steroidal anti-inflammatory drugs and fusidic acid [27].

Topical immune modulators

Substances like Tacrolimus and Picrolimus can positively influence the course of anterior blepharitis, however, one still has to work on a formulation of the preparations for ophthalmologic purposes. The patients must be informed that these substances can produce a feeling of heat and burning, but will not affect the efficacy of the treatment. Also, this therapy should also be applied for a short time only [27].

1.3. Fluoroquinolones

Fluoroquinolones are arguably the best class of antibiotics eye care specialists have ever had to treat and prevent ocular infections. These agents have a broad spectrum of activity; they are bactericidal antibiotics that kill germs rapidly and have a more than 10 years history of being nontoxic when used topically. In addition, this class of antimicrobials showed best ocular penetration of any of the commercially available topical antibiotics [41].

Fluoroquinolones are synthetic fluorinated analogues of nalidixic acid. Nalidixic acid, the first antibacterial quinolone, was introduced in 1963 during chloroquine synthesis. It is not fluorinated and is therefore excreted too rapidly to have systemic antibacterial effects. Quinolones are rapidly bactericidal and are active against a variety of gram-negative and gram-positive bacteria [26].

With a combination of convenience and efficacy, quinolones are a very attractive bactericidal class of drugs. The antibacterial potency of quinolones is determined primarily by their activity against DNA gyrase (topoisomerase II) and topoisomerase IV, two bacterial enzymes with distinct and essential roles in DNA synthesis, and secondarily by their ability to permeate cell membranes and avoid efflux to reach these intracellular targets [26]. Potency also affects the likelihood of development of resistance, since the frequency of selection of mutants at clinically relevant concentrations may be substantially lower for quinolones with a higher therapeutic index.

Frequency of resistance may also be reduced in quinolones with similarly high levels of activity against both DNA gyrase and topoisomerase IV and in those that are poor substrates for bacterial multidrug resistance efflux pumps [63,66].

Infrequent ocular adverse effects associated with quinolones are discomfort, chemosis, hyperemia, eyelid edema, and punctuate epithelial keratitis [26].

Nevertheless, fluroquinolone resistance is a growing problem in ophthalmology and may portend a trend toward declining efficacy of older fluroquinolones (ciprofloxacin and ofloxacin). Newer fluroquinolones such as levoflocaxin, gatifloxacin and moxifloxacin might help to address this problem in two ways. First, their enhanced activity against Gram positive pathogens increases the probability that strains resistant to an older fluroquinolone will be susceptible to one of the newer fluroquinolones. Second, they are less prone to encouraging the development of resistance on a number of fronts, primarily because of their higher activity against Gram positive bacteria, but also for other reasons (higher penetration in case of levofloxacin; resistance to single-step topoisomerase mutations in case of gatifloxacin and moxifloxacin). Primary use of newer fluroquinolones in preference to initial use of older fluoroquinolones is a potential strategy for helping to forestall the development of resistance, but this approach must be coupled with the overall strategy of avoiding indiscriminate use and enduring proper dosing of these antimicrobials [31].

1.3.1 Levofloxacin

Third and fourth generation fluoroquinolones available for topical ophthalmic use are: levofloxacin 0,5% (third generation), gatifloxacin 0,3%; and moxifloxacin 0,5% (fourth generation).The main advantage of these compounds is their similar stronger gram positive activity in comparison with older fluroquinolones [13,41].

Additionally, other discussed and potentially beneficial features shared by some of these antibiotics include enhanced drug delivery into the anterior segment, improved activity against

certain strains of atypical mycobacterium, and lowered likelihood of selection for resistant bacterial strains [25].

Levofloxacin, a synthetic fluorinated carboxyquinolone, is the S (-) isomer of ofloxacin. It has been demonstrated that the principal antimicrobial activity of ofloxacin resides in the S (-) isomer. Levofloxacin is similar to the other fluoroquinolones in physicochemical, pharmacological and toxicological properties, but it demonstrates better antimicrobial activity than ofloxacin and is more soluble in water at neutral pH than the other quinolones *in vitro* [63].

Moxifloxacin and gatifloxacin have an improved spectrum of activity, increased penetration into ocular tissues and delayed propensity to the development of bacterial antibiotic resistance [31,41]. Nevertheless, levofloxacin has higher activity against gram positive pathogens and has shown high intraocular penetration after topical application [75].

1.4. Purpose of the study

The purpose of the current study is to determine the number of days required (following a 1-day, 3-day, and 7-day application) for topical 0.5% levofloxacin to significantly decreasing conjunctival bacterial flora in patients with chronic blepharoconjunctivitis (CBC).

2. Material and Methods

2.1 Design and Ethics

The study was designed as a prospective semi- randomized trial.

It was conducted with voluntary patients to evaluate the efficacy of topical levofloxacin-therapy on bacterial flora in patients with chronic blepharoconjunctvitis. This third generation fluoroquinolone with a broad spectrum and low collateral effects is frequently used for lid and conjunctival infections and also after intraocular surgeries [39,63]. The study was conducted according to the World Medical Association Declaration of Helsinki, under the Policy of "Ethical Principles for Medical Research Involving Human Subjects", adopted by the 18th WMA General Assembly, Helsinki, Finland June 1964 [55].

All patients gave written informed consent for participation in this study. Patients that agreed to participate in the study received an information-leaflet stating details on antibiotic treatment and conjunctival smears.

An application was submitted to the European Clinical Trials Database (EudraCT) and to the Ethics Commission of the Institutional Review Board at Ludwig-Maximilians-University for study approval (in German: Ethikkommision der Medizinischen Fakultät der Ludwig Maximilians Universität München). The research was approved by the Ethics commissions on March 2007, as Project Nr.4032983, with the title: **"Reduction of Conjunctival/Lid Bacterial Count after Topical Application of 0.5% Levofloxacin in Chronic Blepharoconjunctivitis's Patients**". The act was signed by Prof. Dr. G. Paumgarther, Chairman of the Ethics Commission. The insurance of the patients was represented by the ECCLESIA mildenberger HOSPITAL GmbH, Detmold, Germany, and it was supported by Santen GmbH, Germering, Germany and Georg-Hannolore Zimmermann Foundation, Munich, Germany.

2.2 Patient characteristics

Participants were recruited between March 2007 and March 2008 from outpatients attending follow-up visits or first-time appointments at the out-patient center of the Department of Ophthalmology at the University of Munich (Augenklinik der Ludwig-Maximilians-Universität-München).

Inclusion criteria:

Out-patients:

- age 39 years or older
- no surgery patients
- without infectious ocular disease (Control Group)
- with chronic blepharoconjunctivitis for more than 6 weeks

  Definition of Chronic Blepharoconjunctivitis

  - presentation of diffuse conjunctival hyperaemia
  - papillae or follicles
  - minimal mucopurulent discharge
  - conjunctival thickening persistent for more than three weeks

  Severity Code:

  - **Mild:** diffuse conjunctival hyperaemia with either papillae or follicles, minimal discharge, conjunctival thickening, redness and teleangiectasis of the eyelid
  - **Moderate:** plus lash loss, recurrent hordeola
  - **Severe**: plus ulceration at the base of cilia, development of cicatrises

Exclusion criteria:

Out-patients:

- under age 39 years
- reporting to be allergic to levofloxacin
- using topical antibiotics within the last 5 days or systemic antibiotic within the last 30 days
- blepharoconjunctivitis only in one eye
- with acute bleplaroconjunctivitis, dacryocystitis or history of ocular infection within 7 days.

2.3 Method of randomization

At the beginning of the study, a list with 100 numbers was elaborated. The Microsoft-Office-Excel software program (Microsoft, Inc. Seattle, USA) was used to generate random numbers that were assigned to each group. The patients were randomized to either a control or study group. This randomization was distributed in sealed envelopes. Patients learned about their group assignment from their treating ophthalmologist, who opened the envelope and explained the specific treatment according to the group.

In order to compare similarity between the groups, demographic data such as age, gender, eye (right or left), eye anamnesis, general anamnesis; examination and follow up were registered.

