Diploma Thesis

Laboratory diagnosis in infectious keratitis and corneal ulcer. A hospital-based study.

submitted by

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Statutory Declaration

I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organisations that have contributed to the research for this thesis. Due acknowledgement has been made in the text to all other material used.

Throughout this thesis and in all related publications I followed the “Standards of Good Scientific Practice and Ombuds Committee at the Medical University of Graz“.

Graz, May 12th, 2022 *Vincent Constantin Pritzel eh*

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Abbreviations and Definitions

ACAID: Anterior chamber associated immune deviation

APC: Antigen presenting cells

CALT: Conjunctiva associated lymphoid tissue

DTH: Delayed type hypersensitivity

eSwab: Copan Liquid Amies Elution Swab

FAE: Follicle-associated epithelium

ITS: Internal transcribed spacer

LASIK: Laser-assisted in situ keratomileusis

LPCB: Lactophenole Cotton Blue

MALT: Mucosa associated lymphoid tissue

Med Uni Graz: Medical University of Graz

M Cells: Microfold Cells

MMP: Matrix metalloproteinase

MRSA: Methicillin-resistant Staphylococcus aureus

Nd: YAG-laser: Neodymium-doped yttrium aluminium garnet-laser

PAS: Periodic acid-Schiff stain

PCR: Polymerase Chain Reaction

PRK: Photorefractive keratectomy

ROCK inhibitors: Rho kinase inhibitors

SOP: Standart Operation Procedure

Tregs: T regulator cells

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Abstract in German

Titel:

Labordiagnostische Untersuchung der infektiösen Keratitis und des Hornhautulkus.
Eine Krankenhaus-basierte Studie.

Einleitung:

Das Keimspektrum der Keratitis bzw. des Hornhautulkus ist charakteristisch für unterschiedliche geographische Standorte. Bei dieser retrospektiven Studie soll das Keimspektrum der infektiösen Keratitiden bzw. Hornhautulzera für das Versorgungsgebiet der Univ.-Augenklinik Graz (Teile der Steiermark, Burgenland und Kärnten) und die Resistenzen der bakteriellen Erreger dargestellt werden.

Methoden:

Bei dem vorliegenden Forschungsvorhaben wurde ein retrospektives (Single-center) Studiendesign gewählt. Die Daten der Studie wurden aus Datensätzen der Universitäts-Augenklinik, des Landeskrankenhauses Graz extrahiert. Alle Patient*innen, die im Jahr 2018 an der Universitäts-Augenklinik am LKH-Univ. Klinikum Graz eine labordiagnostische Abklärung wegen einer infektiösen Keratitis oder eines Hornhautulkus erhielten, wurden in die Studie eingeschlossen.
Für den Erregernachweis wurden Abstriche von Hornhaut (n=100) und Konjunktiva (n=62) angefertigt und das Sediment der Flüssigkeit aus Kontaktlinsenbehältern (n=23) untersucht. Der Erregernachweis fand direktmikroskopisch, über das Beimpfen von Kulturmedien und mit Hilfe der PCR statt. Beim Nachweis von bakteriellen Erregern erfolgten Resistenzbestimmungen mit Anfertigung von Antibiogrammen. Das Datenmanagement und die statistische Analyse erfolgte mit MS Excel und SPSS.

Ergebnisse:

100 Augen von 100 Patient*innen wurden in die Studie aufgenommen (56 männliche 44 weibliche, Durchschnittsalter: 49±21 Jahre, von 16 bis 93 Jahre, 51 Kontaktlinsenträger, 8 Z.n. Trauma, 9 Z.n. augenchirurgischen Eingriffen).
Bakterien wurden bei 100, Pilze bei 25, Akanthamöben bei 12 und Viren bei 8 Patient*innen nachgewiesen.
Bei der Hornhaut wurden *84 bakterielle Isolate* nachgewiesen. *Koagulase-negative Staphylokokken (40,5%)* bildeten die Mehrheit. Das häufigste bakterielle Isolat war

Propionibacterium acnes (28,6%). Resistenzen gegen 24 Antibiotika wurden nachgewiesen.
Diese Daten wurde auch für die *Bindehaut* und *Kontaktlinsenbehälter* erhoben.
Der Anteil der Patient*innen mit einer Sehschärfe von ≥ 0,5 stieg im Rahmen der Behandlung um 20,5% an.

Schlussfolgerung:
In unsere Studie wurden bakterielle Erreger als häufigste Pathogene identifiziert. Die Bakterielle Keimbesiedlung von Kornea, Konjunktiva und Kontaktlinsenbehältern unterscheidet sich. Die Resistenzen der bakteriellen Isolate gegen Antibiotika wurden zusammengefasst. Eine Verbesserung des Visus wurde durch die angewendete Therapie erzielt. Kontaktlinsen, Traumata und augenchirurgische Eingriffe sind mögliche Risikofaktoren für eine infektiöse Keratitis.

Abstract in English

Title:

Laboratory diagnosis in infectious keratitis and corneal ulcer. A hospital-based study.

Background:

The germ spectrum of keratitis or corneal ulcer is characteristic for different geographical locations. In this retrospective study, the germ spectrum of infectious keratitis or corneal ulcers and the resistances of the bacterial isolates for the care area of the University Eye Hospital Graz (parts of Styria, Burgenland and Carinthia) are presented.

Methods:

A retrospective (single-centre) study design was chosen for the research project. The data of the study are extracted from data sets of the Universitiy Hospital of Ophtalmology, Graz. All patients who were treated for infectious keratitis or ulcer at the University Department of Ophthalmology, LKH-Univ. Klinikum Graz in 2018, were included in the study. Corneal scrapes and direct smears were taken from the cornea (n=100) and conjunctiva (n=62) and contact lenses containers (n=23) were examined for pathogen detection. Pathogen detection was performed by direct microscopy, microbial culture and PCR. When bacterial pathogens were detected, antimicrobial resistance screenings were conducted. Data management and statistical analysis is carried out with MS Excel and SPSS.

Results:

100 eyes of 100 patients were included in the study (56 male 44 female, mean age: 49±21 years, from 16 to 93 years, 51 contact lens wearers, 8 cases after trauma, 9 cases after eye related surgery).

Bacteria were detected in *100*, *fungi in 25*, *Acanthamoeba in 12*, and *viruses in 8 patients*. *Regarding the cornea*, *84 bacterial isolates* were detected. *Koagulase-negative staphylococci (40.5%)* showed the largest category. The most common bacterial isolate was *Propionibacterium acnes (28.6%).* R*esistances against 24 antibiotic agents* were detected.

These data were also acquired for the *conjunctiva* and *contact lens cases*.

The proportion of patients with visual acuity ≥ 0.5 increased by 20,5% during the treatment.

Conclusion:

Our study identified bacterial pathogens as the most frequent pathogens. Bacterial colonization of the cornea, conjunctiva, and contact lens cases differed. Resistances of the presented bacterial isolates to antibiotic agents were summarized.

Improvement in visual acuity was achieved with the applied therapy. Contact lenses, trauma, and eye related surgeries are potential risk factors for infectious keratitis.

1 Introduction

The cornea is essential for the perception of light. Keratitis or corneal ulcers can lead to serious visual impairment or even blindness. Hence, quick and adequate medical interventions are necessary. Knowledge of the pathogens responsible for infectious keratitis and corneal ulcers and the resistances of the causative germs against antibiotics is the basis for adequate therapeutic measures in the treatment of these diseases.

1.1 The anterior segment of the eye

The visual system consists of the visual organ, organum visuale, and central nervous connections. The visual organ includes the eye, oculus (gr.: ophtalmos) and accessory structures, structurae oculi accessoriae, such as eyelids, conjunctiva, the lacrimal system and extraocular muscles. (1)

The eye enables the human to perceive light with a wavelength of 400nm up to 750nm. Three layers surround the internal components of the eyeball. The outer fibrous layer, tunica fibrosa bulbi, consists of the cornea anteriorly and the sclera posteriorly. The choroid, tunica vasculosa bulbi, the middle vascular layer, is continuous with the ciliary body and iris anteriorly and consists of the choroid posteriorly. The inner layer, tunica nervosa bulbi, is formed by the nonvisual retina anteriorly, which covers the internal part of the ciliary body and iris anteriorly. Posteriorly the optic retina builds the optic part. (2,3)

The posterior segment of the eye is formed by the neurosensory perceptive part.

The anterior segment includes eyelids, conjunctiva, cornea, sclera, iris and ciliary body, lens, anterior and posterior chamber. The anterior segment of the eye also forms the refractive (dioptric) part. (2)

The upper and lower **eyelids** serve as protection for the surface of the eyeball. The palpebral fissure is the gap between the eyelids when they are opened. From anterior to posterior, the eyelid´s layers are made up of skin, subcutaneous tissue, voluntary muscle, the septum of the orbita, the tarsal plate, and conjunctiva. (3) Beside sebaceous and sweat glands, associated with the eyelash follicles, tarsal glands are embedded in the tarsal plate. These modified sebaceous glands secrete an oily substance that increases the viscosity of the tears and decrease the rate of evaporation of tears from the surface of the eyeball. (4)

The **Conjunctiva** has a very important role for protection, mediating passive and active immunity. It is a transparent mucous membrane and anatomically divided into the

palpebral conjunctiva, the forniceal conjunctiva and the bulbar conjunctiva. It lines the inner surface of the eyelids and the anterior surface of the eyeball, then terminates at the corneoscleral limbus. Supplied by the anterior ciliary and palpebral arteries, it is richly vascular and there is a dense lymphatic network with drainage to the preauricular and submandibular nodes.

Histologically the non-keratizing epithelium is equipped with mucus-secreting goblet cells. Deep within the densely vascularized stroma, consisting of loose connective tissue, accessory lacrimal glands of Krause and Wolfring are located. The conjunctiva associated lymphoid tissue (CALT) consists of lymphocytes within the epithelial layers, lymphatics and associated blood vessels, with a stromal component of lymphocytes and plasma cells, including follicular aggregates. (5)

The cornea, with its complex structure, has a protective role. With 43 dpt, it is largely responsible for the optical power of the eye. Because the cornea is free of blood vessels, aqueous humour posteriorly and the tears anteriorly, supply and remove nutrients and metabolic products. The first division of the trigeminal nerve makes the cornea the most innervated tissue of the human body and highly sensitive to pain. (5) Because of its central significance, more information about the cornea can be found in a separate chapter.

The sclera forms the outer fibrous layer of the eye. It includes the *lamina cribrosa*, medially located to the posterior pole. The optic nerve´s fibres leave the eyeball through this sieve-shaped structure. (4)

The **iris** forms the eye´s aperture. The diaphragm is adjustable to the intensity of light. Due to a densely pigmented posterior double epithelium, the iris can act as an efficient light stop. At short distance accommodation it constricts, by what the depth of field increases. Two muscles, the musculus dilatator and sphincter pupillae, adjust the pupillary aperture.(4)

The **ciliary body** is confluent with the iris. Its anterior part, the pars plicata, is the source of aqueous humour, it anchors the lens via suspensory ligaments and is responsible for accommodation with the ciliary muscle. Its posterior part, the pars plana mainly matters in clinically routine such as eye surgery, because it´s rare on vulnerable structures. (4)

The **lens** is a part of the optical apparatus and has a higher refractive index, than aqueous fluid or the vitreous body. It is a transparent, crystal clear body and located behind the pupil in the pit of the vitreous body. Zonular fibres act as an anchor for the lens and connect its equator with the pars plana of the ciliary body. When the ciliary muscle contracts, the tension on the lens decreases, and its inherent elasticity leads to an increased

refractive power. No vessels or nerve fibres are located in the lens. By diffusion, it is supplied with nutrients. (4)

The anterior chamber is limited by the back surface of the cornea, the chamber angle, the front surface of the iris and the front surface of the lens in the field of the pupil. In the anterior chamber, there is an immune tolerance, the so-called anterior chamber associated immune deviation (ACAID). The ACAID probably suppresses immune reactions, that could endanger the vision. This kind of immune tolerance can be found in the subretinal space and in the vitreous body. In case of eye injury, antigens may leave this space of immune tolerance and could lead to autoimmune reaction against cells of the uvea or the pigment epithelium. Thereby the other eye can go blind. (4)

The **posterior chamber** is limited by the back surface of the iris´, the sulcus ciliaris, ciliary fibres, ciliary body, front surface of the vitreous body and backspace of the lens. The posterior chamber is significantly smaller than the anterior chamber. The anterior and posterior chambers are filled with aqueous humour, that is produced by the ciliary body. It flows from the posterior chamber to the anterior chamber and passes the pupil. The aqueous humour feeds the cornea and lens. In case of iris diseases, the composition of aqueous humour can change by diffusion of proteins through the blood-retinal barrier.

The **chamber angle** consists of the trabecular meshwork. Through it, most of the aqueous humour reaches the Schlemm´s kanal. (4)

1.2 Tear film physiology

"Overall, the tear film is extremely important for the maintenance of ocular health. Disruption, deficiency or absence of this film may significantly increase the susceptibility to ocular surface desiccation, inflammation and infection, corneal ulceration and perforation, visual impairment and possible blindness." (6)

The neuronally induced blink reflex spreads the tear film over the eye´s surface. For full functionality, the blink reflex must work properly, contact between the outer ocular surface and the eyelid and the integrity of the corneal epithelium must be ensured.

The tear film consists of three layers. Meibomian glands secrete **the outer lipid layer**. It ensures the thickness of the tear film by delaying evaporation of the aqueous layer and acts as a surfactant, to fulfill its main purpose of reducing surface tension. (7)

It plays a big role in tear film stabilization. A non-polar phase consists of cholesterol esters, waxes and triglycerides. A polar phase consists of phospholipids connected by lipocalin to the middle aqueous layer. (7)
The main lacrimal glands and accessory lacrimal glands of Krause and Wolfring secrete the **middle aqueous** layer. It cleans the eye from debris and noxes, transports leukocytes to wounds, contains IgA, lysozyme and lactoferrin, so it has an important antibacterial function. It also supplies the cornea with oxygen and brings optical stability by leveling out unevenness. At ocular inflammation, eye film break-up, or sensory stimulation of the cornea or the conjunctiva, the aqueous layer can be increased by many times, mediated via the fifth cranial nerve. The aqueous layer also contains, electrolytes, mucins, proteins, growth factors and pro-inflammatory interleukins. (5,8)
Mainly conjunctival goblet cells secrete the **inner mucous layer**. It acts as a lubricant and moisturizer for the cornea by making the hydrophobic cornea hydrophilic and ensures the connection between the tear film and cornea. It contains gel-forming or soluble mucins, a glycocalyx forming transmembrane mucins. (5,8)

1.3 Cornea – Structure, histology and physiology

From anteriorly to posteriorly the cornea consists of the epithelium, Bowman layer, stroma, Descemet membrane and endothelium.
In the center, the **cornea** is approximately 500 µm thick. Towards the periphery, the thickness slightly increases.
The anterior part of the cornea is a stratified, squamous and nonkeratinizing **epithelium,** consisting of 4-6 cell layers. The basal layer consists of a single layer of columnar basal cells. With hemidesmosomes, they are attached to the basal membrane below. Wing cells and squamous surface cells build the epithelium as well. (5)
In the palisades of Vogt, which are located in the corneoscleral limbus, **limbal stem cells** (LSC) can be found. The corneoscleral limbus, especially the LSCs localized therein, plays an important role in regeneration of corneal injuries and as a barrier for conjunctival cells, preventing them to disturb the corneal integrity. LCS have a high proliferative potential. That enables them to an efficient corneal regeneration and repair.
Loss or deficiency of LSC might causes disturbance of corneal homeostasis and can result in abnormal wound healing, a condition known as limbal stem cell deficiency (LSCD).

LSCD can lead to conjunctival epithelial ingrowth, neovascularization of the corneal stroma, corneal opacification, and vision loss. (9)
Consisting of collagen fibres, the acellular **Bowman layer** builds the superficial layer of the stroma.
Beside proteoglycan ground substance and fibrocytes, collagen fibres are regularly arranged in the **stroma**, to secure its clarity. In case of violation of the stroma, permanent scars can occur, because there is no ability of regenerating the integrity.
The **Descemet membrane** has the ability to regenerate. It is composed of collagen fibres and builds a modified basement membrane for the endothelium.
The **endothelium** cannot regenerate. It is built of a monolayer of polygonal cells. An important purpose is to actively dehydrate the stroma, to prevent it from swelling and losing its clarity. Over the course of life, the endothelial cells reduce. (5) Meanwhile progress has been made in the treatment of different endothelial diseases like Fuchs disease or corneal edema due to surgical trauma with Rho kinase (ROCK) inhibitors. The usage of Ripasudil and Narsudil for example have shown to result in less apoptosis, better endothelial cell proliferation and increased intercellular adhesion. (10)
Its dense innervation makes the cornea to one of the most sensitive tissues in the body. Conditions, such as abrasion, ulcer, or bullous keratopathy can lead to marked pain. The nasociliary branch of the ophthalmic nerve, that originates from the trigeminal nerve, builds the corneal innervation. Nerve fibres enter the stroma radially, building plexiform structures. Autonomic sympathetic nerve fibres can also be found in the cornea. (11)

1.4 Immune system of the anterior segment

The anterior segment of the eye is exposed to pathogens, allergens and toxic substances daily. An efficient mucosal barrier, composed of complex mechanical and functional immunological defence mechanisms, at the eye´s surface, protects it against injuries. These defence mechanisms not only protect the eye but can even harm the mucosa and deeper layers of tissue, by autoimmune inflammatory processes. Therefore, regulatory mechanisms exist, to control the immune response and to protect the eye of harmful inflammatory processes. That kind of balanced immunity protects all tissues of the eye. It plays an important role for maintaining the eye´s vision.

Innate and acquired immune system are woven. Even if you can classify the immune system into innate immunity and acquired immunity, it makes sense to view it morphologically, step by step. (12)

“The preocular tear film plays a critical role in the eye´s defence against antigenic challenge, as well as in the maintenance of conjunctival integrity, corneal clarity and visual acuity.” (6)
The **tear film** includes antimicrobial peptides, mucins and immunoglobulins.
Antimicrobial peptides, such as lysozymes, lactoferrin, beta-defensin and matrix metalloproteinase 7 (MMP7), have a lethal effect on microorganisms by penetrating their cell walls. They belong to the innate immune system, but also act as chemotactic substances for cells of the acquired immune system, such as antigen-presenting cells (APC) and T Cells. (12,13)
Mucins are secreted by epithelial and mucus-secreting goblet cells. They consist of peptides with many carbohydrate sidechains. Mucins act as the hydrophilic wetting part of the tear film. They interact with microorganisms by making a connection via their epitopes. By encasing pathogens, they prevent them to bind to the epithelial structures and inactivate them. The tear film clearance is increased and pathogens can be derived via the lacrimal ducts more effectively.
Trefoil-Factor-Peptides are expressed in case of inflammation. They connect different mucin molecules and have an influence on the viscosity of the tear film. TFF-Peptides support the epithelial wound healing as well.
Surfactant proteins A-D can bind and inactivate pathogens, by activating phagocytosis and the complement system. They also have an influence on tear film rheology.
Inflammation leads to an increase of **immunoglobulins**, such as sIgA > sIgE > sIgG > sIgM, in descending order. (12,14)

Epithelial cells of the conjunctiva, cornea and lacrimal drainage system form a thin but effective barrier against pathogens by secreting mucins and antimicrobial peptides when contacting them.
They also can express toll-like receptors at contact with pathogens. Toll-like receptors are able to trigger an intracellular inflammation cascade and an associated inflammatory reaction.
Epithelial cells are also in charge of forming MHC II molecules and presenting antigens.

Intraepithelial immune cells contribute to the defense as well. Numerous immune cells, such as CD4+ T cells, CD8+ T cells, Langerhans cells and other APCs are localized especially in the conjunctival epithelium. Plasma cells segregate immunoglobulines.
T cells in the basal membrane try to prevent pathogens to overcome the epithelial barrier as last epithelial obstacle. Here the microorganisms can be recognized, phagocytized or even killed. Antigen presentation, first of all, takes place in the conjunctiva-associated lymphoid tissue (CALT) and regional lymph nodes.
CD4+ Cells and CD8+ cells can be found in caves, formed by Microfold cells (M cells) in the epithelium of the conjunctiva.
(12,15)

Follicle-associated epithelium (FAE) forms the CALT. Mostly in the epithelium, above the FAE, there are the important M cells, which form small folds on their apical side. At their base, they form intraepithelial pockets containing lymphocytes and macrophages. M Cells play an important role for the immune system. They have a sampling function and can phagocytose antigens, pathogens and macromolecular structures. After phagocytosis and sampling, pathogens can be phagocytosed by macrophages and dendritic cells, in the basal pockets or the structures reach the lymphoid follicles for further immunological procedures. (16-20)
Further immunocompetent cells process, present, and transport antigens to regional lymph nodes and mucosa associated lymphoid tissue.
Located in the conjunctival **stroma**, T cells, B cells, and APCs, build diffuse and organized lymphatic tissue. The lymphoid tissue of the eye acts as an immunologically meeting place and takes over sensory and regulatory tasks of the immune system.
Along the entire mucous membrane of the eye, you can find macrophages and lymphocytes, loosely arranged. They build the so called *diffuse* CALT.
Lymphoid follicles, located directly under the conjunctival epithelium, are called the *organized* CALT. These follicles consist of centrally located B cells, surrounded by CD4+, CD8+ T cells, some dendritic cells and regulatory T cells.
If antigens enter the CALT and regional lymph nodes, T cells and B cells are activated and proliferate, then spread in the circulatory system and mature. The conjunctiva is vascularized densely and supplied by blood and lymphoid vessels. At the location of the eye´s inflammation, adhesion molecules at the endothelium of the blood vessels, mark the point of interest for recirculating matured plasma cells and effector cells. (6,12,21)

The CALT contains all the components necessary for a complete immune response.
It is located in the conjunctiva of the upper eye lid rather than in the conjunctiva of the lower eye lid, especially in the palpebral region.
The Mucosa associated lymphoid tissue (MALT) is a tissue, that occurs in all mucous membranes. (19)

Inflammation can be triggered mechanically by rubbing or pressure, toxically by medication or preservatives, chemically by acid or lye, physically by ultraviolet light, infectious, allergic or autoimmune reasons.
To the inflammation regulating mechanisms, belongs the activation of metalloproteinases (MMPs), induction of autoreactive T cells, pro-inflammatory factors such as interleukins and chemokines, angiogenesis, flooding and activation of APCs, formation of immunoregulatory T cells and the expression of chemokine receptors.
(12)

Immune response in the eye can lead to immune mediated tissue destruction and can cause visual aggravation or even vision loss. To prevent the eye of this damage, different phenomena occur, such as immune deviation in the anterior chamber, called **anterior chamber associated immune deviation (ACAID)**.
When antigens enter the anterior chamber, a systemic immune response is launched via cytotoxic CD8+ T cell precursors and non-complement-fixing IgG producing B cells. ACAID ensures a prevention of delayed type hypersensitivity (DTH) development by downregulation of CD4+ T cells. ACAID inducing APCs take up antigens, migrate to the spleen after entering the trabecular meshwork and the blood vessels. In the spleen T regulator cells (Tregs) are generated. Tregs prevent the initial immune response, DTH and inhibit B cells.
Similar immune deviation phenomena are induced when antigens enter other parts of the eye, such as vitreous cavity or the subretinal space.
Clinically, ACAID plays an important role in corneal transplantation, infectious keratitis and other immunopathological conditions. (12,22)

1.5 Infectious keratitis

Inflammatory corneal disease is amongst the most common reasons for visual loss. A changing germ spectrum and changing risk factors throughout the years can be found. Triggering factors for infectious keratitis seem to be the use of contact lenses and refractive surgeries. Probably because of the widespread use of highly effective antibiotics like fluoroquinolones, the bacterial spectrum seems to change. (23)

The pathogen distribution, causative for infectious keratitis or bacterial ulcer differs, depending on geographic location. In a peer-review, Ameet Shah et al. published an analysis of geographic variations in microbial keratitis. The frequency of culture positive organisms around the world differs clearly. (24) Factors as gross national income seem to have an influence on pathogen distribution as well. (25)

"The highest proportion of staphylococcal ulcers was found in a study from Paraguay, while the highest proportion of Pseudomonas ulcers was reported in a study from Bangkok. The highest proportions of fungal infections were found in studies from India and Nepal." Statistically significant correlations between gross national income and percentages of bacterial and streptococcal isolates were demonstrated. (24)

In a microbiolocial review from Daniel Tena et al. bacterial keratitis was predominant by far, followed by viral keratitis and fungal keratitis. (25)

1.5.1 Bacterial keratitis

Worldwide inflammatory corneal disease is the main reason for monocular blindness. Bacteria are the main cause for infectious keratitis with 80%. (23)

In various countries worldwide, different climatic conditions, economic power, socio-cultural imprints and medical infrastructures can be found. These factors lead to varying data on frequency and risk factors of bacterial keratitis. (24)

Important bacteria leading to infectious keratitis include Gram-positive cocci, such as coagulase-positive Staphylococcus aureus, coagulase-negative Staphylococci like Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus viridans and in rare cases, anaerobe Streptococci. Furthermore, diplococcus such as Gram-negative Neisseria gonorrhoeae and Neisseria meningitidis, Gram-positive rod-shaped bacteria such as Corynebacterium spp., Gram-negative rod-shaped bacteria such as Pseudomonas aeruginosa, Serratia marcescens, Acinetobactcer spp., Escherichia coli, Klebsiella spp., and other Gram-negtative bacilli play an important role. (26)

Throughout the world most frequent risk factors for bacterial keratitis are contact lenses, refractive surgery and ocular trauma. Further risk factors are lid deformities, dry eye, corneal sensitivity disorders, long-term use of topical steroids and usage of systemic immunosuppressants. (27-29)

Contact lenses belong to one of the main risk factors for inflammatory corneal disease. In a study, conducted in 2012 by Yildiz et al., 44% of over 500 patients with infectious keratitis were contact lens related. (30)

Staphylococcus aureus and Pseudomonas aeruginosa belong to the most frequent germs in the context of risk factor contact lenses. Further germs are Serratia spp., Moraxella lacunata, Bacillus subtilis, Streptococci and Corynebacterium spp.. (31)

Soft contact lenses, or contact lenses with a prolonged wearing time, can increase the risk of microbial keratitis by a factor of 20, a Dutch study showed. (32)

With the increase of refractive surgery procedures in the last years, the risk of infectious corneal disease increased and gained importance as a risk factor for inflammatory corneal disease. (33)

The incidence of bacterial keratitis is 1/1000 in laser-assisted in situ keratomileusis (LASIK) and 1/5000 for photorefractive keratectomy (PRK). (34)

It is assumed that mainly contaminated surgical instruments, microkeratomes and therapeutic contact lenses are causative for the infections. (35)

Although there seems to be a decrease of infectious keratitis in Europe and the USA after trauma, injuries of the cornea still are an important risk factor. In a review by Bourcier et al., up to 21% of 300 cases with infectious keratitis were traced back to corneal injury. (23,24,36)

Clinical features of bacterial keratitis could include foreign body sensation, light sensitivity, lacrimation, circumlimbal/diffuse injection, white opacities in the stroma, oedema, epithelial defects and possible anterior uveitis.

Complications may include an extension of the inflammation to the limbus or sclera, perforation of the cornea, endophthalmitis or panophthalmitis.

Important examinations include early corneal scrapes for stainings and culture, culture of lenses, solutions, and cases. If herpetic disease is considered, a swab for PCR should be taken. (37)

Basically, a high-dose, time-limited local antibiotic therapy combined with anti-inflammatory treatment should be given. The therapy should reduce or eradicate the bacterial pathogens, reduce the inflammatory response and support wound healing. (23)

Initially, contact lens wear should be stopped. In case of lacking compliance or distinct infection, hospitalisation may be necessary. (37)

Systemic therapy usually is only used when there is an infection of the interior of the eye or tissue involvement around the cornea. With topical administration, higher concentrations can be reached on the corneal surface and depending on the pharmacological properties of the drug, with topical administration higher concentrations also can be reached in the stroma of the cornea, compared to systemic therapy. This fact is due to the non-vascularized cornea. (38-40)

Initially, after the diagnosis has been made, intensive therapy can be carried out within the first hour with an application of a suitable antibiotic every 5 minutes. After the first hour, the antibiotic can be applied every 30 to 60 minutes for 2 days, after which the drop interval can be reduced to 2 hours. The therapy regimen also depends on the clinical findings. (23)

Widely used therapy options are the monotherapy with fluoroquinolones or the dual therapy with cephalosporins in combination with aminoglycosides. The different therapeutic approaches do not seem to have any difference in effectiveness, yet there are differences in other categories. Fluoroquinolones seem to have a lower risk for chemical conjunctivitis and ocular discomfort. Compared to the dual therapy mentioned earlier, among fluoroquinolones, ciprofloxacin seems to increase the risk of white corneal pricipitates. (41)

Which kind of antibiotic preperation is chosen, also depends on the region, availability of the substance, cost, spectrum covered and toxicity of the substance. (42,43)

Fluorochinolones, such as moxifloxacin are often used as a monotherapy. As a dual therapy the cephalosporin cefazolin and the aminoglycoside tobramycin or the cephalosporin cefuroxime and the aminoglycoside gentamicin often are used in everyday clinical practice. In a study by Kowalski et al. no significant in vitro coverage advantage was found when comparing these approaches. (44)

The benefits of corticosteroids have been controversial for a long period of time. (45-47) However, subgroups such as patients with very poor visual acuity, central large ulcers and deep ulcers seem to benefit from early supportive corticosteroid therapy within the first 2 to 3 days. (48)

There are also approaches that combine steroid therapy with corneal collagen cross-linking therapy, whereby inflammatory cells should be inhibited and pathogens should be reduced. (42,49)

Further therapeutic measures in case of corneal thinning or perforation may include therapeutic contact lenses, tissue adhesives, amniotic membrane and reconstruction. (37)

1.5.2 Fungal keratitis

Fungal keratitis is a sight threatening disease of the eye.
It is important that fungal keratitis is recognised and treated early to prevent severe progression with corneal damage, endophthalmitis, visual decay or even blindness. (25,50)
Different factors, such as region, climate, occupation, contact lens wear, trauma, immunodeficiency, ocular surface disease and use of corticosteroids, shape the risk for fungal infection of the cornea and also influence, which type of fungus is more likely to cause keratitis.
Among the fungi that can cause corneal infections, there are more than 100 species. They can be divided into monomorhic and dimorphic fungi. Among monomorphic fungi, monomorphic filamentary fungi such as Fusarium, Aspergillus and monomorphic yeasts such as Candida species belong to the the main triggers for fungal keratitides. (51-53)
Treatment can be extremely difficult, because the depth of penetration of the available drugs into the deeper layers of the cornea, is often limited. This fact creates the problem that substances can develop their effect poorly in deeper layers of the cornea. Even the diagnosis usually is extremely difficult. It is scarcely possible to cultivate fungi in a culture. They rarely show positive cultures and do not have a clear clinical appearance. (54)
As mentioned earlier, different factors influence which fungus is most likely to lead to fungal keratitis. In a study conducted by Tuft et al., Candida was identified as the most common fungus and Aspergillus as the second most common fungus in the United Kingdom. (52)
Fusarium and yeasts, including Candida, were identified as the most common fungal pathogens in a study conducted in the USA by Gower et al. (53)
Aspergillus and Fusarium were identified as the most common fungal pathogens in a study from India conducted by Tilak et al. and the situation in China is illustrated in a study by Wang et al., in which Fusarium was identified as the most common fungus followed by the moulds Aspergillus and Alternaria. (55,56)
Even in different regions of different countries, fungal species differ in their frequency and distribution, regarding fungal keratitis.