2.4 Distribution of Groups

In this prospective semi-randomized control trial, 60 patients diagnosed with bilateral CBC were enrolled in the study following Institutional Review Board approval. These patients were randomized to three different treatment regimens: a “no treatment” group received no antibiotic (n = 20); a “levofloxacin only group” treated with 0.5% topical levofloxacin in both eyes 4 times a day for 7 days (n = 20); a “combined group” instructed to scrub their eyelid margins with a moistened

cotton tip in addition to the application of 0.5% topical levofloxacin in both eyes 4 times a day for 7 days (n = 20). The negative control group consisted of volunteers (n = 40) without CBC. (Table 1)

Table 1 Distribution of Groups

Negative control group	Patients without infectious disease of the eye
No treatment group(Positive Control)	Patients with CBC will randomly receive no treatment for seven days.
Levofloxacin only group	Patients with CBC will randomly receive topical 0.5% levofloxacin on both eyes four times per day for seven days.
Combined group	Patients with CBC will randomly receive topical 0.5% levofloxacin on both eyes four times per day for seven days. Additionally patients will be instructed to scrub their eyelid margins with a moistened cotton tip

2.5 Microbiological Evaluation

Bacterial cultures were obtained from the inferior conjunctiva of both eyes using a Culture Swab EZ (BD-BBL™ Collection and Transport System, Becton,Dickinson and Company, USA) moistened with sterile thioglycolate broth (bioMerieux®), while avoiding contact with the eyelashes. Cultures were obtained from both eyes at baseline (D0) for all patients. Additional cultures were obtained from both eyes of CBC patients in the no treatment, levofloxacin and combined groups on day one (D1), day three (D3) and day seven (D7) after starting treatment. (Table 2)

Table 2 Description of the time points when Conjunctival Cultures were obtained.

Time	Definitions
D0	First smear before antibiotics (Baseline)
D1	Smears one day after the therapy beginning
D3	Smears three days after therapy beginning
D7	Smears seven days after therapy beginning

Collection of specimens from the conjunctival sac was performed by rotation of a thioglycolate moistened swab through the inferior fornix from the nasal to the temporal side, covering all sides of the swab. Special care was taken

- not to touch the eyelid margins or lashes.
- not to depress the fornix as this causes slight pain an excessive sample
- not to touch the cornea as this causes slight pain in addition to the minimum risk of corneal excoriation.

The specimens were immediately inoculated first onto blood agar (BAG) for microaerophilic and aerobic bacteria using one side of the swab, then onto chocolate agar (CHOCO) plates for anaerobic bacteria (anaerobic GENbag, bioMerieux®) using the opposite side; by this technique, bacteria collected should have been distributed equally on both solid culture media and finally the swab was placed in thioglycolate broth. Thioglyolate broth, being an enrichment culture media, was used for detection of even small amounts of common aerobic-microaerophilic and anaerobic bacteria. All culture media were incubated for 7 days at 37ºC. Bacteria were isolated and quantified. Numbers of colony-forming-units (CFUs) of aerobic and microaerophilic bacteria were counted on solid culture media on BAG after three days and of anaerobic bacteria on CHOCO after five days, respectively. This was performed using a magnifying glass and by counting all separately CFUs of bacteria on solid culture media. The thioglycolate

culture was considered to be of “positive growth” if the broth became cloudy within 7 days of incubation and “sterile” if after 7 days the medium maintained its clear and citrine transparent color.

The decision of when to take a culture out of the incubator was dependent on the growth of bacteria. Bacterial growth was graded according to the volume of the liquid medium that was occupied by visible bacterial growth. Colony growth patterns were graded from one cross (+), indicating barely visible small colonies, to three crosses (+++), where at least 2/3 of the medium was occupied by bacterial growth. When positive growth of (++) or more was observed, positivity of growth was registered and bacteria were indentified. If after the 7 days, a positivity of one + was found, the culture was considered positive and the bacteria were identified. The identification of organisms and antibiogram were performed with the Vitek 2 Compact system (bioMérieux®). Only for streptococci, the isolated bacteria were tested for antibiotics susceptibilities via the Kirby-Bauer disk-diffusion technique for the reason that the Vitek 2 Compact system is not equipped for this task. At D0 to D7, cultures were obtained without topical anesthetic to optimize bacterial growth by eliminating any preservative that may affect bacterial growth. The researchers collecting the samples and culturing the microorganisms were masked as to the treatment groups of the patients.

2.6 Statistics

Statistical analysis was performed using the Analyse-It software program (Analyse-It Software, Leeds, England). Primary variable to assess the treatment efficacy was culture positive rate of thioglycolate broth after seven days. For each CBC study group, a sample size of 20 patients (40 eyes) was planned. This was based on the assumption of a culture positive rate from conjunctival swabs in thioglycolate broth of 80% in the no treatment CBC group as compared to 40% in the treatment groups. At an alpha-value of 0.05 this would give the study a power of 95%. A Chi-squared test was used to compare the number of positive thioglycolate broth cultures for each

group of patients while a Mann-Whitney test was used to compare the colony forming units (CFU) of bacteria isolated on blood and chocolate agars.

Used Materials

Company	Culture media	Reagents	Laboratory materials	Equiment/Instruments
bioMerieux® Nürtingen Germany	Blood agar (BAG) Chocolate agar (CHOCO) McConkey (MKC) Chromogenic culture (CPS) Thioglyolate broth	Gram Stain Kits Saline solutions 0,45 % Saline solutions 0,9 %	anaerobic GENbag Ecouvillons Swabs	Vitek 2 Compact system GP Karte AST-P554 Karte GN Karte AST-N021 Karte Densicheck Kalibrator Densicheck Dispensette Pipettenspitzen
Becton,Dickinson and Company, (BD) USA	Müller Hinton agar		Culture Swab EZ Sensi Disc Antibiotic Susceptibility Test: penicillin, oxacillin cefuroxim, ceftazidim cefotaxim, imepenem, meropenem,ciprofloxacin levofloxacin,ofloxacin, ciprofloxacin,norfloxacin, moxifloxacin,gatifloxacin neomycin,amicacin tobramicin,gentamicin erytromicinazitromycin, vancomycin,tetracyclin chloramphenicol,mezlocillin	
Bio Rad France	Stock Culture			
Nunc. Part of Thermo Fisher Scientific. Denmark			Disposable inoculating loops and needles	
Memmert GmbH Schwabach Germany				Incubator
ZEISS Microscopy & Imaging Carl Zeiss MicroImaging GmbH, Germany.				Microscopy

3. Results

3.1 Patient characteristics

Patients were enrolled in the study from March 2007 through March 2008 at the Ophthalmology Department of the Ludwig-Maximilians University in Munich, Germany.

Amongst the 60 enrolled CBC patients, 52 completed the study. Conjunctival cultures were not obtained in 4 patients (2 in the no treatment group und 2 in the combined treated group) and 4 patients had at least one protocol violation (2 patients each in the Levofloxacin only treated group and 2 in the combined group). As a result, there were 20 patients in the "no treatment group," 17 in the "levofloxacin only group" and 15 in the "combined group."

Among the 52 remaining patients with CBC, the mean age was 62.2 years (67,3% female patients) (Table 3). The negative control group had a slightly lower mean age of 60 years (53% female patients). The most commonly type of CBC was moderate (Figure 1) (44, 2 %), following with mild (42, 3%) and severe (13, 5 %).

Table 3 Demographic data

	Age in years	Gender				Type of CBC					
	Mean	F	%	M	%	Mild	%	Moderate	%	Severe	%
Control Group n=40	60	21	53	19	48	N/A	N/A	N/A	N/A	N/A	N/A
Study Group n= 52 No treatment group n=20 Levofloxacin only group n=17 Combined group n=15	62,2	35	67,3	17	32,7	22	42,3	23	44,2	7	13,5

Figure 1. Show one patient with moderate chronic blepharoconjunctivitis (CBC). Lid margins and conjunctival flora on blood and chocolate agar.

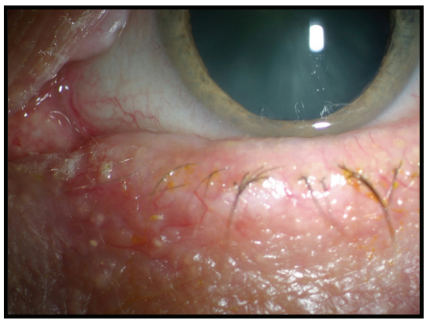


Fig. 1.1 Left eye, prior to any application of antibiotic the patient with CBC presented in the anterior lid margin: mandarosis, diffuse conjunctival hyperaemia and telangiectasie.