The lack of vessels in the corneal tissue and the phenomenon of immune tolerance of the eye have a protective effect against undesirable immune responses that could impair vision. But the particular nature of the cornea also means that once fungi have broken through the epithelial barrier, they can easily colonize the cornea. (4,57)
Fungal infection of the cornea often occurs when the integrity of the cornea is disrupted by injury, allowing the pathogens to reach the stroma with the help of matrix metalloproteinases and serine proteases. When the epithelium is destroyed, the pathogens can advance into deeper layers or infect surrounding structures. The damage then takes its course. Ulceration, perforation of the cornea, endophtalmitis, scleritis or panendophtalmitis can be the result. (58,59)
In rare cases, the medical history and clinical presentation of different fungal species may differ. (60)
However, it is often difficult to distinguish fungal keratitis from bacterial keratitis. Trauma, with or without foreign bodies, may be present. Typical symptoms may include blurred vision, pain, redness, discharge and blepharospasm. Ulcers with elevated slough, linear textures drawn by hyphae, reaching beyond the ulcer edge, and satellite stromal lesions may be seen with a slit lamp. (54,61)
The assessment consists of reviewing often very different clinical features ranging from mild to advanced symptoms and clinical features.
More typical for yeast infections is a local infection, sudden onset, widening stromal infltrates, and minor ulcerations.
Typical for filamentary fungal infections usually are dastardly as well. Initially no symtoms could appear, slight stromal infiltrates or mild inflammation of the anterior chamber may occur. Later satellite lesions or feathery branching infiltrates could occur. Advanced ulceration, penetration of deeper corneal layers and the Descemet's membrane, endothelial white plaques, and advanced anterior chamber inflammations with occuring hypopyon are typical for marked inflammation. (37) Clinical features like viscous, glutinous pyramidal hypopyon, or hypopyon in form of a cone is known as Behrens-Baumann-sign 1 and highly suspicious for fungal etiology. Corneal landshaped infiltrates with satellites, without or with a small epithelial defect, is known as Behrens-Baumann-sign 2. (62)

Important examinations include early corneal scrapes for culture and stainings like Gram stainings, Giemsa stainings, PAS stainings or calcofluor stainings, confocal microscopy or

histopathological examination of corneal biopsy and PCR can be considered. (37)
Commonly used topical antifungals include azoles or imidazoles such as clotrimazole, polyenes such as amphotericin B or natamycin, and triazoles such as voriconazole. Application can be topical, systemic or as intracameral and intracorneal injections.
For the first 72 hours, the antifungal agent should be administered hourly. (37,63)
Voriconazole is a newer antifungal agent that is increasingly used topically, because it covers a broad spectrum and penetrates well into deep layers of the cornea. (63-65)
Natamycin against filamentous fungi and amphotericin B against yeast like fungus are in common use. Azoles may be given in addition or as an alternative in persistent cases. (63)
As a systemic therapy, antifungal agents such as ketoconazole, itraconazole, fluconazole and voriconazole may be used for ulcerations larger than 5 mm, penetrating more than half the stroma, for severe infections, bilateral infections, involvement of the sclera or limbus or endophthalmitis, paediatric cases, post keratoplasty infections and in case of imminent perforation of ulceration. (37,66)
Nevertheless, the systemic use of antimycotics remains controversial. (67)
Further therapeutic measures against fungal keratitis, such as contact lens-based drug delivery, corneal collagen crosslinking, photodynamic therapy and surgical measures such as keratoplasty may follow. (63)

1.5.3 Viral Keratitis

Viral Keratitis is a frequent cause for infectious Keratitis. (68)
Alpha-herpesvirus herpes simplex virus (HSV) is the most common representative beside other causatives like alpha-herpesvirus varicella-zoster virus (VZV), beta-herpesvirus cytomegalovirus (CMV) and the gamma-herpesvirus Epstein-Barr virus (EBV).
Throughout the world the risk for developing a herpes simplex keratitis is around 1%. (69,70) The type 1 herpes simplex virus plays an important role in ocular infection. The type 2 herpes simplex virus is more relevant as a trigger for infection of the genitalia. (71)
Up to 65% of the US population is infected with the herpes simplex virus type 1, globally the infection rate is up to two-thirds. (72) As a persistent pathogen, high recurrence rates are present. They range from 9,6% after one year to 76% after 10 years. (73) The virus arrives through the spot of infection at the trigeminal ganglion and persists in it. A reactivation of an inflammation is probably caused by factors such as, psychological stress, fatigue, immunosuppression and UV exposure and happens by retrograde returning of the

virus from the trigeminal ganglion to its original spot of infection or other body parts, for example the cornea. (71,74,75) Affected cells have a higher number of Toll-like receptors. Epithelium, stroma or endothelium can be affected by the inflammatory micro-environment, triggered by different cytokines and the infection may even lead to ulcerations. (76,77) The herpes simplex keratitis can be classified into epithelial, stromal, interstitial, endothelial or neurotrophic keratitis, depending on the infected tissue and cell types. The stromal herpes simplex keratitis can be classified into a *necrotizing* with viral replication in the stroma and into a *non-necrotizing type*, also known as *immune stromal keratitis*, without viral replication.

It can be diagnosed with the slit lamp, depending on clinical appearance and can appear as granular spots, punctate lesions, dendritic lesions or geographic ulcer with reduced corneal sensation. (78-81)

High vaccination rates in children, led to a significantly decreased incidence of alpha virus VZV infections. (82) Nevertheless, mostly later in life, an infection is possible and may appear clinically as chickenpox or shingles.

The upper respiratory epithelium mostly is the location of the primary infection. (83)

The transmission of the virus mainly occurs via respiratory aerosol, droplet inhalation or contact with infectious lesions. (84)

Up to 72% of individuals, who suffer from VZV infection, show symptoms of viral eye infection. (85)

After latency in the trigeminal ganglion, VZV can cause herpes zostes ophthalmicus as far as reactivation occurs in the ophthalmic nerve. (86)

The sight-threatening condition herpes zoster ophtalmicus describes a re-infection of the trigeminal nerve that supplies somatosensory innervation to the eye surface, eyelid, forehead and nose. Common symptoms are pain, tearing and foreign body sensation. (87)

Clinically periorbital vesicular rash is seen in most cases of herpes zoster ophthalmicus. In some cases, the patients develop keratitis, which clinically can appear as pseudo-dendrites, punctate keratitis or grey elevated pleomorphic lesions, corneal ulcers and anterior corneal stromal infiltration or disciform distributions. (86,88,89)

Less frequent but also important to mention for differential diagnosis in ambiguous cases, are the Epstein-Barr Virus, Coxsackievirus, Rhabdovirus and Adenovirus. (81,90-93)

The beta-herpesvirus CMV plays an important role as congenital and intrauterine infection with a transmission rate from a mother with a primary CMV infection to her child from up to 40%. A CMV test is positive for 1% of newborns in the US and the virus can lead to

serious visual impairment and other impairments such as worsened hearing, disorders in the development of psychomotor skills and even death of the child. (94-96) For immunocompetent hosts, only endothelial infection is reported. (97) The endothelial CMV keratitis can be classified in linear, sectoral, disciform and diffuse keratitis. (98) It appears clinically similar as the endothelial HSV keratitis and can show liniary arranged, sometimes pigmented precipitates or central circular corneal edema, increased intraocular pressure, loss of endothelial cells or anterior uveitis. (81,98-100)

Clinical features, depending on the type of virus, could be epithelial, stromal or endothelial involvement. Regarding HSV blepharokeratoconjunctivitis with periorbital vesicular rash, follicular conjunctivitis, and preauricular lymphadenopathy, mostly self-limiting, could occur. Further foreign body sensation, pain, blurred vision, lacrimation, erosion, ulceration, opacities, corneal vascularization, reduced corneal sensation up to painlessness, lipid exudation, scarring, anterior chamber activity, central or paracentral disc of corneal oedema, Descemet's folds or Wessely ring could occur. (37)

Clinical features for zoster opthalmicus could include viral prodrome, preherpetic neuralgia rash or Hutchinson's sign. The infection may be disseminated in immunocompromised patients. Moreover, due to the negative effect on the corneal nerves, the cornea is vulnerable to bacterial and fungal keratitis. Anterior uveitis and retinitis can occur as well. Important examinations include conjunctival and corneal swabs for molecular diagnosis. (37)

A commonly used drug is aciclovir, a DNA polymerase inhibitor. Topical or systemic administration is possible. (101)

Some analogues that have appeared on the market, are ganciclovir, valacyclovir and trifuridine. Aciclovir and its analogues are considered the standard therapy.

Glucocorticoids such as dexamethasone or loteprednol are applied as well. If the therapy is not sufficient, cyclosporine can also be applied. (81)

If the patient's condition remains the same for a longer period of time or even worsens in the case of proven herpes keratitis, resistance can be assumed, which primarily occurs in immunocompromised patients. (102,103)

The treatment regimen and therapy intensity also depend on the severity of the infection and epithelial, stromal or endothelial involvement.

In case of corneal melting, corneal gluing, amniotic membrane transplants, corneal transplantation may be performed. (104-106)

1.5.4 Protozoan keratitis

Keratitis caused by Acanthamoeba is a particular challenge for ophthalmologists, due to the variability of its clinical presentation, late diagnosis, a lack of response to available drugs and a prolonged clinical course. It was first identified in the early 1970s. (107)
The increasing use of contact lenses for refractive correction, resulted in an increased incidence of Acanthamoeba keratitis in the mid-1980s. (108,109)
Acanthamoeba are free-living amoeboid protozoa and can be found in soil, swimming pools, air-conditioning systems and tap water, world wide. (110)
They can cause keratitis and as opportunistic pathogens, the unicellular parasites can cause encephalitides and cutaneous acanthamoebiasis. (111)
Acanthamoeba occur as vegetative trophozoites (10-25µm) and highly resilient cysts (8-12µm). The trophozoites feed on bacteria, especially enterobacteriaceae, algae and yeasts. (112,113)
Under adverse conditions like lack of food, changes in temperature and pH, the pathogens can transform to cysts, which are resistant to numerous biocides, chlorination, and antibiotics. They also survive low temperatures down to 0°C and high doses of UV and γ-radiation. These characteristics can make them highly persistent in the cornea, in spite of distinctive drug therapy. (114-117)
The current classification scheme is based on morpholigcal criteria and the basis of 12 rDNA sequence types (T1-T12) by Pussard and Pons and Stothard et al. (111,118,119)
While Acanthamoeba should also be considered in patients with keratitis who do not wear contact lenses, the ocular prosthetic, especially soft contact lenses with extended wear time, are the greatest risk factor for developing Acanthamoeba keratitis, by far. The information, given in the literature varies greatly, but the number of new cases per year was about 1 per 30,000 contact lenses wearers, for the United Kingdom and Hongkong, in the 1990s. (120-123)
Additional risk factors for contact lens wearers include bathing with contact lenses, cleaning contact lenses with tap water, previous injury, exposure to contaminated water and low socioeconomic background. (124-126)
Initially, adhesion of trophozoites to the corneal epithelium occurs. This process is mediated by the mannose-binding protein, expressed by Acanthamoeba. Later, the pathogens penetrate into deeper corneal layers, with the help of different proteinases (127-129)

Symptoms can include no symptoms at all to foreign body sensation, lacrimation and strong pain. Early clinical signs can include grey-dirty epithelium, dendrites, stromal infltrates, reduced corneal sensation or similar appearence to viral keratitis. An occurance of ring ulcers in late stage is possible.
Important examinations include corneal scrapes for PCR and culture, culture and stainings of lenses, solutions, and cases. Confocal microscopy or corneal biopsy could also be performed if available. (37,130)
In vivo confocal laser microscopy is increasingly used for non-invasive diagnostics in case of suspected Acanthamobea keratitis and the differentiation of other etiologies for infectious keratitis. For the efficient use a lot of user experience is needed. (131-134)
Abrasion of the cornea is an important step in the treatment of acanthamoebic keratitis. On the one hand, the material obtained can be used for further diagnostic measures, on the other hand, abrasion can lead to a reduction of germs in the case of superficial infection. (129) In a study by Brooks et al., in two cases, dendritiform epithelial irregularities, caused by Acanthamoeba, could be resolved via abrasion without further antiamoebic treatment. (135)
Drug therapy should be given quickly for at least three to four weeks. Some recommendations include a duration of 6-12 months, depending on the course of the disease. The first 3 days of therapy, the application should be implemented at least nine times a day but preferably every hour, day and night. After 3 days the application can be reduced to every three hours. Generally, a combination of different therapeutic agents is used, which is mainly due to the highly resistant cystoid form of Acanthamoeba. (136)
The biguanides polyhexanide and chlorhexidine are often used. They are effective against both trophozoites and cysts. (121)
A triple therapy such as the combination of a biguanide such as polyhexanide or chlorhexidine with different diamidines such as propamidine isothionate and an aminoglycoside antibiotic such as neomycin is common. (136,137)
Polyhexanide und chlorhexidine are broad-spectrum antimicrobial agents and widely used for the desinfection of skin and mucosa. Chlorhexidine is primarily bacteriostatic, polyhexanide disorganizes the cytoplasmic membrane of microbes, leading to increased membrane permeability. Consisting of highly positive charged molecules, both substances penetrate the amoeba and lead to cell lysis and death. (138,139)
During treatment, attention should be paid to toxic kerathopathy and changes in intraocular pressure. Cycloplegics may also be used. The use of steroids in keratitis caused by

Acanthamoeba is controversial. Surgical intervention can be performed in case of perforation of the cornea or spread of inflammation into the paracentral stromal areas of the cornea. Therapeutic penetrating keratoplasty may be considered, but bipedicle conjunctival flap and cryopreserved amniotic membrane graft have also shown effects. (136,140,141)
Newer therapeutic approaches include the treatment with phototherapeutic keratectomy and cross-linking. (129,142,143)

1.6 Contact lenses - An important risk factor

The risk to fall ill with infectious keratitis is 9.3 times higher in contact lens wearers compared to non-contact lens wearers, which was proven by a Northern California study conducted by Jeng et al.. Especially in industrialised countries, contact lenses are one of the biggest risk factors for infectious keratitis. This fact is well known and could also be exemplified in the study mentioned above. (144)
Especially contact lenses, that lead to corneal hypoxy, can increase the risk for infectious keratitis. Interestingly, an injured surface of the corneal epithelium alone does not necessarily increase the risk but the combination of injury and hypoxy causing contact lenses. An injury may be caused mechanically by the lens itself or by lens-induced hypoxia. (145)
Contact lens wear may alter the protective components of the tear film due to reduced tear exchange during blinking. Particularly under soft contact lenses, a stagnation of tear fluid can occur. This can cause pathogens to accumulate between the inner surface of the contact lenses and the epithelium of the cornea, creating conditions that allow germs to better adhere to the corneal surface. There is also a change in the biochemistry of the tear film and reduced desquamation of the cells of the corneal epithelium. (146)
The type of contact lens used is considered to be a predisposing factor that may favour contact lens associated keratitis. For example, the risk is increased when wearing soft contact lenses compared to wearing rigid, gas-permeable lenses. Other predisposing factors include poor contact lens hygiene and poor contact lens case hygiene, using contact lens solutions not prescribed by a professional, cosmetic lenses and orthokeratological lenses. (30,147,148)
In the context of contact lens-associated keratitis, Pseudomonas aeruginosa and