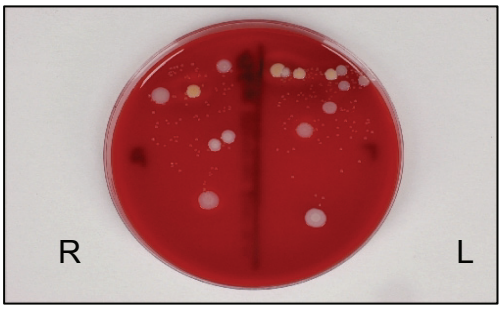


Fig. 1.1.1 Culture on blood agar plate (right and left eye) with coagulase – negative S*taphylococcus (*big white colony) and *Corynebacterium sp*. (small white mate colony)

Fig. 1.1.2 Culture on chocolate agar plate at the right eye with *propionibacterium acnes* (small pink colony)and at the left eye with coagulase – negative S*taphylococcus (*big white colony) and *Propionibacterium*

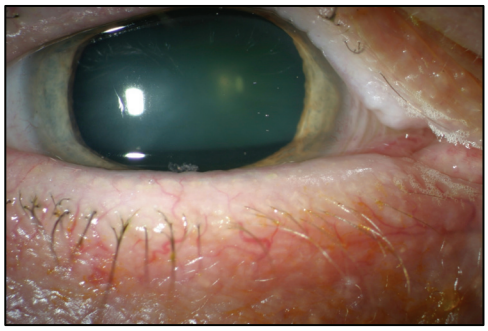


Fig. 1.2 Right eye after one day of treatment the patient presented a mild improvement of the signs**.**

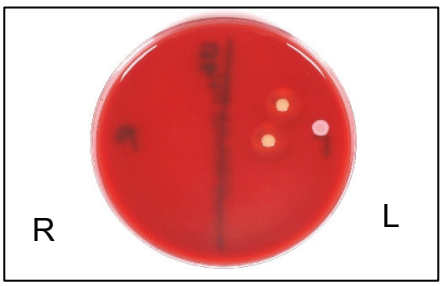


Fig. 1.2.1 Culture on blood agar plate at the right eye sterile and the left eye with coagulase – negative S*taphylococcus (*big white colony)

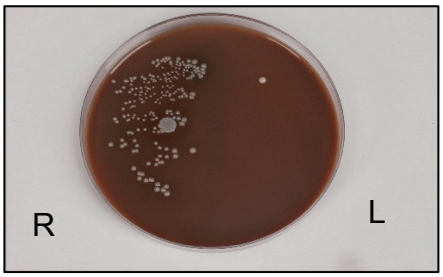


Fig. 1.2.2 Culture on chocolate agar plate at the right eye with *Propionibacterium acnes* (small pink colony)and at the left eye just one colony of *Propionibacterium acnes*

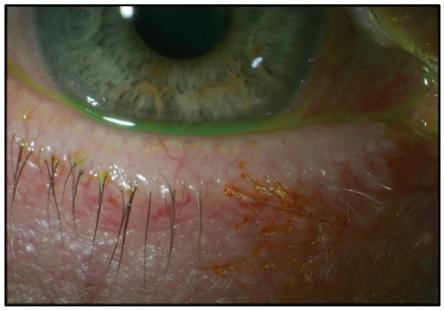


Fig. 1.3 Right eye, after three day of treatment the patient presented a moderade improvement of the signs.

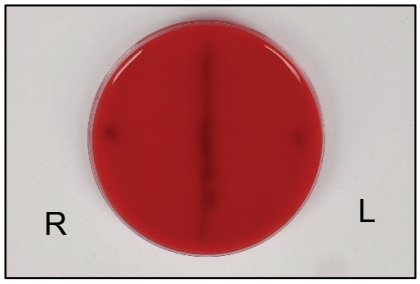


Fig. 1.3.1 Culture on blood agar plate (right and left eye) sterile

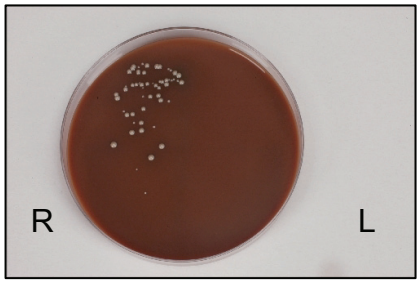


Fig. 1.3.2 Culture on chocolate agar plate at the right eye with *Propionibacterium acnes* (small pink colony) and at the left eye sterile

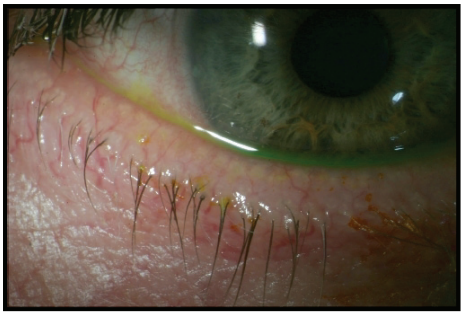


Fig. 1.4 Right eye, after seven day of treatment the patient presented a marked improvement of the signs

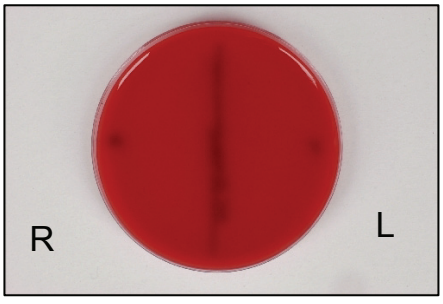


Fig. 1.4.1 Culture on blood agar plate (right and left eye) sterile

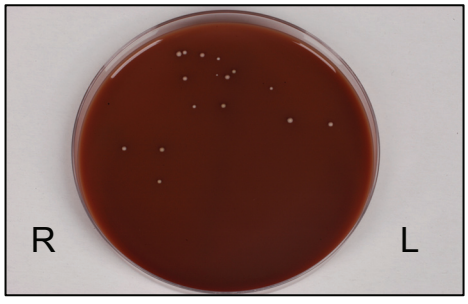


Fig. 1.4.2 Culture on chocolate agar plate (right and left eye) with a few colony of *Propionibacterium acnes* (small pink colony)

3.2 Bacteria

Baseline culture results from both eyes demonstrated 105 bacteria isolated from the 52 CBC patients, compared to 46 bacteria isolated from 40 negative control patients. The most common bacteria isolated were coagulase-negative *Staphylococcus*, representing 70.5% (Table 4) of bacteria recovered from CBC patients and 82.6% in the negative control group. The other bacteria isolated, in decreasing order of frequency, included *Propionibacterium acnes, Staphylococcus aureus*, *Streptococcus sp*, *Corynebacterium sp*, Gram-negative rods, and *Micrococcus sp*.

Table 4: Distribution of Microorganisms Isolated at baseline (D0).

	No CBC		No Treatment		Levo only		Combined		CBC Patients Overall	
Bacteria	**Number**	**%**	**Number**	**%**	**Number**	**%**	**Number**	**%**	**Number**	**%**
Coagulase negative ***Staphylococcus***	38	82.6%	28	65.1%	28	80.0%	18	66.7%	74	70.5%
Staphylococcus aureus	1	2.2%	2	4.7%	2	5.7%	3	11.1%	7	6.7%
α- hemolytic ***Streptococcus***	0	0.0%	4	9.3%	0	0.0%	1	3.7%	5	4.8%
Corynebacterium sp.	0	0.0%	3	7.0%	1	2.9%	1	3.7%	5	4.8%
Propionibacterium acnes	7	15.2%	4	9.3%	1	2.9%	2	7.4%	7	6.7%
Micrococcus sp.	0	0.0%	1	2.3%	0	0.0%	1	3.7%	2	1.9%
Gram-negative bacteria	0	0.0%	1	2.3%	3	8.6%	1	3.7%	5	4.8%
Total	46	100.0%	43	100.0%	35	100.0%	27	100.0%	105	100.0%

The thioglycolate broth culture results demonstrated that at baseline, 100 out of 106 eyes (95%) of patients with CBC had positive cultures, compared to only 46 out of 80 eyes (58%) from patients in the negative control group ($P < 0.0001$). Baseline positive culture in thioglycolate broth demonstrated significant differences between No CBC group [n = 46/80, (58%)] and No treatment group [n = 37/40, (88%), $P = 0{,}0012$]; Levo only group [n = 34/34, (100%), $P > 0.0001$] and Combined [n = 29/30, (97%), $P = 0{,}0002$]. After one day (D1) application of topical levofloxacin, the eyes of Levo only group showed a significantly reduced number of positive conjunctival cultures from 34/34 (100%) (D0) to 23/34 (68%) (D1), on day 3 (D3) 19/34 (56%) and after **7** days (D7) 10/34 (29%). Comparing No treatment group and Levo only group, a significant difference

was observed at all time-points (P = 0, 0117, P = 0, 0036, P = < .0001) (Figure 2 and Table 5). Antibiotic treatment resulted in a lower number of positive thioglycolate broth cultures. There were significantly fewer positive cultures following 3 and 7 days of antibiotics for the levofloxacin and combined group compared to untreated CBC eyes. The addition of eyelid scrub in the combined group did not seem to have any beneficial effect over the use of antibiotic alone. Although eyes treated with a combination of antibiotics and eyelid scrub had a higher culture positive rate (50%) compared to antibiotics alone (29%), this difference is not statistically significant (P = 0.1533).

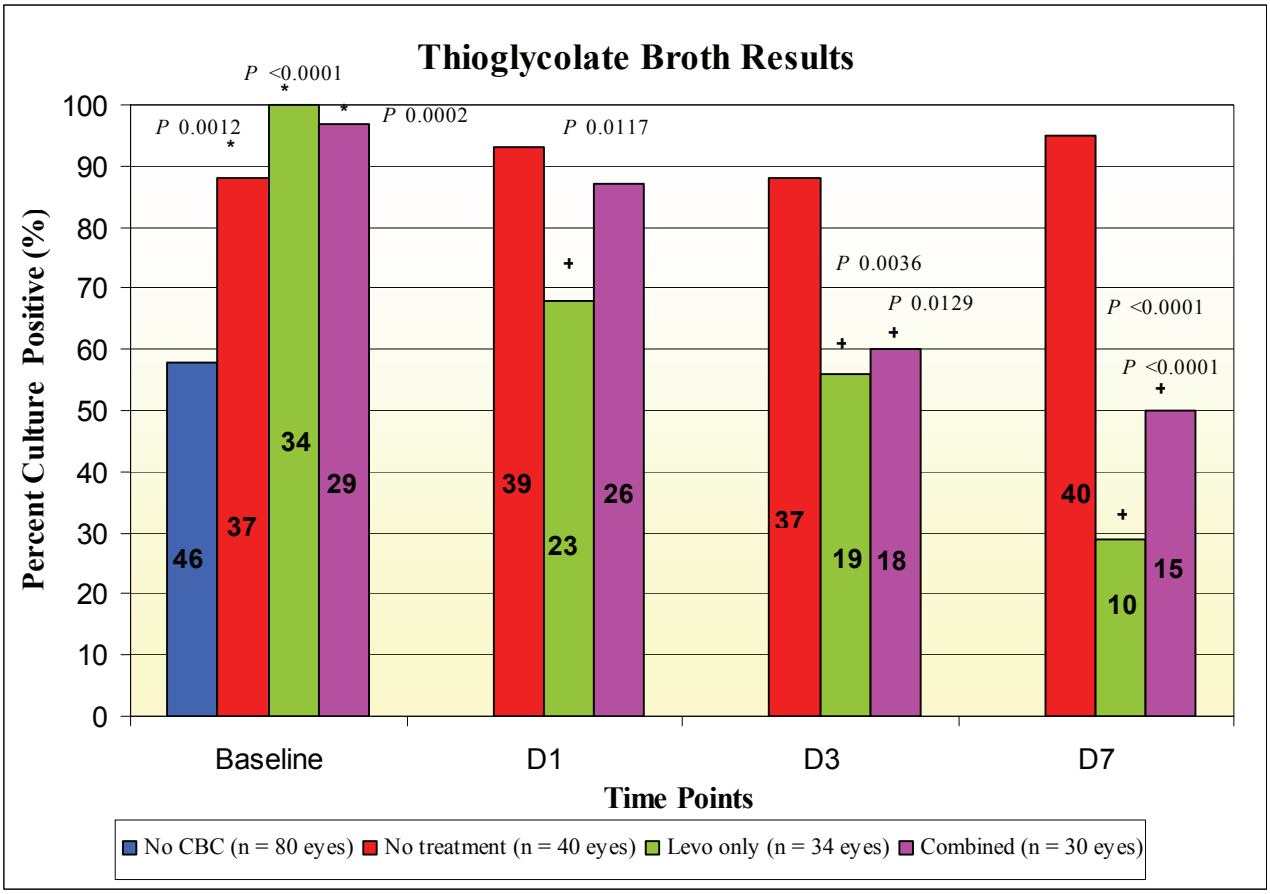


Figure 2: Percentage of Eyes with Positive Cultures in Thioglycolate Broth at Each Time Point of Culture Collection. CBC indicates patients with chronic blepharoconjunctivitis, therefore, "No CBC" were patients without chronic blepharoconjunctivitis. Patients with chronic blepharoconjunctivitis were randomized to the following groups: No treatment = no antibiotic; Levo only = topical levofloxacin 4 times a day; Combined = eyelid scrub in addition to topical levofloxacin 4 times per day. Culture collection time points were the following: **Baseline**, prior to application of any antibiotics; **D1** – one day following antibiotic application for the levo only and combined group; **D3** – three days following antibiotic application; **D7** – seven days following antibiotic application. * Indicates significant difference compared to No CBC group.
+ Indicates significant difference compared to no treatment group.

The blood agar cultures demonstrated a similar pattern as the thioglycolate broth cultures (Figure 3 and Table 5). Patients with CBC had significantly more positive blood agar cultures (83 out of 106 eyes, 78%) than those without disease (30 out of 80 eyes, 38%) ($P < 0.0001$). At each of the time points following antibiotic administration, patients in the levofloxacin and combined groups had significantly fewer positive cultures ($P < 0.05$) than those who did not receive antibiotic (no treatment group).