Acanthamoeba spp. are frequently cited as causative germs. In a US-American study, contact lenses were also named as one of the main risk factors for fungal keratitis. (149) Interestingly, the conjunctival microbiome is very similar in healthy non-contact lens wearers, healthy contact lens wearers and contact lens wearers who suffer from infectious keratitis. This suggests that the conjunctival microbiome is not a risk factor for contact lens-associated infectious keratitis. Nevertheless, the conjunctival microbiome may contain opportunistic pathogens that can trigger infectious keratitis or promote contact lens-associated keratitis. (150,151)

1.7 Antimicrobial resistance

In recent decades antibiotics were increasingly used in agriculture due to commercial pressure. Antibiotics also were inappropriately used in the treatment of infections and generously prescribed due to financial incentives or ease of use. Antibiotic resistance has therefore become a serious problem in health care. (152)
From a medical and economic perspective the issue is interesting, as a report commissioned by the UK government suggests that if the growth of drug-resistant germs is not slowed down, it is estimated that up to 10 million lives per year could be at risk and costs to the economy of up to $100 trillion could arise until the year 2050. Antibiotics are indispensable in many common surgical interventions and in the treatment of all types of infections. Antibiotic resistance is also becoming increasingly relevant in ophthalmology. (153,154)
Kaye et al. were able to show that in infectious keratitis, whose causative germs were P. aeruginosa, S. aureus and Enterobacteriaceae spp., the sensitivity to fluoroquinolones decreased and the time to healing was prolonged. (154) Decreased sensitivity towards antibiotics may, for example, lead to an increased risk of corneal perforation. (155)
Important mechanisms by which bacteria build resistance include genetic mutations and horizontal gene transfer. (152,156)
The gold standard for the treatment of infectious keratitis is the topical use of cephalosporine in combination with aminoglycosides or monotherapy with fluoroquinolones, depending on the severity of the disease and preference of the treating physician. (41)
The use of antibiotics in the form of eye drops can achieve high concentrations of active

substances in the eye. Although the high concentrations may reduce the occurrence of antibiotic resistance, there are studies on infectious keratitis that mention resistance in the USA, China and India. (157-159)

There are clear trends towards resistance to antibiotics commonly used in clinical practice for infectious keratitis, such as fluoroquinolones, cephalosporins (e.g. ceftazidime), aminoglycosides (e.g. gentamicin, tobramycin) or glycopeptides (e.g. vancomycin). (160)

A good overview of incidence, in vitro antibiotic susceptibility and resistance of bacteria causative for infectious keratitis in the period from July 2007 to October 2019 is provided by a UK study from Nottingham, conducted by Ting et al. The study identified Pseudomonas aeruginosa, Stapylococcus aureus, Streptococci and coagulase-negative germs as the most common causative agents for infectious keratitis between the year 2007 to 2019. In addition, a significant increase in penicillin resistance was found in Gram-positive bacteria from 3.5% to 12.7% and Gram-negative bacteria from 52.6 to 65.4%. (161) To the currently widely used broad spectrum antibiotics, such as cephalosporins and aminoglycosides as dual therapy, as well as fluoroquinolones as monotherapy, most germs tested were sensitive in the study. But none the less there seems to be a clear rising trend towards resistance. (161)

In a study from southern China by Lin et al., 52.72% bacteria, 57.60% fungi and 10.32% coinfections were identified between the year 2010 and 2018. The most common bacterial pathogens were Gram-positive cocci, of which coagulase-negative staphylococcus formed the biggest part of the bacteria isolated. Of the coagulase-negative germs and among all bacterial isolates in total, stapylococcus epidermidis was detected most frequently. As a Gram-negative germ, among all bacterial isolates in total, Pseudomonas aeruginosa was detected second most. (159)

For Gram-positive cocci, the sensitivity to cephalosporins was about 90%, which was a relatively high value compared to fluoroquinolones.

Gram-negative bacilli showed the highest sensitivity to fluoroquinolones.

To cephalosporins, Gram-negative bacilli showed relatively low sensitivity. Against aminoglycosides, a sensitivity of approx. 70% was generally shown.

In the study, there seems to be a general trend towards an increase in resistance to antibiotics, as more than half of the antibiotics tested, showed reduced sensitivity.

For example, the sensitivity of Gram-positive cocci to levofloxacin, tobramycin, caefazolin, ceftazidine, chloramphenicol and cefuroxime decreased between 2010 and 2018. Multy drug resistance is also an increasing problem. (159)

Pseudomonas aeroginosa exhibits high resistance to ciprofloxacin, moxifloxacin and aminoglycosides in a study from North India, conducted by Acahrya et al. (158)
In the context of eye infections, US studies showed that many Staphylococci spp. and Streptococci spp. were resistant to antibiotics, especially methicillin. Multy drug resistance was found in 2/3 of methicillin-resistant Staph. aureus (MRSA) and methicillin-resistant coagulase-negatives. Among MRSA, resistances to fluoroquinolones could also be detected. (151,157,162)

2 Materials and Methods

The present retrospective, hospital-based study, included 100 patients (100 eyes) with infectious keratitis or corneal ulcer, attending the Department of Ophthalmology and were investigated for infectious aetiology at the Department of Ophthalmology of the Medical University of Graz, Austria, over a period of one year (01/01/2018 to 07/01/2019). In 3 cases, both eyes were affected. Because of the low clinical expression and to ensure clarity of the study, the second affected eyes were not included in this evaluation.

During the year 2018 a large collection of laboratory diagnostic data of patients with infectious keratitis and corneal ulcer was established by physicians, employees of the Laboratory of Microbiology and Bacteriology of the Universitiy Hospital of Ophthalmology and staff members of the Institute of Hygiene and Microbiology of the Styrian Hospital Society (KAGes). These data build the basis of the thesis.

For the thesis, studies from the data base PubMed were chosen as well as medical literature and specialist ophthalmologist literature from the library of the University Eye Hospital Graz. Standard Operating Procedures (SOPs) from the Laboratory for Microbiology and Bacteriology of the University Eye Hospital Graz and SOPs from the Institute of Hygiene and Microbiology of the Styrian Hospital Society (KAGes) were used.

The data were acquired via laboratory journals of the Laboratory of Microbiology and Bacteriology of the University Eye Hospital Graz and the data collection system EyMed. With the software EyMed, access to general patient information such as ophthalmological examination data, clinical history, visual acuity, medication, microbiological reports, antibiograms, surgery reports, admission reports, release reports, outpatient reports and eye photographs, is possible.
When data were incomplete, or difficulties in data documentation occured, folders from the central data archive were used, to complete the documentation process.
Microbiological reports included reports from smears of the cornea, conjunctiva and contact lenses cases. Clinical samples were used for laboratory diagnostics, such as stainings, microbial cultures, antibiograms, and PCR.

Keratitis is a clinical diagnosis. When keratitis was suspected, clinical specimens were collected by hockey knife scrapings from the cornea, swabs from cornea and conjunctiva, contact lenses cases or contact lenses themselves, usually before therapy starts.
After the clinical specimen were collected, further diagnostic steps were implemented.

Gram staining, Periodic acid-Schiff staining (PAS) and Lactophenole Cotton Blue (LPCB) staining took place in the Laboratory of Microbiology and Bacteriology of the University Eye Hospital Graz.
Laboratory diagnostics, such as bacterial culture and bacterial susceptibility testing took place in the Institute of Hygiene and Microbiology of the Styrian Hospital Society (KAGes).
PCR diagnostics were conducted in the Diagnostic and Research Institute of Pathology of the Medical University of Graz.
Only for PCR diagnostics of suspected Acanthamoeba infection, tissue samples were sent to the Institute of Medical Parasitology of the Medical University of Vienna.

The documentation process started with a list of patient names, who attended the University Eye Hospital Graz, due to keratitis or corneal ulcer. The list was handed out by the Laboratory of Microbiology and Bacteriology of the Univesitiy Eye Hospital Graz. Every patient, who attended laboratory diagnostics, is documented in a laboratory journal from the Laboratory of Microbiology and Bacteriology of the University Eye Hospital Graz. The laboratory journal served as a source for the patients included in the list.
Every patient finally was assigned to a sequential number and registered in anonymous form in an excel chart.

The following attributes were documented for each patient in the Excel chart:

- Sequential number, date of birth, sex, affected eye, date of sample collection, date of initial visit, days until diagnosis, referred by, medical history, visual acuity, ocular findings, contact lenses, eye trauma, surgical procedure, occurrence of hypopyon, first medical treatment
- Corneal scrapings: Gram-, PAS-, LPCB staining results
- Corneal scrapings/swabs: pathogens of bacterial and fungal cultures
- Corneal scrapings/swabs: pathogen N°1, N°2, N°3 of antibiogram and susceptibility testing

- Corneal PCR-testing results (HSV 1/2, VZV, fungi, Acanthamoeba)
- Conjunctival swabs: pathogens of bacterial cultures
- Conjunctival swabs: pathogen N°1, N°2, N°3 of antibiogram and susceptibility testing
- Sediment of the contact lenses container´s liquids: Gram-, PAS-, LPCB staining results
- Sediment of the contact lenses container´s liquids: pathogen of the microbial and fungal cultures
- Sediment of the contact lenses container´s liquids: pathogen N°1, N°2, N°3 of antibiogram and susceptibility testing
- In-patient stay (yes or no)
- In-patient stay (period in days)
- Therapy: local antibiotic therapy, local antimycotic therapy, local Acanthamoeba therapy, local cortison therapy, systemic therapy (antibiotic agents/antiviral therapy/pain killers)
- Date of the end of therapy
- Time in days until corneal erosion/ulcer regenerated
- Surgical procedure (yes or no)
- Type of surgical procedure
- Photography at the beginning of therapy
- Photography at the end of therapy

All data were registered and administered with the computer program Excel (Version 16.30) and statistically analysed by the statistics programme SPSS (SPSS Statistics 27). Descriptive statistics was used to present the data.

Corneal scrape/swab

The procedure was carried out at the Universitiy Eye Hospital Graz. For corneal scrapes, a Hockey knife was used. The clinical sample then was spread on a clean, labelled microscopic slide and brought to the Laboratory of Microbiology and Bacteriology of the University Eye Hospital Graz for further diagnostic steps, such as Gram staining, Periodic acid-Schiff (PAS) stain and Lactophenole Cotton Blue (LPCB) staining.
A clinical sample via Hockey Knife for Brain Heart Infusion Broth was taken as well.

Another clinical sample was taken from the cornea via a sterile collection nylon fiber swab and was kept in a collection and transport system, called Copan Liquid Amies Elution Swab (eSwab). The sample then was transported to the Institute of Hygiene and Microbiology of the Styrian Hospital Society (KAGes), for further laboratory diagnostic steps, such as culture and antibiograms. ESwab is a collection and transport system, often used in clinical routine for laboratory diagnosis. The maintenance medium consists of inorganic phosphate buffer, calcium and magnesium salts, sodium chloride and sodium thioglycolate. The collection and transport system can be used for aerobes, anaerobes, bacterial antigens, viral antigens, Chlamydia antigens and nucleic acids.
For PCR diagnostics, a part of the clinical sample was transported in a sterile Eppendorf tube to the Diagnostic and Research Institute of Pathology of the Medical University of Graz.
Only for PCR diagnostics of suspected Acanthamoeba infection, tissue samples were sent to the Institute of Medical Parasitology of the Medical University of Vienna in a sterile Eppendorf tube.

Conjunctival swab

The procedure was carried out at the Universitiy Eye Hospital Graz.
A sterile collection nylon fiber swab was used for the conjunctival smear. The material then was kept in the transport system Copan Liquid Amies Elution Swab (eSwab) and afterwards, for further laboratory diagnostic steps like microbial culture and antibiograms, transported to the Institute of Hygiene and Microbiology of the Styrian Hospital Society (KAGes).

Gram staining

The examination was carried out at the Laboratory of Microbiology and Bacteriology of the Universitiy Eye Hospital Graz.
For microscopic examination of bacteria, Gram staining was performed of different clinical samples, such as corneal direct smears, bacterial culture and samples of the sediment of the contact lenses container´s liquids.
The sample was spread on a microscopic slide, air dried and heat fixed. The staining process was performed in different steps, such as applying Crystal Violet, Lugolsche Solution, decolouriser and drying. After the procedure, differentiation between gram

positive bacteria and Gram negativ bacteria via light microscopy is possible. Gram positive bacteria appear blue-black, Gram negative bacteria appear red.

Periodic acid-Schiff (PAS) staining

The examination was carried out at the Laboratory of Microbiology and Bacteriology of the Universitiy Eye Hospital Graz.

For microscopic examination of fungal pathogens, Periodic acid-Schiff stain was performed of different clinical samples, such as corneal direct smears and samples of the sediment of the contact lenses container´s liquids.

The staining process was performed in different steps, such as spreading the corneal scrape on a micrsocopic slide, immersing slides in Periodic acid solution, washing in tap water and distilled water, immersing slides in Schiff´s Reagent, again washing, counterstaining in Hematoxylin Solution, washing in tap water and distilled water, dehydration in a series of alcohol in ascending concentration and fixation of the stained sample.

PAS- positive substances appear pink to violet, carbohydrates and basal membranes appear purple, cell nuclei appear blue, Proteins and cytoplasm appears yellow.

Lactophenol Cotton Blue (LPCB) staining

The examination was carried out at the Laboratory of Microbiology and Bacteriology of the Universitiy Eye Hospital Graz.

For microscopic examination of Acanthamoeba cysts or trophozoites, Lactophenol Cotton Blue (LPCB) staining was performed of different clinical samples, such as corneal scraping smears with a hockey knife and the sediment of the contact lenses container´s liquids. The staining process was performed by immersing the airdried sample in Lactophenol Cotton Blue Solution and covering the container with a glass lid. The LPCB stainings of the corneal scrapes should not be microscopically examined immediately. The samples should only be examined microscopically after a few hours or after the colour has been left to soak overnight.

Trophozoites (10-25µm) appear dark blue, cysts (8-12µm) appear dark blue with birefringent cover. (113)

Examination of the sediment of contact lenses container´s liquids

The examination was carried out in the Laboratory of Microbiology and Bacteriology of the Universitiy Eye Hospital Graz and at the Institute of Hygiene and Microbiology of the Styrian Hospital Society (KAGes).

A microscopic examination of the sediment of the contact lenses container´s liquids was performed. The procedure was performed for reviewing the presence of bacteria, fungus and Acanthamoeba cysts or trophozoites. The contact lenses were taken out of the cases. The storage liquid was transferred with a sterile pipette to a cryotube and centrifugated. The centrifuged liquid afterwards was discarded and Gram staining, LBCB staining and PAS staining was performed of the sediment.

The contact lenses´ condition was checked for deposits and cracks via a microscope.

For the proof of Acanthamoeba cysts on the contact lenses´ surfaces, an LPCB staining was performed of contact lenses surface smears by deducting the contact lenses´ inner surfaces on slides and performing LPCB staining.

Furthermore, microbial culture and antimicrobial susceptibility testing was permormed in the Institute of Hygiene and Microbiology of the Styrian Hospital Society (KAGes).

PCR - Acanthamoeba

The examination was carried out at the Institute of Medical Parasitology of the Medical University of Vienna.

For Acanthamoeba detection, culture and PCR diagnostic was performed of clinical material (corneal scrapes/ contact lenses, contact lenses cases, contact lenses cases transport medium). The samples were sent from the University Eye Hospital Graz to the Institute of Medical Parasitology of the Medical University of Vienna.

For culture, depending on the type of sample and the sample´s size, the pellet of the transport medium of the clinical material or a part of the clinical material itself, was inoculated onto a nonnutrient agar plate, covered with a 24h old culture of E.coli in bacterial broth. The plates were incubated for 7 days at 30 °C and checked for amoeba growth every 24 h via phase contrast microscopy. For genotyping, the growing amoebae were harvested from culture plates and resuspended in 0.9% NaCl for DNA isolation. DNA was isolated from the cultures and the clinical material, respectevly, with a standard commercial isolation kit like QIAmp DNA mini Kit (Qiagen GmbH, Germany), following the manufacture´s protocol.

PCR for the diagnostics of Acanthamoeba DNA was performed according to the protocol of Schroeder et al. (163)
A fragment of the 18S rRNA was amplified with a JDP1 and JDP 2 primer pair.
Amplicons were separated by agarose-gel electrophoresis and visualized using GelRed.
Respective bands were extracted from the gel with a DNA band purification kit. (163,164)

PCR - HSV/VZV, PCR - Fungus

The examination was carried out at the Diagnostic and Research Institute of Pathology of the Medical University of Graz.
For the PCR diagnosis of Varicella-zoster virus and Herpes simplex virus 16s PCR sequencing was performed.
DNA extraction of the corneal scraping was performed with a Magnapure Compact and Maxwell® 16 LEV Blood DNA Kit (Promega, REF: AS1290).
The clinical samples were analysed with quantitative real-time-PCR with fluorescence resonance energy transfer (FRET) - suitable hybridisation probes. Therefor artus ® HSV – 1/2 LC PCR Kit (96), V1 (REF: 4500065), artus ® HSV – 1/2 LC PCR Kit (24), V1 (REF: 4500063), artus® VZV LC PCR Kit (96), V1 (REF: 4502065), artus® VZV LC PCR Kit (24), V1 (REF: 4502063) were used.
In fluorescence resonance energy transfer (FRET) energy is transferred from a stimulated dye to a nearby second dye. Donor and acceptor need to be close to each other. For quantification, two marked probes, which bind to a special nucleotide sequence, were added to the reaction mix. The first probe is marked with a fluorescent dye at the 3´-end. The second probe is marked with LC-Red 640 at the 5´-end. LC-Red 640 is a special dye, emitting light with a wavelength of 640 nm.
Both probes bind to the single strand DNA in the annealing phase of the PCR reaction. When the light source of the LightCycler hits the fluorescent dye, the resulting energy is transmitted to the LC-Red640-molecule, which emits the energy in the form of light, with a wavelength of 640 nm.
In the elongation phase, both probes are displaced by the Tag-polymerase. In the annealing phase of the following cycle, they bind to the DNA single strand again.
Beside DNA amplification of the target sequence, the fluorescence signal increases proportional to the amount of target DNA.
For the PCR diagnosis of fungus, internal transcribed spacer (ITS) sequencing was performed with Ion Torrent Kit, after PCR according to White et al.. (165)

The further procedure was performed as described above, for PCR diagnosis of VZV and HSV.

Culture and Antimicrobial Susceptibility Testing - Cornea and contact lens cases sediments

The examination was carried out at the Institute of Hygiene and Microbiology of the Styrian Hospital Society (KAGes).

For bacterial culture of the corneal smears and contact lens cases samples, the clinical material was inoculated on Thioglycolat-Bouillons for seven days at 36 +/- 1 °C under aerobe and anaerobe conditions. The Bouillons were checked for microbial growth every 24 hours. In case of turbidity, gram-staining was performed. As soon as gram staining was positive, new inoculation was performed, depending on gram staining results.

After 7 days, inoculation of the initial bouillons on a chocolat polyvitex plate (aerobe) for 24 hours and inoculation on a Schaedler agar plate (anaerobe) for 48 hours was performed. In case bacterial growth was verified, identification of the germ followed.

Microscopic germ identification was performed followed by pathogen identification. Pathogen identification was performed by MALDI-TOF, a mass spectrometry technique, or biochemical methods.

Resistance assessment was performed either automated via VITEK-2 or manually via disk diffusion test. The Interpretation of resistances was made according to the current EUCAST guidelines.

Culture and Antimicrobial Susceptibility Testing - Conjunctiva

The examination was carried out at the Institute of Hygiene and Microbiology of the Styrian Hospital Society (KAGes). For bacterial culture of the conjunctival smear, the clinical material was inoculated on Mc Konkey, Candida albicans agar, Columbia-CAN-Agar, Chocolat Polyvitex plate, Schaedler agar, CSL-Boullion (Trypticase-soja-Bouillon), Thioglykolat Bouillon for 48 hours at 36 +/- 1°C aerobe and anaerobe. Aerobe cultural medium were checked after 24 hours, anaerobe cultural media were checked after 48 hours, for microbial growth.

Identification of the germ followed.

Microscopic germ identification was performed followed by pathogen identification. Pathogen identification was performed by MALDI-TOF, a mass spectrometry technique, or biochemical methods.

Resistance assessment was performed either automated via VITEK-2 or manually via disk diffusion test. The Interpretation of resistances was made according to the current EUCAST guidelines.

3 Results

A total of 100 patients with infectious keratitis or corneal ulcer, attending the University Eye Hospital Graz and were investigated for infectious aetiology over a period of one year (01/01/2018 to 07/01/2019), were included in the study on the topic laboratory diagnosis in infectious keratitis and corneal ulcer.

3.1 Cornea

3.1.1 Positive test results

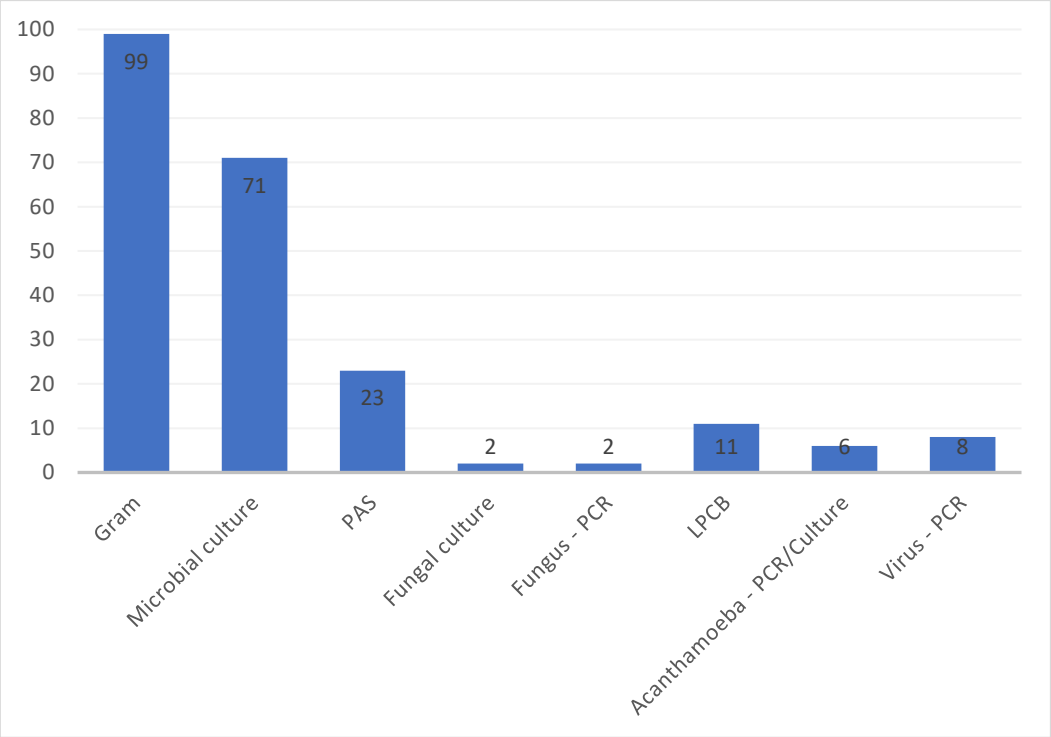

Figure 1: Cornea – Positive test results

Positive test results of all laboratory diagnostic methods used for pathogen detection of the cornea. The vertical axis represents 100 patients. The horizontal axis represents the positive performed diagnostic methods.

According to Figure 1, Gram stainings showed the most positive test results with 99 cases in the form of stained bacteria. Second most positive test results showed microbial cultures in 71 cases in the form of microbial growth. PAS stainings showed the third most positive test results with 23 cases in the form of stained/suspected fungal components, followed by LPCB stainings with 11 positive test results in the form of stained/suspected Acanthamoeba cysts or trophozoites, virus PCR with 8 positive test results and Acanthamoeba PCR/Culture with 6 positive test results in the form of either positive PCR

test results or positive culture results or both. Fungal culture and fungal PCR diagnostics both showed positive test results in 2 cases.

Gram stainings and/or *microbial cultures* for the detection of bacteria, were positve in *100 patients*.
PAS stainings and/or *fungal cultures and/or fungal PCR* for the detection of fungus, were positive in *25 patients*.
LPCB stainings and/or *Acanthamoeba PCR/culture* for the detection of Acanthamoeba, were positive in *12 patients*.
Virus PCR for the detection of viruses, was positive in *8 patients*.
Bacteria were detected in 100 patients, *fungi in 25 patients*, *Acanthamoeba in 12 patients*, and *viruses in 8 patients*.

3.1.2 Gram staining

Table 1: Cornea - Gram staining performed/not performed

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Not performed	1	1.0	1.0	1.0
	Performed	99	99.0	99.0	100.0
	Total	100	100.0	100.0	

Absolute numbers and percentages of patients in which corneal scrapes and Gram stainings were performed.

Gram stains were performed for the detection of bacteria.
Table 1 shows that corneal scrapes and subsequent *Gram stains were performed in 99 (99%) patients/eyes* in total. *In 1 case (1%) Gram stain* was *not performed*.

Table 2: Cornea – Gram staining positive/negative

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Positive	99	99.0	100.0	100.0
Missing	System	1	1.0		
Total		100	100.0		

Positive/negative Gram staining results (in absolute numbers and percentages) of the percentage of patients in which corneal scrapes and Gram stainings were performed.

Gram stainings showed *positive test results* in the form of stained microbia *in 99* cases *(100%)*. Gram staining was *negative* in the form of *missing stained microbia in 0* of the cases *(0%)*.

3.1.3 Microbial culture

Table 3: Cornea – Microbial culture performed/not performed

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Performed	100	100.0	100.0	100.0
	Not performed	0	0	0	0
	Total	100	100.0	100.0	

Absolute numbers and percentages of patients in which a corneal scrape and microbial culture was performed.

Table 3 shows that corneal scrapes and subsequent *microbial cultures were performed in 100 (100%) patients/eyes* in total.

Table 4: Cornea – Microbial culture positve/negative

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Negative microbial culture	29	29.0	29.0	29.0
	Positive microbial culture	71	71.0	71.0	100.0
	Total	100	100.0	100.0	

Positive/negative microbial culture results (absolute numbers and percentages) of the percentages of patients in which a corneal scrape and microbial culture was performed.

Microbial culture showed *positive test results* in the form of *bacterial growth in 71* cases *(71%)*. Microbial culture was *negative* in the form of *missing microbial growth in 29* of the cases *(29%)*.

Cornea - Microbial culture - Prevalence of bacteria isolated from corneal scrapes (bacterial isolates categorized)

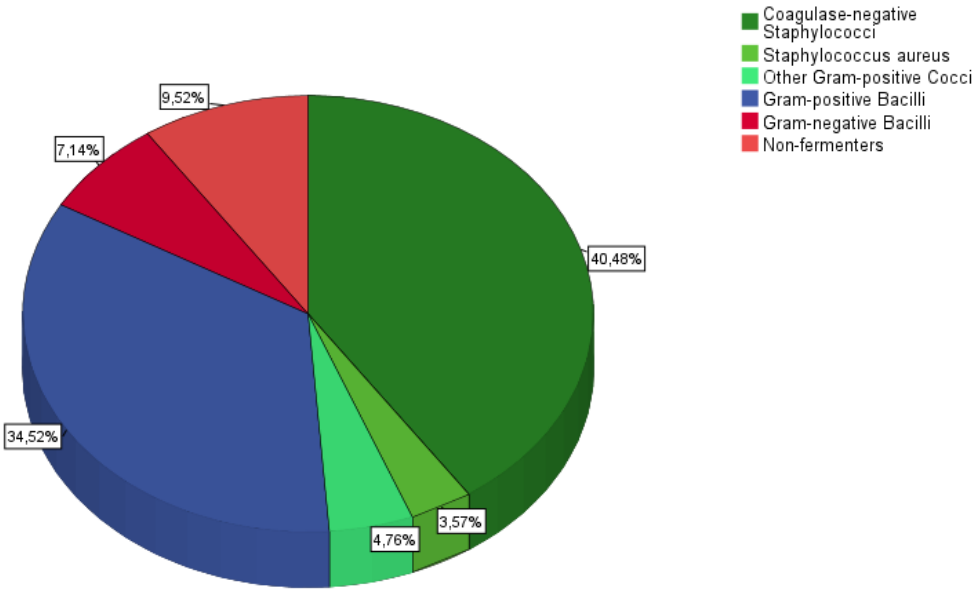

Figure 2: Cornea - Microbial culture - Prevalence of bacteria isolated from corneal scrapes (bacterial isolates categorized)

Prevalence of all corneal bacteria (summarized in categories) that were detected via microbial culture.

Table 5: Cornea - Microbial culture - Prevalence of bacteria isolated from corneal scrapes (bacterial isolates categorized)

		Answers		Percent of the cases
		N	Percent	
Cornea – Bacterial isolates categorized	Coagulase-negative staphylococci	34	40.5%	47.9%
	Staphylococcus aureus	3	3.6%	4.2%
	Other Gram-positive cocci	4	4.8%	5.6%
	Gram-positive bacilli	29	34.5%	40.8%
	Gram-negative bacilli	6	7.1%	8.5%
	Non-Fermenters	8	9.5%	11.3%
Total		84	100.0%	118.3%

Prevalence of all corneal bacteria (summarized in categories) that were proven via microbial culture.

Figure 2 and *Table 5* show the prevalence of all bacterial isolates detected in *71 positive microbial cultures* that were inoculated with the material of corneal scrapes.
In total 84 (100%) bacterial isolates were detected via microbial cultures.

For the purpose of clarity, the bacterial isolates were grouped into categories like *coagulase-negative Staphylococcus*, *Staphylococcus aureus*, *other Gram-positive cocci*, *Gram-positive bacilli*, *Gram-negative bacilli* and *non-fermenting bacteria*. (*Table 6*)
Among Gram-positive cocci *coagulase-negative staphylococci* formed the majority with *34* isolates *(40.5%)*, *Staphylococcus aureus* showed *3* isolates *(3.6%)* and summarized as *other Gram-positive cocci,* 4 isolates (4.8%) were detected. (Figure 2, Table 5)
The second largest proportion formed *Gram-positive bacilli* with *29 isolates (34.5%). Gram-negative bacilli* made up the proportion of 6 isolates (7.1%) and *non-fermenting bacteria made up a proportion of 8 isolates (9.5%)*. (Figure 2, Table5)

Table 6: Cornea - Microbial culture - Bacterial isolates categorized - Overview

Coagulase-negative staphylococci	Staphylococcus capitis
	Staphylococcus caprae
	Staphylococcus epidermidis
	Staphylococcus gallinarum
	Staphylococcus hominis ssp. hominis
	Staphylococcus lugdunensis
	Staphylococcus xylosus
Staphylococcus aureus	Staphylococcus aureus
Other Gram-positivi cocci	Alloiococcus otitis
	Kocuria rosea
	Streptococcus dysgalact. ssp. equisimiliis
	Streptococcus mitis
Gram-positive bacilli	Bacillus cereus
	Bacillus simplex
	Corynebacterium jeikeium
	Lactobacillus fermentum
	Propionibacterium acnes
Gram-negative bacilli	Citrobacter koseri
	Klebsiella oxytoca
	Klebsiella pneumoniae ssp. pneumoniae
	Moraxella nonliquefaciens
	Morganella morganii ssp. morganii
	Serratia marcescens
Non-Fermenters	Pseudomonas aeruginosa

All corneal isolates detected via microbial culture. The isolates are summarized in categories.