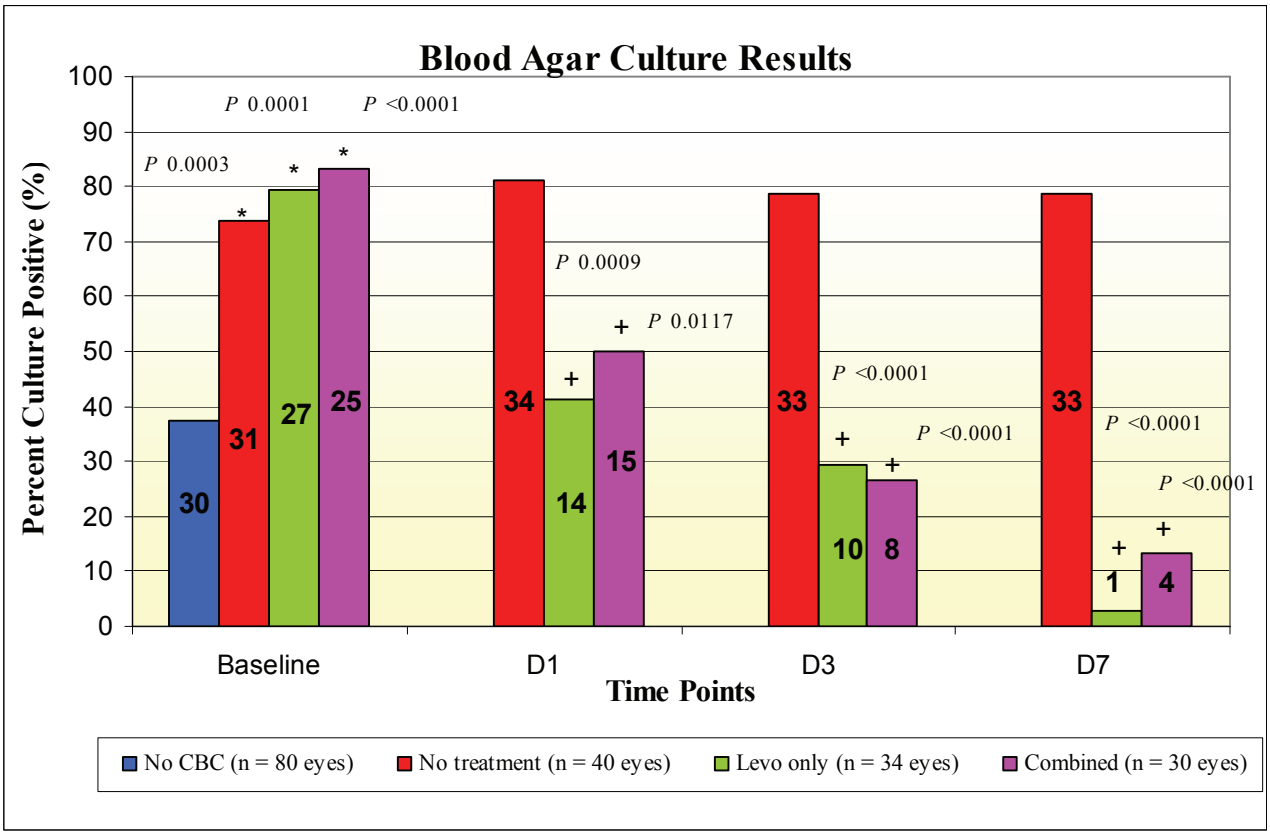


Figure 3: Percentage of Eyes with Positive Cultures in Blood Agar at Each Time Point of Culture Collection. CBC indicates patients with chronic blepharoconjunctivitis, therefore, "No CBC" were patients without chronic blepharoconjunctivitis. Patients with chronic blepharoconjunctivitis were randomized to the following groups: No treatment = no antibiotic; Levo only = topical levofloxacin 4 times a day; Combined = eyelid scrub in addition to topical levofloxacin 4 times per day. Culture collection time points were the following: **Baseline**, prior to application of any antibiotics; **D1** – one day following antibiotic application for the levo only and combined group; **D3** – three days following antibiotic application; **D7** – seven days following antibiotic application. * Indicates significant difference compared to No CBC group.
+ Indicates significant difference compared to no treatment group.

Figure 4 and Table 5 summarizes the culture results on chocolate agar. As with the thioglycolate broth and blood agar results, patients with CBC had a significantly higher number of positive cultures (66 out of 106 eyes, 62%) than those without CBC (30 out of 80 eyes, 38%) (P = 0.0086). Again, similar to the thioglycolate and blood agar findings, treatment with antibiotics resulted in a fewer number of positive cultures compared to eyes that did not receive antibiotics.

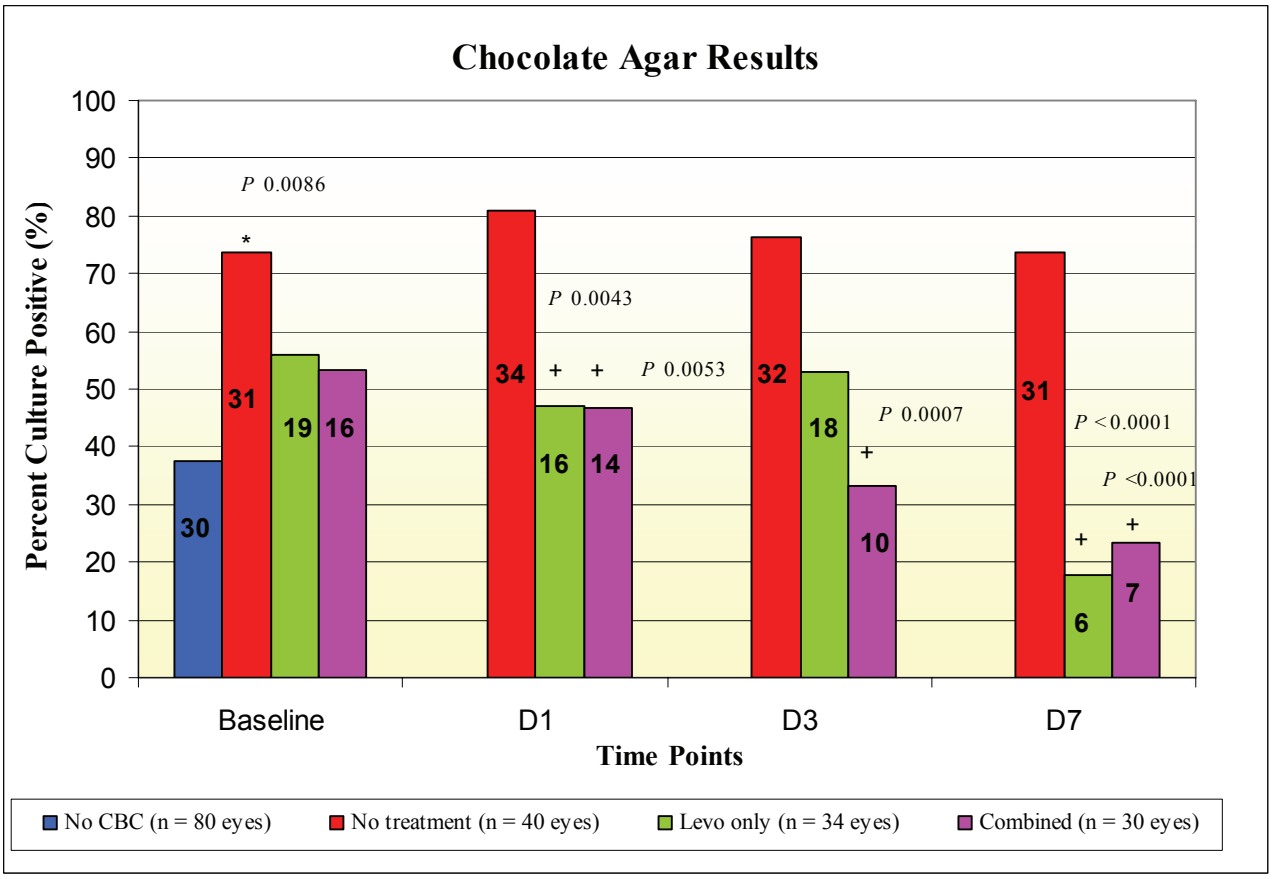


Figure 4: Percentage of Eyes with Positive Cultures for Anaerobic Bacteria in Chocolate Agar at Each Time Point of Culture Collection. CBC indicates patients with chronic blepharoconjunctivitis, therefore, "No CBC" were patients without chronic blepharoconjunctivitis. Patients with chronic blepharoconjunctivitis were randomized to the following groups: No treatment = no antibiotic; Levo only = topical levofloxacin 4 times a day; Combined = eyelid scrub in addition to topical levofloxacin 4 times per day. Culture collection time points were the following: **Baseline**, prior to application of any antibiotics; **D1 –** one day following antibiotic application for the levo only and combined group; **D3 –** three days following antibiotic application; **D7 –** seven days following antibiotic application. * Indicates significant difference compared to No CBC group. + Indicates significant difference compared to no treatment group.

Table 5 : Statistical Comparisons of Number of Patients with Positive Cultures and Quantity of Bacteria Isolated.

Comparison	Thioglycolate broth	Blood Agar Positive	Blood Agar CFU	Chocolate Agar Positive	Chocolate Agar CFU
Baseline					
No CBC vs. No treatment	**0.0012**	**0.0003**	**<0.0001**	**0.0086**	**0.0001**
No CBC vs. Levo only	**<0.0001**	**0.0001**	**<0.0001**	0.1081	**0.0289**
No CBC vs. Combined	**0.0002**	**<0.0001**	**<0.0001**	0.1997	**0.0498**
No treatment vs. Levo only	0.1060	0.7643	0.4207	0.6106	0.1322
No treatment vs. Combined	0.3871	0.5023	0.5198	0.4899	0.3816
Levo only vs. Combined	0.9497	0.9361	0.7302	0.9624	0.8931
D1					
No treatment vs. Levo only	**0.0117**	**0.0009**	**<0.0001**	**0.0043**	**0.0005**
No treatment vs. Combined	0.6379	**0.0117**	**0.0019**	**0.0053**	**0.0122**
Levo only vs. Combined	0.1344	0.6484	0.1008	0.8262	0.7756
D3					
No treatment vs. Levo only	**0.0036**	**<0.0001**	**<0.0001**	0.0600	**0.0091**
No treatment vs. Combined	**0.0129**	**<0.0001**	**<0.0001**	**0.0007**	**0.0007**
Levo only vs. Combined	0.9368	0.9722	0.9389	0.1850	0.2225
D7					
No treatment vs. Levo only	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
No treatment vs. Combined	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Levo only vs. Combined	0.1533	0.2805	0.1327	0.8003	0.6876

Values in bold are significant at a threshold of $P = 0.05$. Comparison of number of positive cultures was done by a χ^2 test while comparison of CFU's was made with a Mann-Whitney test.

In addition to analysing the blood and chocolate agar plates for positive cultures, the median number of colony forming units (CFU's) was determined (Table 6). There were significantly more CFU's on both blood and chocolate agar in CBC patients compared to those without CBC at baseline ($P < 0.05$). In comparing the CFU at baseline among the untreated and the treated groups, there were no statistically significant differences ($P = 0.5023$ - 0.9361). Following treatment, patients in groups receiving antibiotic drops consistently had fewer CFU's isolated at each time point of culture collection ($P < 0.05$) compared to eyes without antibiotic treatment, with or without eyelid scrub.

No adverse reactions attributable to the study medication occurred

Table 6: Median Colony Forming Units (CFU) Cultured on Blood and Chocolate Agars

	Blood Agar				Chocolate Agar			
	Baseline	**D1**	**D3**	**D7**	**Baseline**	**D1**	**D3**	**D7**
No CBC	0	N/A	N/A	N/A	0	N/A	N/A	N/A
No treatment	6.5*	7	8	5.5	2.5*	3.5	3	4.5
Levo only	3*	0^{+}	0^{+}	0^{+}	1*	0^{+}	1^{+}	0^{+}
Combined	8*	1^{+}	0^{+}	0^{+}	1*	0^{+}	0^{+}	0^{+}

CBC indicates patients with chronic blepharoconjunctivitis, therefore, "No CBC" are patients without chronic blepharoconjunctivitis. Patients with chronic blepharoconjunctivitis were randomized to the following groups: No treatment = no antibiotic; Levo only = topical 0.5% levofloxacin 4 times a day; Combined = eyelid scrub in addition to topical levofloxacin 4 times per day. Culture collection time points were the following: **Baseline –** prior to application of any antibiotics; **D1 –** one day following antibiotic application for the levo only and combined group; **D3 –** three days following antibiotic application; **D7 –** seven days following antibiotic application. *Indicates significant difference compared to patients without CBC (negative control group), $^{+}$ indicates significant difference compared to no treatment group (patients with CBC who did not receive antibiotic treatment).

Figures 5 and 5.1 show the results of antibiotic susceptibility testing (using one eye from each patient at Baseline) for the most common bacteria isolated in this study (coagulase-negative *Staphylococcus)*. Comparing the results for "No CBC-group" and "CBC group", there was a statistically significant difference for Mezlocillin ($P< 0.002$) and Erythromycin ($P< 0.05$), only.

Figure 5.

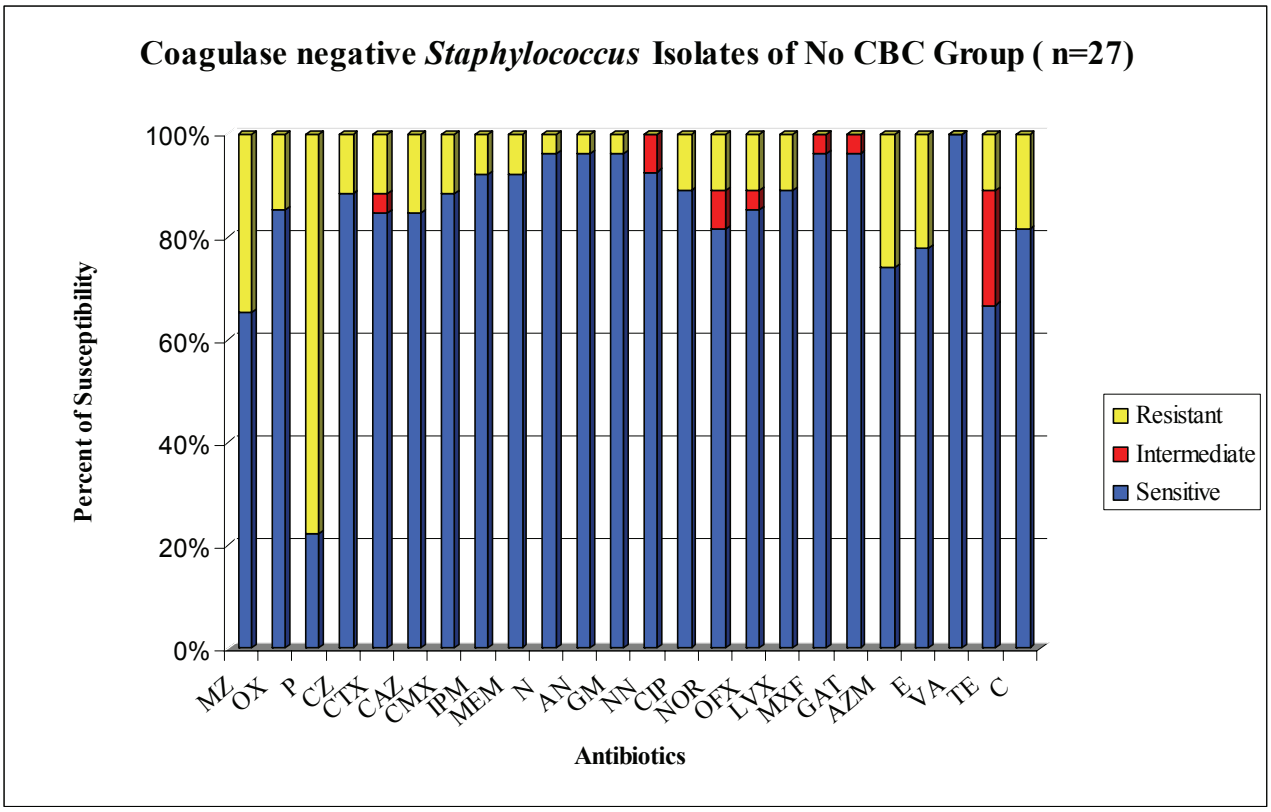
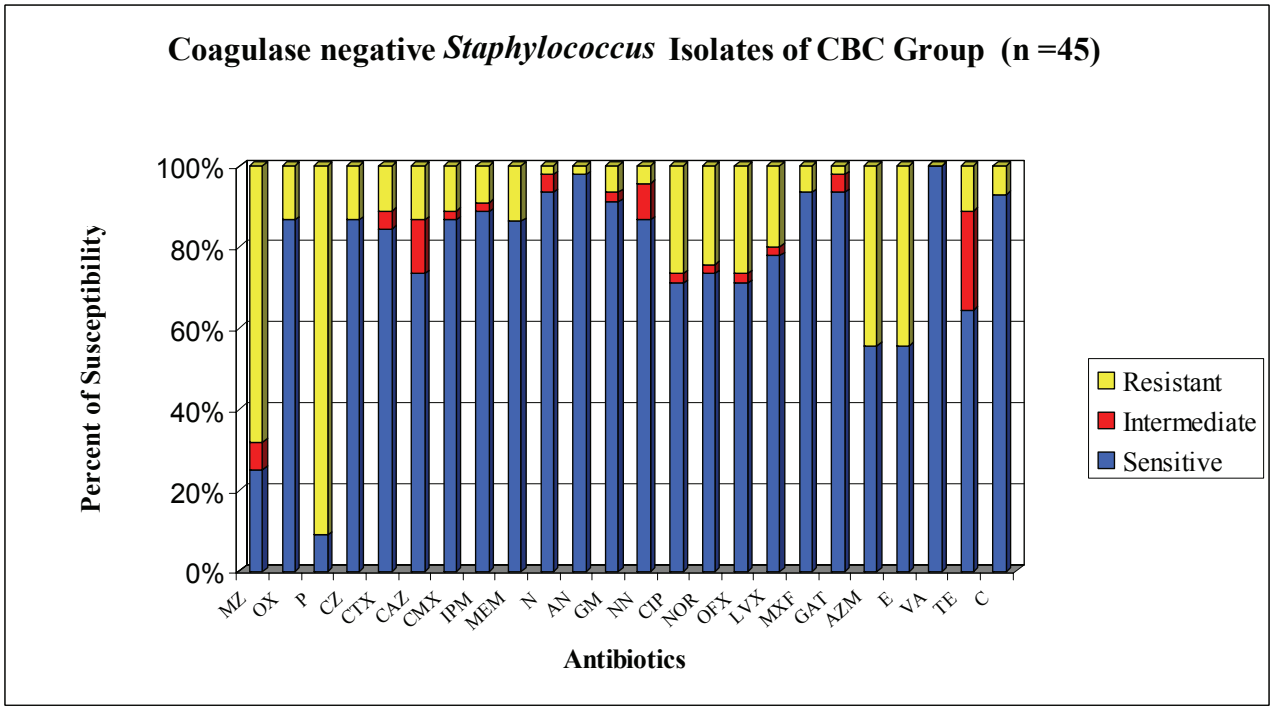