Table 6 could serve the reader as an aid for the taxonomy of corneal isolates and does not consider any frequencies.

Table 7: Cornea - Microbial culture - Prevalence of bacteria isolated from corneal scrapes (individual bacterial isolates)

		Answers		Percent oft he
		N	Percent	cases
Cornea – Bacterial isolates	Alloiococcus otitis	1	1.2%	1.4%
	Bacillus cereus	2	2.4%	2.8%
	Bacillus simplex	1	1.2%	1.4%
	Citrobacter koseri	1	1.2%	1.4%
	Corynebacterium jeikeium	1	1.2%	1.4%
	Klebsiella oxytoca	1	1.2%	1.4%
	Klebsiella pneumoniae ssp. pneumoniae	1	1.2%	1.4%
	Kocuria rosea	1	1.2%	1.4%
	Lactobacillus fermentum	1	1.2%	1.4%
	Moraxella nonliquefaciens	1	1.2%	1.4%
	Morganella morganii ssp. morganii	1	1.2%	1.4%
	Propionibacterium acnes	24	28.6%	33.8%
	Pseudomonas aeruginosa	8	9.5%	11.3%
	Serratia marcescens	1	1.2%	1.4%
	Staphylococcus aureus	3	3.6%	4.2%
	Staphylococcus capitis	5	6.0%	7.0%
	Staphylococcus caprae	1	1.2%	1.4%
	Staphylococcus epidermidis	22	26.2%	31.0%
	Staphylococcus gallinarum	1	1.2%	1.4%
	Staphylococcus hominis ssp. hominis	2	2.4%	2.8%
	Staphylococcus lugdunensis	2	2,4%	2.8%
	Staphylococcus xylosus	1	1.2%	1.4%
	Streptococcus dysgalact. ssp. equisimiliis	1	1.2%	1.4%
	Streptococcus mitis	1	1.2%	1.4%
Total		84	100.0%	118.3%

Prevalence of all corneal bacteria (individual bacterial isolates) that were proven via microbial culture.

Table 7 shows the prevalence of every single bacterial isolate, listed in alphabetical order. The most frequently detected bacterium is the anaerobic *Gram-positive bacillus Propionibacterium acnes with 24 isolates (28.6%).*

The second most frequently detected bacterium is the *coagulase-negative, Gram-positive coccus Staphylococcus epidermidis with 22 isolates (26.2%).*

The third most frequently occurring bacterium is the non-fermenting, Gram-negative bacillus Pseudomonas aeruginosa with 8 isolates (9.5%), followed by the coagulase-negative, Gram-positive coccus Staphylococcus capitis with 5 isolates (6%) and the coagulase-positive, Gram-positive coccus Staphylococcus aureus with 3 isolates (3.6%).

3.1.4 PAS staining

Table 8: Cornea – PAS staining performed/not performed

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Not performed	2	2.0	2.0	2.0
	perforemd	98	98.0	98.0	100.0
	Total	100	100.0	100.0	

Absolute numbers and percentages of patients in which corneal scrapes and PAS stainings were performed.

PAS stainings were performed for the detection of fungal components.
Table 8 shows that corneal scrapes and subsequent *PAS stainings were performed in 98 (98%) patients/eyes* in total. *In 2 cases (2%) PAS stainings were not performed*.

Table 9: Cornea – PAS staining positive/negative

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Negative PAS staining	75	75.0	76.5	76.5
	Positive PAS staining	23	23.0	23.5	100.0
	Total	98	98.0	100.0	
Missing	System	2	2.0		
Total		100	100.0		

Positive/negative PAS staining results (in absolute numbers and percentages) of the percentage of patients in which corneal scrapes and PAS stainings were performed.

Table 9 shows that PAS stainings were positive in the form of *stained fungal components in 23 cases (23.5%)*. PAS stainings were *negative* in the form of missing stained fungal components in 75 of the cases (76.5%).

3.1.5 Fungal culture

Table 10: Cornea – Fungal culture performed/not performed

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Not performed	4	4.0	4.0	4.0
	Performed	96	96.0	96.0	100.0
	Total	100	100.0	100.0	

Absolute numbers and percentages of patients in which corneal scrapes and fungal cultures were performed.

Table 10 shows that corneal scrapes and subsequent *fungal cultures* were *performed in 96 (96%) patients/eyes* out of 100 patients (100%) in total. In *4 cases (4%) fungal culture was not performed*.

Table 11: Cornea – Fungal culture positive/negative

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Negative fungal culture	94	94.0	97.9	97.9
	Positive fungal culture	2	2.0	2.1	100.0
	Total	96	96.0	100.0	
Missing	System	4	4.0		
Total		100	100.0		

Positive/negative fungal culture results (in absolute numbers and percentages) of the percentage of patients in which corneal scrapes and fungal cultures were performed.

Table 11 shows that fungal cultures were *negative* in the form of *missing fungal growth in 94* cases *(97.9%)*. Fungal cultures showed *positive test results* in the form of *fungal growth in 2* cases *(2.1%)*.

In total 2 fungal isolates (100 %) were detected.

One of the fungal isolates detected, was the filamentary fungus *Fusarium proliferatum* (50%) and the second fungal isolate detected, was the yeast like fungus *Candida albicans (50%)*.

3.1.6 PCR - Fungus

Table 12: Cornea – PCR fungus performed/not performed

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Not performed	96	96.0	96.0	96.0
	Performed	4	4.0	4.0	100.0
	Total	100	100.0	100.0	

Absolute numbers and percentages of patients in which fungal PCR diagnostics were performed.

PCR diagnostics were performed for the detection of fungi.
Table 12 shows that corneal scrapes and subsequent *PCR diagnostics* for the detection of fungi *were performed in 4 (4%) patients/eyes* in total. *In 96 cases (96%) PCR* for the detection fungus was *not performed*.

Table 13: Cornea – PCR fungus positive/negative

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Negative	2	2.0	50.0	50.0
	Positive	2	2.0	50.0	100.0
	Total	4	4.0	100.0	
Missing	System	96	96.0		
Total		100	100.0		

Positive/negative fungal PCR results (in absolute numbers and percentages) of the percentage of patients on which corneal scrapes and fungal PCR diagnostics were performed.

Table 13 shows that *PCR* diagnostics for the detection of fungi was *positive in 2 cases (50.0%)*. *PCR* diagnostics for the detection of fungi showed *negative test results* in 2 of the cases (50.0%).
Among the 2 detected fungi (100%) one isolate was Candida albicans (50%) and the second isolate (50%) was Fusarium proliferatum.

The 2 positive fungal cultures *(Table 11)* and the 2 detected fungi via fungal PCR *(Table 13*) were assigned to 4 different patients.

3.1.7 LPCB staining

Table 14: Cornea – LPCB staining performed/not performed

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Not performed	43	43.0	43.0	43.0
	performed	57	57.0	57.0	100.0
	Total	100	100.0	100.0	

Absolute numbers and percentage of patients in which corneal scrapes and LPCB stainings were performed.

LPCB stainings were performed for the detection of Acanthamoeba cysts or trophozoites. *Table 14* shows that corneal scrapes and subsequent *LPCB* stainings *were performed in 57 (57%) patients/eyes* in total. *In 43 cases (43%) LPCB* stainings were *not performed*.

Table 15: Cornea – LPCB staining positive/negative

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Negative	46	46.0	80.7	80.7
	Positive	11	11.0	19.3	100.0
	Total	57	57.0	100.0	
Missing	System	43	43.0		
Total		100	100.0		

Positive/negative LPCB staining results (in absolute numbers and percentages) of the percentage of patients in which corneal scrapes and LPCB stainings were performed.

Table 15 shows that LPCB stainings were *positive* in the form of *stained* Acanthamoeba cysts or trophozoites *in 11 cases (19.3%)*. LPCB stainings were *negative* in the form of missing stained Acanthamoeba cysts or trophozoites in 46 of the cases (80.7%).

3.1.8 PCR/Culture - Acanthamoeba

Table 16: Cornea – Acanthamoeba PCR/Culture performed/not performed

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Not performed	87	87.0	87.0	87.0
	Performed	13	13.0	13.0	100.0
	Total	100	100.0	100.0	

Percentages of patients in which Acanthamoeba PCR and/or Acanthamoeba cultures were performed.

Table 16 shows that corneal scrapes and subsequent PCR diagnostics for the detection of Acanthamoeba cysts or trophozoites and Acanthamoeba cultures were performed *in 13 (13%) patients/eyes* in total. *In 87 cases (87%)* PCR diagnostics for the detection of Acanthamoeba cysts or trophozoites and Acanthamoeba cultures were not performed.

Table 17: Cornea – Acanthamoeba PCR/Culture positive/negative

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Negative	7	7.0	53.8	53.8
	Positive	6	6.0	46.2	100.0
	Total	13	13.0	100.0	
Missing	System	87	87.0		
Total		100	100.0		

Positive/negative Acanthamoeba PCR and/or Acanthamoeba culture results (in absolute numbers and percentages) of the percentage of patients in which corneal scrapes and Acanthamoeba PCR and/or Acanthamoeba cultures were performed.

Table 17 shows that *PCR* diagnostics *f*or the detection of Acanthamoeba cysts or trophozoites and Acanthamoeba culture showed *positive test results in 6 cases (46.2%)*. *PCR* diagnostics *f*or the detection of Acanthamoeba cysts or trophozoites and Acanthamoeba culture showed *negative test results* in 7 of the cases (53.8%).

3.1.9 PCR – HSV/VZV

Table 18: Cornea – PCR HSV/VZV performed/not performed

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Not performed	70	70.0	70.0	70.0
	Performed	30	30.0	30.0	100.0
	Total	100	100.0	100.0	

Absolute numbers and percentage of patients in which corneal scrapes and PCR diagnostics for HSV/VZV were performed.

PCR diagnostics was performed for the detection of HSV or VZV.
Table 18 shows that corneal scrapes and subsequent *PCR diagnostic* for the detection of HSV or VZV *was performed in 30 patients/eyes (30%)* in total. *In 70 cases (70%) PCR* for the detection of HSV or VZV was *not performed*.

Table 19: Cornea – PCR HSV/VZV positive/negative

		Frequency	Percent	Valid Percent	Cumulative percent
Valid	Negative	22	22.0	73.3	73.3
	Positive	8	8.0	26.7	100.0
	Total	30	30.0	100.0	
Missing	System	70	70.0		
Total		100	100.0		

Positive/negative PCR test results for HSV/VZV (in absolute numbers and percentages) of the percentage of patients in which corneal scrapes and PCR diagnostic for HSV/VZV were performed.

Table 19 shows that PCR diagnostics for the detection of HSV or VZV was *positive in 8 cases (26.7%)*. *PCR* diagnostics for the detection of HSV or VZV showed *negative test results* in 22 of the cases (73.3%).
Among the 8 positive test results (100%) all of the detected viruses (100%) were typed as type 1 herpes simplex virus.

3.2 Conjunctiva

3.2.1 Microbial culture

Table 20: Conjunctiva – Microbial culture performed/not performed

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Not performed	38	38.0	38.0	38.0
	Performed	62	62.0	62.0	100.0
	Total	100	100.0	100.0	

Absolute numbers and percentages of patients in which conjunctival smears and microbial cultures were performed.

Table 20 shows that conjunctival smears and subsequent *microbial cultures were performed in 62 (62%) patients/eyes* in total. *In 38 cases (38%) microbial* cultures were *not performed*.

Table 21: Conjunctiva – Microbial culture positive/negative

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Negative	45	45.0	72.6	72.6
	Positive	17	17.0	27.4	100.0
	Total	62	62.0	100.0	
Missing	System	38	38.0		
Total		100	100.0		

Positive/negative microbial culture results (absolute numbers and percentages) of the percentage of patients in which conjunctival smears and microbial cultures were performed.

Table 21 shows that microbial cultures were *positive* in the form of *bacterial growth in 17* cases *(27.4%)*. Microbial cultures were *negative* in the form of *missing microbial growth in 45* of the cases *(72.6%)*.

Conjunctiva - Microbial culture - Prevalence of bacteria isolated from conjunctival smears (pathogens categorized)

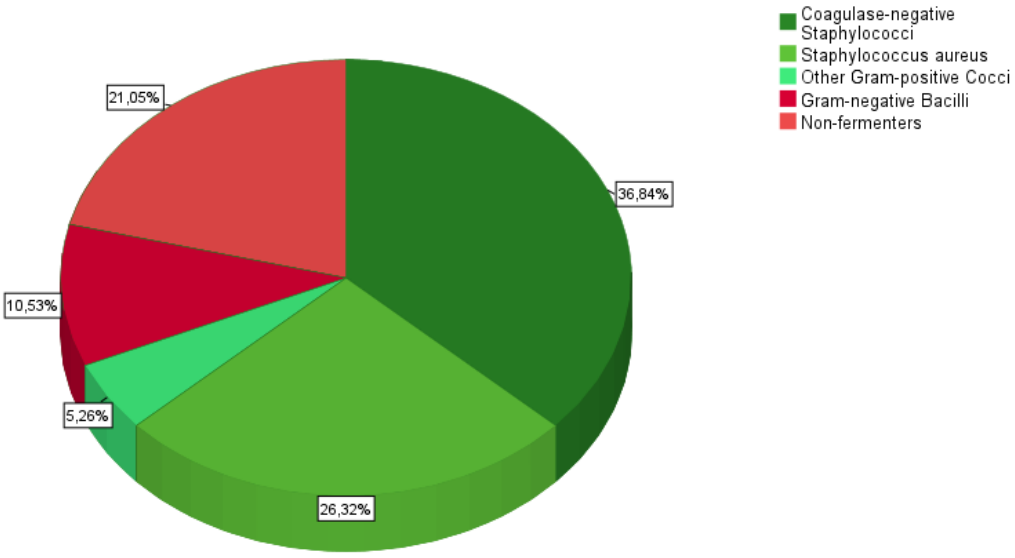

Figure 3: Conjunctiva - Microbial culture - Prevalence of bacteria isolated from conjunctival smears (bacterial isolates categorized)

Prevalence of all conjunctival bacteria (summarized in categories) that were detected via microbial culture.

Table 22: Conjunctiva - Microbial culture - Prevalence of bacteria isolated from conjunctival smears (bacterial isolates categorized)

		Answers		Percent of the cases
		N	Percent	
Conjunctiva – Bacterial isolates categorized	Coagulase-negative Staphylococci	7	36.8%	41.2%
	Staphylococcus aureus	5	26.3%	29.4%
	Other Gram-positive cocci	1	5.3%	5.9%
	Gram-positive bacilli	0	0%	0%
	Gram-negative bacilli	2	10.5%	11.8%
	Non-Fermenters	4	21.1%	23.5%
Total		19	100.0%	111.8%

Prevalence of all conjunctival bacteria (summarized in categories) that were proven via microbial culture.

Figure 3 and *Table 22* show the prevalence of all bacterial isolates detected at *17 positive microbial cultures* that were inoculated with the material of conjunctival smears.
In total 19 bacterial isolates (100%) were detected via microbial cultures.

For the purpose of clarity, the bacterial isolates were grouped into categories like *coagulase-negative staphylococci*, *Staphylococcus aureus*, *other Gram-positive cocci*, *Gram-positive bacilli*, *Gram-negative bacilli* and *non-fermenting bacteria*. (Figure 3, Table 22, Table 23) *Gram positive cocci* with *13 (68.4%)* isolates represented the majority of bacteria detected. Among Gram-positive cocci *coagulase-negative staphylococci* formed the majority with *7* isolates *(36.8%)*, followed by *Staphylococcus aureus* with *5* isolates *(26.3%)*. Summarized as *other Gram-positives, 1* isolate *(5.3%)* was detected.
Not a single *Gram-positive bacillus isolate 0 (0%) was detected.*
Gram-negative bacteria made up the proportion of *6 (31.6%)* isolates, among which *Gram-negative bacilli* made up a proportion of 2 isolates (10.5%) and *non-fermenting bacteria made up a proportion of 4 isolates (21.1%)*.

Table 23: Conjunctiva - Microbial culture - Bacterial isolates categorized - Overview

Coagulase-negative staphylococci	Staphylococcus epidermidis
	Staphylococcus hominis ssp. hominis
	Staphylococcus lugdunensis
Staphylococcus aureus	Staphylococcus aureus
Other Gram-positivi cocci	Streptococcus dysgalact. ssp. dysgalacticae
Gram-positive bacilli	/
Gram-negative bacilli	Citrobacter koseri
	Serratia marcescens
Non-Fermenters	Pseudomonas aeruginosa

All conjunctival isolates detected via microbial culture. The isolates are summarized in categories.

Table 23 could serve the reader as an aid for the taxonomy of conjunctival isolates and does not consider any frequencies.

Table 24: Conjunctiva - Microbial culture - Prevalence of bacteria isolated from conjunctival smears (individual bacterial isolates)

		Answers		Percent of the cases
		N	Percent	
Conjunctiva – Bacterial isolates	Citrobacter koseri	1	5.3%	5.9%
	Pseudomonas aeruginosa	4	21.1%	23.5%
	Serratia marcescens	1	5.3%	5.9%
	Staphylococcus aureus	5	26.3%	29.4%
	Staphylococcus epidermidis	4	21.1%	23.5%
	Staphylococcus hominis ssp. hominis	2	10.5%	11.8%
	Staphylococcus lugdunensis	1	5.3%	5.9%
	Streptococcus dysgalact. ssp. dysgalacticae	1	5.3%	5.9%
Total		19	100.0%	111.8%

Prevalence of all conjunctival bacteria (individual bacterial isolates) that were proven via microbial culture.

Table 24 shows every single bacterial isolate, listed in alphabetical order.

The most frequently detected bacterium is the *Gram-positive coccus Staphylococcus aureus with 5 isolates (26.3%).*

The second most frequently detected bacteria are the *non-fermenting Gram-negative bacillus Pseudomonas aeruginosa with 4 isolates (21.1%)* and the *Gram-positive coccus Staphylococcus epidermidis with 4 isolates (21.1%)*

The third most frequently occurring bacterium is the coagulase-negative, Gram-positive Staphylococcus hominis ssp. hominis with 2 isolates (10.5%).

3.3 Contact lens cases

3.3.1 Positive test results

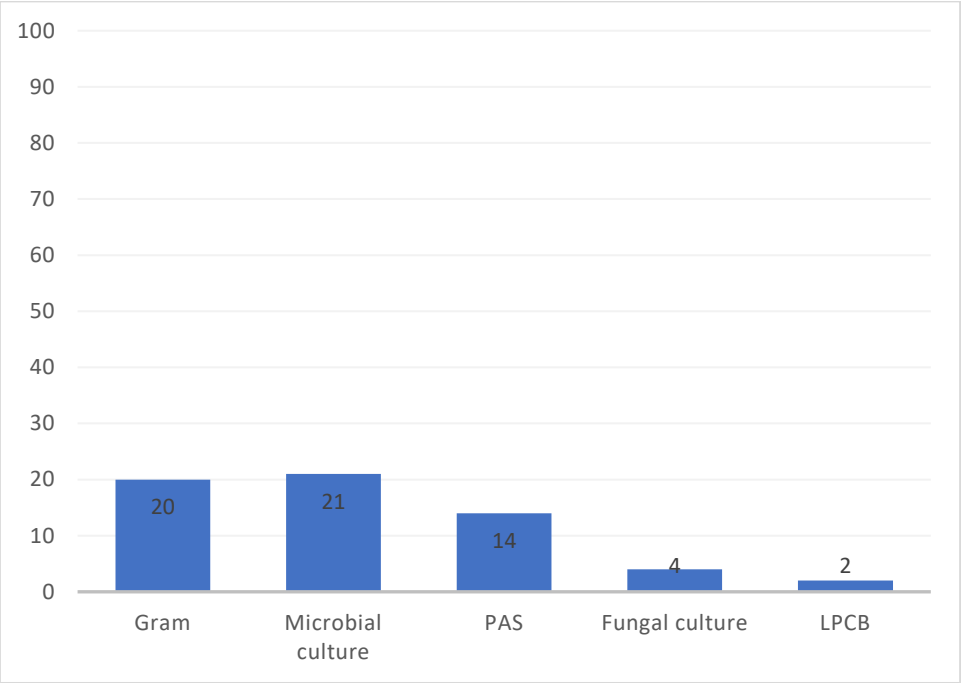

Figure 4: Contact lens cases – Positive test results

Positive test results of all laboratory diagnostic methods used for pathogen detection of the contact lens cases. The vertical axis represents 100 patients. The horizontal axis represents the performed diagnostic methods.

According to Figure 4, microbial cultures showed the most positive test results with 21 cases in the form of bacterial growth. Second most positive test results showed Gram stainings in 20 cases in the form of stained bacteria. PAS stainings showed the third most positive test results with 14 cases in the form of stained/suspected fungal components, followed by fungal culture with 4 positive test results.

LPCB stainings showed 2 positive test results in the form of stained/suspected Acanthamoeba cysts or trophozoites.

3.3.2 Gram staining

Table 25: Contact lens cases – Gram staining performed/not performed

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Not performed	79	79.0	79.0	79.0
	Performed	21	21.0	21.0	100.0
	Total	100	100.0	100.0	

Absolute numbers and percentage of patients at which sample collection of the sediment of contact lens liquids out of contact lens cases and Gram stainings were performed.

Gram stainings were performed for the detection of bacteria.

Table 25 shows that sample collection of the sediment of the contact lens liquids and subsequent *Gram stainings were performed at 21 (21%) patients/contact lens cases* in total. *In 79 cases (79%) Gram stainings* were *not performed*.

Table 26: Contact lens cases – Gram staining positive/negative

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Negative Gram staining	1	1.0	4.8	4.8
	Positive Gram staining	20	20.0	95.2	100.0
	Total	21	21.0	100.0	
Missing	System	79	79.0		
Total		100	100.0		

Positive/negative Gram staining results (in absolute numbers and percentages) of the percentage of patients in which sample collection of the sediment of contact lens liquids out of contact lens cases and Gram staining was performed.

Table 26 shows that Gram stainings were *positive* in the form of stained microbia *in 20* cases *(95.2%)*.

Gram staining was *negative* in the form of *missing stained microbia in 1* of the cases *(4.8%)*.

3.3.3 Microbial culture

Table 27: Contact lens cases – Microbial culture performed/not performed

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Not performed	78	78.0	78.0	78.0
	Performed	22	22.0	22.0	100.0
	Total	100	100.0	100.0	

Absolute numbers and percentages of patients in which sample collection of the sediment of contact lense liquids out of contact lens cases and microbial culture was performed.

Table 27 shows that sample collection of the sediment of contact lens liquids from contact lens cases and subsequent *microbial cultures* were *performed in 22 (22%) patients/contact lens cases* in total. *In 78 cases (78%)* sample collection of the sediment of contact lens liquids and subsequent *microbial cultures were not performed*.

Table 28: Contact lens cases – Microbial culture positve/negative

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Negative	1	1.0	4.5	4.5
	Positive	21	21.0	95.5	100.0
	Total	22	22.0	100.0	
Missing	System	78	78.0		
Total		100	100.0		

Positive/negative microbial culture results (absolute numbers and percentages) of the percentages of patients in which sample collection from the sediment of contact lens cases liquids and microbial culture was performed.

Table 28 shows that microbial culture was *negative* in the form of *missing microbial growth in 1* case *(4.5%)*. Microbial cultures showed *positive test results* in the form of *bacterial growth in 21* cases *(95.5%)*.

Contact lens cases - Microbial culture - Prevalence of bacteria/fungi isolated from contact lens cases (isolates categorized)

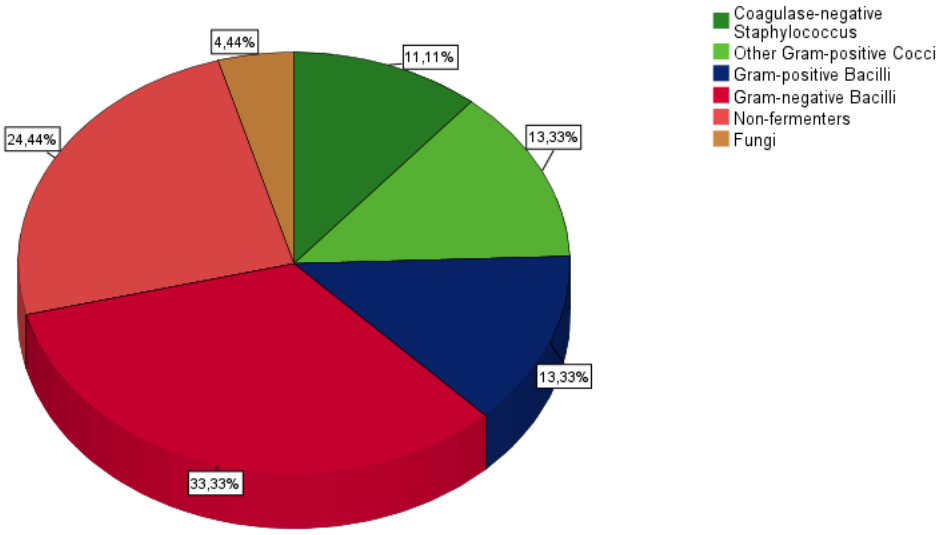

Figure 5: Contact lens cases - Microbial culture - Prevalence of bacteria/fungi isolated from contact lens cases (isolates categorized)

Prevalence of all bacteria/fungi from contact lens cases (summarized in categories) that were detected via microbial cultures.

Table 29: Contact lens cases - Microbial culture - Prevalence of bacteria/fungi isolated from contact lens cases (isolates categorized)

		Answers		Percent of the cases
		N	Percent	
Contact lens cases – Bacterial isolates categorized	Coagulase-negative Staphylococcus	5	11.1%	23.8%
	Staphylococcus aureus	0	0%	0%
	Other Gram-positive coccus	6	13.3%	28.6%
	Gram-positive bacilli	6	13.3%	28.6%
	Gram-negative bacilli	15	33.3%	71.4%
	Non-fermenters	11	24.4%	52.4%
	Fungi	2	4.4%	9.5%
Total		45	100.0%	214.3%

Prevalence of all contact lens cases isolates (summarized in categories) that were proven via microbial culture.

Figure 5 and *Table 29* show the prevalence of all bacterial isolates detected in *21 positive microbial cultures* that were inoculated with material of sediments from contact lens cases liquids.

In total 45 (100%) bacterial isolates were detected via microbial cultures.
For the purpose of clarity, the bacterial isolates were grouped into categories like *coagulase-negative staphylococci*, *Staphylococcus aureus*, *other Gram-positive cocci*, *Gram-positive bacilli*, *Gram-negative bacilli* and *non-fermenting bacteria*. *(Table 30)*
With the sum of 26 isolates (57.7%), all Gram-negative bacilli represented the majority of bacteria detected. *(Table 30)*
The category Gram-negative bacilli made up a proportion of 15 isolates (33.3%), especially *non-fermenters made up a proportion of 11 isolates (24.4%).*
Gram-positive bacilli made the part of 6 isolates (13.3%).
Another big part made up Gram-positive cocci with a proportion of 11 isolates (24.4%).
Among Gram-positive cocci the category other Gram-positive cocci show a proportion of 6 isolates (13.3%), coagulase-negative staphylococci built a proportion of 5 isolates (11.1%).
Additionally, 2 fungal isolates (4.4%) were detected via microbial culture.

Table 30: Contact lens cases - Microbial culture – Bacterial/fungal isolates categorized - Overview

Coagulase-negative staphylococci	Staphylococcus epidermidis
	Staphylococcus xylosus
Staphylococcus aureus	/
Other Gram-positivi cocci	Enterococcus faecalis
	Kocuria kristinae
	Streptococcus mitis
	Streptococcus salivarius
	Streptokokken der "Viridans"-Gruppe
Gram-positive bacilli	Bacillus cereus
Gram-negative bacilli	Achromobacter denitrificans
	Achromobacter xylosoxidans
	Citrobacter freundii
	Delftia acidovorans
	Elizabethkingia meningoseptica
	Enterobacter cloacae
	Haemophilus sp.
	Klebsiella oxytoca
	Ochrobactrum anthropi
	Serratia marcescens
	Sphingomonas paucimobilis
Non-Fermenters	Acinetobacter lwoffii
	Acinetobacter radioresistens
	Acinetobacter ursingii
	Pseudomonas aeruginosa
Fungi	Aspergillus Flavus
	Candida tropicalis

All contact lens cases isolates detected via microbial culture. The isolates are summarized in categories.

Table 30 could serve the reader as an aid for the taxonomy of contact lens cases isolates and does not consider any frequencies.

Table 31: Contact lens cases - Microbial culture - Prevalence of bacteria/fungi isolated from contact lense cases (individual bacterial/fungal isolates)

		Answers		Percent of the
		N	Percent	cases
Contact lens cases – Bacterial isolates	Achromobacter denitrificans	1	2.2%	4.8%
	Achromobacter xylosoxidans	1	2.2%	4.8%
	Acinetobacter lwoffii	1	2.2%	4.8%
	Acinetobacter radioresistens	1	2.2%	4.8%
	Acinetobacter ursingii	1	2.2%	4.8%
	Aspergillus Flavus	1	2.2%	4.8%
	Bacillus cereus	6	13.3%	28.6%
	Candida tropicalis	1	2.2%	4.8%
	Citrobacter freundii	1	2.2%	4.8%
	Delftia acidovorans	1	2.2%	4.8%
	Elizabethkingia meningoseptica	1	2.2%	4.8%
	Enterobacter cloacae	3	6.7%	14.3%
	Enterococcus faecalis	1	2.2%	4.8%
	Haemophilus sp.	1	2.2%	4.8%
	Klebsiella oxytoca	4	8.9%	19.0%
	Kocuria kristinae	1	2.2%	4.8%
	Ochrobactrum anthropi	1	2.2%	4.8%
	Pseudomonas aeruginosa	7	15.6%	33.3%
	Serratia marcescens	1	2.2%	4.8%
	Sphingomonas paucimobilis	1	2.2%	4.8%
	Staphylococcus epidermidis	4	8.9%	19.0%
	Staphylococcus xylosus	1	2.2%	4.8%
	Streptococcus mitis	2	4.4%	9.5%
	Streptococcus salivarius	1	2.2%	4.8%
	Streptokokken der "Viridans"-Gruppe	1	2.2%	4.8%
Total		45	100.0%	214.3%

Prevalence of all contact lens cases bacteria/fungi (individual bacterial/fungal isolates) that were proven via microbial culture.