Figure 5.1



4. Discussion

Chronic blepharoconjuctivitis (CBC) is one of the most common conditions seen in the ophthalmologist's office; but, it is difficult to treat effectively. The disease is an extremely complex condition that manifests in several different and overlapping arrays of signs and symptoms [48].

Despite the high prevalence of this condition, blepharitis has been a poorly understood clinical entity, and has posed a considerable diagnostic and therapeutic challenge to practicing eye care providers. It has been well established that microorganisms play a significant role in the pathogenesis of blepharitis. An understanding of the importance of ocular microflora, meibomian secretions as well as their composition, and the tear film in the development of blepharitis has been crucial for discovering more effective regimens for treating this disease [48].

The Fluoroquinolones have become widely used antibacterial agents in the treatment of ocular infections. They have good activity against Gram-negative and Gram-Positive bacteria, in addition to an unsurpassed ocular penetration [38,63]. Newer generation fluoroquinolones provide excellent efficacy against coagulase-negative staphylococci and S*treptococcus* group D despite a high number of multiresitant bacteria [37].

Our results demonstrated that topical 0.5% levofloxacin is effective in reducing conjunctival bacterial flora in patients with chronic blepharoconjunctivitis. There was a significant decrease in the number of bacteria present on the conjunctiva following a one-day application of topical levofloxacin [1,2]. The proportion of eyes with positive thioglycolate cultures was significantly decreased following a three-day application of topical antibiotic compared to untreated eyes, with further reduction after seven days of continued antibiotic use. Our results are similar to previous reports of bacteria eradication with ciprofloxacin in the treatment of blepharitis [9,21,50].

Prior to antibiotic treatment, patients with blepharitis were more likely to harbor bacteria on their conjunctiva compared to patients without blepharitis. The most common bacteria isolated in our study were coagulase-negative *Staphylococcus* for both patient groups, with or without chronic

blepharoconjunctivitis. However, normal control patients were more likely to have coagulase-negative *Staphylococcus* as part of their normal bacterial flora whereas patients with blepharitis were more likely to have other bacteria such as *Propionibacterium acnes, Staphylococcus aureus*, and *Streptococcus sp* [4,24]. These findings are also consistent with published research by McCulley and Dougherty in addition to others [16,44].

Coagulase negative Staphylococcus in patients with CBC showed a higher rate of resistance against certain antibiotics in comparison to patients without CBC. One reason might be the selection of resistant bacteria by previous antibiotic treatment in the CBC group.

Our study did not demonstrate any additional beneficial effect of eyelid scrub in combination with topical antibiotics regarding bacterial eradication compared to topical antibiotics alone. In contrast, eyelid hygiene is frequently recommended for the treatment of blepharitis, although there are few studies demonstrating its beneficial effects [20,65,72].

The most likely explanation for the lack of efficacy of eyelid scrub in reducing bacterial count is the fact that we did not instruct the patients to use any antibacterial soap, as opposed to a study by Avisar *et al.* [6] which demonstrated the efficacy of a detergent.

Additionally, eyelid scrub may have even caused further release of bacteria from the lid margin skin and glands [47,65]. Nonetheless this procedure may continue to play an important role in the treatment of blepharitis by mechanisms other than reducing bacterial flora, such as relieving the inspissated meibomian glands [46,59].

There are several important caveats regarding our study. First, the number of patients in each study group was not equal due to study incompletion for 8 patients. Despite the fewer number of patients completing the study than anticipated, significant differences were found between the antibiotic-treated and the non-treatment group at the final time-point measured at day 7. Second, cultures were obtained from both eyes. Therefore, the number of bacteria and response to treatment may be exaggerated when comparing patients with and without CBC, as well as the treatment

versus no treatment group. Finally, the clinical response for the treatment of CBC was not assessed in this study.

Despite the above mentioned drawbacks, there are important clinical implications of our study. Topical levofloxacin is effective in reducing bacterial flora in patients with chronic blepharoconjunctivitis. Given that the cause of blepharitis is multifactorial, including inflammation of the eyelids due to bacterial lipases, eliminating or reducing bacterial flora may contribute to the treatment of blepharitis [17].

Furthermore, patients with blepharoconjunctivitis who are scheduled for intraocular surgery may benefit from a course of topical antibiotic prior to surgery to reduce the bacterial count. It has been reported that patients with blepharitis have a higher risk of developing postoperative endophthalmitis [51,58].

In summary, patients with chronic blepharoconjunctivitis are more likely to harbor bacteria on their conjunctiva. Topical levofloxacin 0.5% is effective in eradicating these bacteria in patients with blepharoconjunctivitis. Eyelid scrub did not provide additional benefit in bacterial eradication in patients already treated with topical antibiotics. Further studies are necessary to clarify the role of bacteria in the pathophysiology of blepharitis in order to develop an effective treatment for this chronic condition.

5. Summary

The conjunctival flora in patients with CBC has been reported to be comprised of a greater number of bacteria compared to normal individuals.

Patients with CBC who undergo intraocular surgery may be at higher risk for developing postoperative endophthalmitis due to an increased eyelid and conjunctiva bacterial load. Treatment of CBC with antibiotics to reduce bacterial load may be particularly important prior to surgery.

The most common bacteria isolated for all groups at baseline were coagulase-negative *Staphylococcus*. Seven patients did not complete the study. Conjunctival cultures were not obtained in 4 patients (2 in the no treatment group und 2 in the combined group) and 4 patients had at least one protocol violation (2 in the Levofloxacin treated group and 2 in the combined group). The remaining 52 patients with CBC had a significantly higher rate of positive thioglycolate broth cultures (94%) compared to a 58% positive culture rate in patient without CBC ($P < 0.0001$). Treatment with at least 3 days of topical antibiotic in patient with CBC resulted in a significant reduction ($P < 0.05$) in the number of thioglycolate positive cultures (≤ 60%) compared to non-treated eyes (≥ 88%). Following a minimum of a 1 day application of antibiotic, the median colony-forming unit was 0-1 compared to 3-8 for eyes without antibiotic treatment ($P < 0.05$). Scrubbing of the eyelids did not provide further benefit compared to antibiotic treatment alone.

CBC eyes have a significantly higher number of positive cultures compared to eyes without CBC. The application of topical 0.5% levofloxacin for at least 3 days provided significant reduction in the number of positive cultures as well as the number of bacteria harbored on the conjunctival surface.

In summary, patients with chronic blepharoconjunctivitis are more likely to harbor bacteria on their conjunctiva. Topical levofloxacin 0.5% is effective in eradicating these bacteria in patients with blepharoconjunctivitis. Eyelid scrub did not provide additional benefit in bacterial eradication

in patients already treated with topical antibiotics. Further studies are necessary to clarify the role of bacteria in the pathophysiology of blepharitis in order to develop an effective treatment for this chronic condition.

Zusammenfassung

In Studien konnte bereits gezeigt werden, dass die konjunktivale Flora bei Patienten mit chronischer Blepharokonjunktivitis (CBC) im Vergleich zu einer gesunden Kontrollgruppe eine größere Anzahl an Bakterien aufweist. Patienten mit CBC, die sich intraokularen Operationen unterziehen, haben ein höheres Risiko eine postoperative Endophthalmitis zu entwickeln, was auf eine höhere Anzahl an Bakterien (Keimlast) an Augenlidern und Konjunktiva zurückzuführen ist. Eine präoperative Behandlung der CBC zur Reduktion der Keimzahl könnte daher einen bedeutenden Faktor zur Reduktion postoperativer Infektionen darstellen.

Der häufigste Keim, der bei allen Gruppen vor Therapiebeginn isoliert werden konnte, waren Koagulase negative Staphylokokken. Insgesamt wurden 60 Patienten in die Studie eingeschlossen. Am Ende wurden sieben Patienten aus folgenden Gründen in der Auswertung ausgeschlossen: Verpasste Bindenhautabstriche zu irgendeinem Zeitpunkt bei 4 Patienten (2 in der unbehandelten Gruppe und 2 in der kombiniert behandelten Gruppe) und wenigstens eine Protokollverletzung bei 4 Patienten (2 in der allein mit Levofloxacin behandelten Gruppe und 2 in der kombiniert behandelten Gruppe). Die restlichen 52 Patienten mit CBC hatten eine signifikant höhere Rate an positiven Thioglycolat Bouillon Kulturen (94%) verglichen mit Patienten ohne CBC (58%, $P < 0.0001$). Eine mindestens 3-tägige Behandlung von Patienten mit CBC mit einem topischen Antibiotikum führte zu einer signifikanten Reduktion ($P < 0.05$) der Anzahl an Thioglykolat positiven Kulturen ($\leq 60\%$) im Vergleich zu nicht behandelten Augen ($\geq 88\%$).

Nach mindestens 1-tägiger Applikation des topischen Antibiotikums, lag die mittlere koloniebildende Einheit (KBE) bei 0-1 verglichen mit 3-8 bei nicht behandelten Augen ($P < 0.05$).

Eine additive Lidkantenpflege brachte keinen zusätzlichen Vorteil verglichen mit der topischen Antibiotikabehandlung alleine.

Augen mit CBC wiesen im Vergleich zu gesunden Augen eine signifikant höhere Anzahl positiver Kulturen auf. Die Anwendung von 0.5% Levofloxacin für mindestens 3 Tage führte zu einer signifikanten Reduktion der Anzahl an positiven Kulturen und der Keimzahl auf der konjunktivalen Oberfläche.

Zusammenfassend lässt sich festhalten, dass Patienten mit Blepharokonjunktivitis mehr Bakterien auf der konjunktivalen Oberfläche aufweisen. Die topische Anwendung von Levofloxacin 0.5% bei Patienten mit chronischer Blepharokonjunktivitis führte zu einer effizienten Eradikation dieser Bakterien. Lidkantenpflege zusätzlich zur topischen Antibiotikatherapie erbrachte keinen additiven Effekt. Weitere Studien sind notwendig, um die Rolle von Bakterien in der Pathophysiologie der Blepharitis zu klären und eine effiziente Behandlung dieser chronischen Augenerkrankung entwickeln zu können.

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8. Resume

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AUSBILDUNG	▪ **Grundschule:** Unidad Educativa Desarrollo Educativo Industrial (D.E.I) Puerto Ordaz, Venezuela. 1983 – 1988. ▪ **Gymnasium:** Colegio Loyola Gumilla. Puerto Ordaz. Venezuela. 1989-1993. Schulzeugnis: Bachiller en Ciencias. ▪ **Auslandsaufenthalte:** Schüleraustausch, 11. Klasse, Labenwolf-Gymnasium . Nürnberg, Deutschland. Sep. 1994 – Juli. 1995. Mit AFS (American Field Service) Interkulturelle Begegnungen. e.V. ▪ **Universität**: Universidad de Oriente. Núcleo Bolívar. Escuela de Medicina. Ciudad Bolívar.Venezuela. 1994 - 2002. Abschlusszeugnis: Titulo Médico Cirujano.
BERUFSERFAHRUNG	▪ **Landarzt:** Ambulatorio Urbano Tipo I Sierra III. Upata. Instituto de Salud Pública del Estado Bolívar. Venezuela. 01.12.2002 – 30.11.2003. ▪ **Praktikumärztin (Ärztin im Internen Turnus) :** Hospital Raúl Leoni, San Félix, Instituto Venezolano de los Seguros Sociales. Venezuela. 01.12. 2003 – 30.12.2004. ▪ **Assistenzarzt:** Chirurgie. Hospital Uyapar, Puerto Ordaz, Instituto Venezolano de los Seguros Sociales. Venezuela. 01.01.2005 – 27.04.2006. ▪ **Zur Zeit:** Assistenzärztin als Stipendiaten in der Augenklinik der Ludwig Maximilians Universität München. (seit Juni 2008)

FORSCHUNG

- **Titel:** Prevalencia de Linfoma Hodgkin y No Hodgkin en el Hospital Universitario Ruiz y Páez de Ciudad Bolívar. Período 1996-1999. Veröffentlich in: ***XVII Jornadas científicas, Tecnológicas y Educativas de Guayana, AsoVAC*** Capítulo Oriental. Nov. 2001. Ciudad Bolívar. Venezuela.

- **Titel:** Lipoma Gigante. A propósito de un caso. Hospital Uyapar. Servicio de Pediatría. Período 2002-2003. Veröffentlich in ***XII Congreso Venezolano de Cirugía Pediátrica.*** Asociación Venezolana de Cirugía Pediátrica. Okt. 2003. Caracas. Venezuela.

- **Titel:** Efectos de la Terapia Reductora de Lípidos sobre el Estado de Ánimo y la Función Cognitiva en Ancianos Institucionalizados en el Asilo San Vicente de Paúl. Ciudad Bolívar. 2001-2002. Veröffentlich in ***Revista del Colegio Venezolano de Neuropsicofarmacología. (Nationale Zeitschrift)*** Volumen 5. Número 1. Año 2003. Pág: 09-18. Caracas. Venezuela.

- **Titel:** Reduction of Conjunctival Flora by Presurgical Irrigation with Povidone- Iodine in Cataract Surgery Patients. Department of Ophthalmology, Fundación Visión, Asunción, Paraguay. Department of Ophthalmology, Ludwig-Maximilians-University, Munich, Germany. November 2005 – July 2006. Poster Presentation. **The Association for Research in Vision and Ophthalmology (ARVO) Annual Meeting May 2007. Fort Lauderdale, Florida.**

- **Titel:** Postoperative Endophthalmitis: Incidence and Prognosis over a 5 year Survey. Department of Ophthalmology, Ludwig-Maximilians-University, Munich, Germany. Poster Presentation. **The Association for Research in Vision and Ophthalmology (ARVO) Annual Meeting May 2007. Fort Lauderdale, Florida.**

- **Titel:** Bacterial Contamination of Needle's Point after Intravitreal Injections. Department of Ophthalmology, Ludwig-Maximilians-University, Munich, Germany. Poster Presentation in **The American Academy of Ophthalmology. Annual Meeting November 2007. New Orleans, USA.**

- **Titel:** Efficacy of 0.5% Levofloxacin Therapy against Aerobic-Anaerobic Bacterial Flora in Chronic-Blepharoconjunctivitis Patients: A Prospective Semi-Randomized Study. Department of Ophthalmology, Ludwig-Maximilians-University, Munich, Germany. Poster Presentation. **The Association for Research in Vision and Ophthalmology (ARVO) Annual Meeting May 2008. Fort Lauderdale, Florida.** (Thema der eigenen Dissertationsarbeit).

- **Titel:** A prospective study determining the efficacy of topical 0.5% levofloxacin on bacterial flora of patients with chronic blepharocon-junctivitis. Yactayo-Miranda Y, Ta CN, He L, Kreutzer TC, Nentwich MM, Kampik A, Mino de Kaspar H.; **Graefes Arch Clin Exp Ophthalmol. 2009 Feb 11.** (Thema der eigenen Dissertationsarbeit).

WEITERBILDUNG

39.-Ablatio-Kurs (Wacker-Kurs) Fortbildungskurs zur Prophylaxe, Diagnostik und Therapie der Ablatio Retinae, 28./29. Juni 2007 in der Augenklinik der Universität München.