Table 31 shows the prevalence of every single bacterial isolate, listed in alphabetical order. The most frequently detected bacterium is *the non-fermenting Gram-negative bacillus Pseudomonas aeruginosa with 7 isolates (15.6%).*

The second most frequently detected bacterium is the *Gram-positive Bacillus cereus with 6 isolates (13.3%).*

The third most frequently occurring bacterium is the Gram-negative bacillus Klebsiella oxytoca and the coagulase-negative, Gram-positive coccus Staphylococcus epidermidis with 4 isolates (8.9%) each.
Beside bacterial isolates, 2 fungal isolates were detected as well. The first isolate (2.2%) was the filamentary fungus *Aspergillus flavus, the second isolate (2.2%) was the yeast like fungus Candida tropicales.*

3.3.4 PAS staining

Table 32:Contact lens cases – PAS staining performed/not performed

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Not performed	80	80.0	80.0	80.0
	Performed	20	20.0	20.0	100.0
	Total	100	100.0	100.0	

Absolute numbers and the percentage of patients in which a sample collection of the sediment of contact lens liquids out of contact lens cases and PAS stainings were performed.

PAS stainings were performed for the detection of fungal components.
Table 32 shows that sample collection of the sediment of the contact lens liquids and subsequent *PAS stainings were performed in 20 (20%) patients/contact lens cases* in total. *In 80 cases (80%) PAS stainings* were *not performed*.

Table 33:Contact lens cases – PAS staining positive/negative

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Negative	6	6.0	30.0	30.0
	Positive	14	14.0	70.0	100.0
	Total	20	20.0	100.0	
Missing	System	80	80.0		
Total		100	100.0		

Positive/negative PAS staining results (in absolute numbers and percentages) of the percentage of patients in which sample collection of the sediment of contact lens liquids out of contact lens cases and PAS stainings were performed.

Table 33 shows that PAS stainings were *positive* in the form of *stained fungal components in 14 cases (70%)*. PAS stainings were *negative* in the form of missing stained fungal components in 6 of the cases (30%).

3.3.5 Fungal culture

Table 34: Contact lens cases – Fungal culture performed/not performed

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Not performed	77	77.0	77.0	77.0
	Performed	23	23.0	23.0	100.0
	Total	100	100.0	100.0	

Absolute numbers and percentages of patients in which a sample collection of the sediment of contact lens liquids out of contact lens cases and fungal cultures were performed.

Table 34 shows that sample collection of the sediment of the contact lens liquids and subsequent *fungal cultures* were *performed at 23 patients/contact lens cases (23%)* out of 100 patients in total. In *77 cases (77%) fungal cultures were not performed*.

Table 35: Contact lens cases – Fungal culture positive/negative

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Negative culture results	19	19.0	82.6	82.6
	Positive culture results	4	4.0	17.4	100.0
	Total	23	23.0	100.0	
Missing	System	77	77.0		
Total		100	100.0		

Positive/negative fungal culture results (in absolute numbers and percentages) of the percentage of patients in which a sample collection of the sediment of contact lens liquids out of contact lens cases and fungal cultures were performed.

Table 35 shows that Fungal cultures were *negative* in the form of *missing fungal growth in 19* cases *(82.6%).* Fungal culture showed *positive test results* in the form of *fungal growth in 4* cases *(17.4%).*

In total 5 fungal isolates (100 %) were detected.
2 of the fungal isolates detected were yeast like fungus (40%). One of the yeast like fungal isolates, was Candida parapsilosis *(20%) the second yeast like isolate was* Candida tropicalis (20%).

3 of the fungal isolates (60%) detected were filamentous fungi. One of the filamentous isolates was Fusarium proliferatum (20%), two of the filamentous isolates were Aspergillus flavus (40%).

3.3.6 LPCB staining

Table 36: Contact lens cases – LPCB staining performed/not performed

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Not performed	80	80.0	80.0	80.0
	Performed	20	20.0	20.0	100.0
	Total	100	100.0	100.0	

Absolute numbers and the percentage of patients in which a sample collection of the sediment of contact lens liquids out of contact lens cases and LPCB stainings were performed.

LPCB stainings were performed for the detection of Acanthamoeba cysts or trophozoites. *Table 36* shows that sample collection of the sediment of the contact lens liquids and subsequent *LPCB* stainings *were performed in 20 (20%) patients/contact lens cases* in total. *In 80 cases (80%) LPCB* stainings were *not performed*.

Table 37: Contact lens cases – LPCB staining positive/negative

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Negative LPCB	18	18.0	90.0	90.0
	Positive LPCB	2	2.0	10.0	100.0
	Total	20	20.0	100.0	
Missing	System	80	80.0		
Total		100	100.0		

Positive/negative LPCB staining results (in absolute numbers and percentages) of the percentage of patients in which sample collection of the sediment of contact lens liquids out of contact lens cases and LPCB stainings were performed.

Table 37 shows that LPCB stainings were *positive* in the form *stained* Acanthamoeba cysts or trophozoites *in 2 cases (10%)*. LPCB stainings were *negative* in the form of missing stained Acanthamoeba cysts or trophozoites in 18 of the cases (90%).

3.4 Microbial culture - Isolates compared - Cornea - Conjunctiva - Contact lens cases

Table 38: Comparison of the isolates of the cornea, conjunctiva and contact lens cases

Patient	Cornea - isolates	Conjunctiva - isolates	Contact lens cases - isolates
Patient 1	Bacillus cereus	NMC	Bacillus cereus
Patient 2	NMC	NMC	Staphylococcus epidermidis
			Aspergillus Flavus
Patient 3	Staphylococcus epidermidis	NMC	Bacillus cereus
Patient 4	Pseudomonas aeruginosa	Pseudomonas aeruginosa	Pseudomonas aeruginosa
			Serratia marcescens
Patient 5	NMC	NMC	Enterobacter cloacae ssp. cloacae
Patient 6	Staphylococcus epidermidis	NMC	Staphylococcus epidermidis
	Propionibacterium acnes		Sphingomonas paucimobilis
Patient 7	NMC	NMC	Bacillus cereus
			Ochrobactrum anthropi
			Candida tropicalis
Patient 8	Pseudomonas aeruginosa	Pseudomonas aeruginosa	Pseudomonas aeruginosa
		Staphylococcus lugdunensis	

Patients and their related culture results of the cornea, the conjunctiva and contact lens cases. (NMC=Negative microbial culture)

Table 38 shows that in 8 cases out of 100, *microbial cultures of the cornea*, *microbial cultures of the conjunctiva* and *microbial cultures* of the *contact lens cases* were performed concurrently. Hence, a direct comparison of the culture results was possible regarding these patients.

Microbial cultures of patient No.1 showed concordance in categories cornea and contact lens cases with Bacillus cereus as detected isolate. Microbial culture of the conjunctiva showed negative test results in the form of missing microbial growth.

With negative test results in the form of missing microbial growth, the microbial cultures of patient No.2 showed concordance in the categories cornea and conjunctiva. Microbial

culture of the contact lens cases on the other hand showed growth of Staphylococcus epidermidis and Aspergillus flavus.

Microbial culture results of patient No.3 differed in all categories. The corneal culture showed Staphylococcus epidermis, conjunctival culture showed negative test results and the contact lens culture showed Bacillus cereus.

Microbial cultures of patient No. 4 showed concordance in all three categories cornea, conjunctiva and contact lens cases with the bacterium Pseudomonas aeruginosa. Addiotionally, beside Pseudomonas aeruginosa the microbial culture of contact lens cases showed growth of Serratia mercescens.

Microbial cultures of patient No.5 both showed negative test results in the categories cornea and conjunctiva. Microbial culture of the contact lens case showed growth of Enterobacter cloacae.

Microbial cultures of patient No. 6 showed concordance in the categories cornea and contact lens cases, with the growth of Staphylococcus epidermidis. Moreover, the corneal culture showed growth of Propionibacterium acnes and the culture of contact lens cases showed growth of Sphingomonas paucimobilis as second isolates. The culture results of the conjunctiva were negative.

Corneal and conjunctival cultures of patient No. 7 both were negative. On the other hand, the contact lens cases showed growth of even three isolates like Bacillus cereus, Ochrobactrum anthropi, and Candida tropicalis.

All three cultures of patient No. 8 showed growth with the bacterial isolate Pseudomonas aeruginosa. Additionally, as second isolate conjunctival culture showed growth of Staphylococcus lugdunensis.

3.5 Resistances

3.5.1 Cornea

Table 39: Cornea - Resistances

Antibiotic agents	Coagulase-negative staphylococci (34)	Staphylococcus aureus (3)	Other Gram-positive cocci (4)	Gram-positive bacilli (29)	Gram-negative bacilli (6)	Non-Fermenters (8)
Amoxicillin/Clavulanic acid	6/24				2/2	
Ampicillin				1/1	5/5	
Ampicillin/Sulbactam	6/24				2/2	
Aztreonam						*8/8
Cefotaxim				3/3		
Cefoxitin					1/2; *1/2	
Cefuroxim	4/22				2/2	
Ciprofloxacin				1/1		
Clindamycin	*14/28	*1/3		1/1		
Erythromycin	13/24					
Fosfomycin	10/30					
Fusidic acid	6/23					
Gentamicin	5/22					
Imipenem	6/24					
Levofloxacin	5/22					
Linezolid	*1/5					
Metronidazole				25/25		
Moxifloxacin	5/22			1/1	2/2	
Oxacillin	5/22					
Penicillin		2/3		5/5		
Teicolanin	7/22	1/3				
Tetracyclin	6/30; *2/30		1/1			
Tigecyclin					*1/1	
Trimethoprim/Sulfonamide	2/22					

All resistances of the bacterial isolates of the cornea that were detected via microbial culture.

In the first line, the bacterial isolates are categorized in coagulase-negative staphylococci, Staphylococcus aureus, other gram-positive cocci, gram-positive bacilli, gram-negative bacilli and non-fermenters. Added in the brackets behind each categorie, you can find the number of corneal isolates, detected via microbial culture.

In the first column, all tested antibiotic agents with proven resistances or proven intermediate resistances, are listed according to alphabetical order. Intermediate susceptibility test results are marked with an asterisk (*). Among the listed antibiotics, only agents that showed a proven resistance can be found. In the case of susceptibility, a tested antibiotic agent was not listed.

Empty colored cells could either mean that no isolate of a category was tested for susceptibility of a specific antibiotic agent or certain isolates were tested but showed susceptibility/no resistance. The depiction should give an idea of existing resistances.

The number in the brackets behind each categorie represents the number of detected isolates. This number does not match the number of the registered resistances in each cell. This is because not every bacterial isolate in a particular category was tested for every assigned antibiotic agent.

The first number in each cell represents the absolute frequency of isolates that show resistance to the assigned antibiotic agent. The second number in each cell represents the absolute frequency of isolates that were tested for resistance of the assigned antibiotic agent.

Acccording to *Table 39*, the chosen depiction should not tempt the clinician to choose an antibiotic for clinical usage that shows few resistances, because empty coloured cells could either mean that no isolate of a category was tested for susceptibility of a specific antibiotic agent or certain isolates were tested but showed susceptibility/no resistance. The agent is *per se* not appropriate for the treatment of the respective organism/group, because some bug-drug combinations have no relevance for the clinical use. The depiction should give an idea of existing resistances.

Table 39 shows all resistances of the bacterial isolates of the cornea that were detected via microbial culture.
Among 34 detected *coagulase-negative staphylococci* isolates, the table shows resistances/intermediate resistances to 16 different antibiotic agents.
As an example, 24 coagulase-negative Staphylococci isolates were tested for the susceptibility of Amoxicillin/Clavulanic acid of which 6 isolates showed resistances.
In the table the susceptibility of Ampicillin/Sulbactam shows the same number ratio.
22 isolates were tested for the susceptibility of Cefuroxim of which 4 isolates showed resistances, 28 isolates were tested for susceptibility of Clindamycin of which 14 isolates showed intermediate resistances, 24 isolates were tested for susceptibility of Erythromycin of which 13 isolates showed resistances, 30 isolates were tested for susceptibility of Fosfomycin of which 10 isolates showed resistances, 23 isolates were tested for susceptibility of Fusidic acid of which 5 isolates showed resistances, 22 isolates were tested for susceptibility of Gentamicin of which 5 isolates showed resistances, 5 isolates were tested for susceptibility of Linezolid of which 1 isolates showed intermediate resistance. 22 coagulase-negative Staphylococci isolates were tested for the susceptibility of Moxifloxacin and Oxacillin of which 5 isolates each showed resistances, 22 isolates were tested for susceptibility of Teicoplanin of which 7 isolates showed resistances. 30 isolates were tested for susceptibility of Tetracyclin of which 6 isolates showed resistances and 2 isolates showed intermediate resistances, 22 isolates were tested for susceptibility of Trimethoprim/Sulfonamide of which 2 isolates showed resistances.
Among 3 detected *Staphylococcus aureus* isolates, the table shows resistances to 3 different antibiotic agents. Three isolates were tested for the susceptibility of Clindamycin of which 1 isolate showed intermediate resistance, 3 isolates were tested for the susceptibility of Penicillin of which 2 isolates showed resistances and 3 isolates were tested for the susceptibility of Teicoplanin of which 1 isolate showed resistance.

Among 4 detected other *Gram-positive cocci* the table shows resistance aiganst 1 antibiotic agent. One isolate was tested for the susceptibility of Tetracyclin and showed resistance.
Among 29 detected *Gram-positive bacilli* isolates, the table shows resistances to 7 different antibiotic agents. One isolate was tested for the susceptibility of Ampicillin and showed resistance. Three isolates were tested for the susceptibility of Cefotaxim of which all isolates showed resistances. One isolate respectively, was tested for the susceptibility of Ciprofloxacin, Clindamycin and Moxifloxacin and all of them showed resistance to the assigned antibiotic agent. All 25 isolates that were tested for the susceptibility of Metronidazole showed resistances and all 5 isolates tested for the susceptibility of Penicilline showed resistances.
Among 6 detected *Gram-negative bacilli* isolates, the table shows resistances/intermediate resistances to 7 different antibiotic agents.
As examples, 5 isolates were tested for the susceptibility of Ampicillin of which all isolates showed resistances, 2 isolates were tested for the susceptibility of Cefoxitin of which 1 isolate showed resistance and 1 isolate showed intermediate resistance. Two isolates were tested for the susceptibility of Moxifloxacin of which both isolates showed resistance.
Among 8 detected *non-fermenters*, the table shows 8 intermediate resistances to Aztreonam.

3.5.2 Conjunctiva

Table 40: Conjunctiva - Resistances

Antibiotic agents	Coagulase-negative staphylococci (7)	Staphylococcus aureus (5)	Other Gram-positive cocci (1)	Gram-positive bacilli (0)	Gram-negative bacilli (2)	Non-Fermenters (4)
Amoxicillin/Clavulanic acid	1/4				1/1	
Ampicillin	1/4				2/2	
Ampicillin/Sulbactam					1/1	
Aztreonam						* 2/3
Cefoxitin					*1/1	
Cefuroxim	1/4				1/1	
Clindamycin	1/1					
Erythromycin	3/5					
Fosfomycin	1/2					
Gentamicin	1/4					
Imipenem	1/4					
Moxifloxacin					1/1	
Oxacillin	1/4					
Penicillin		2/5				
Tetracyclin	1/4		1/1			
Tigecyclin					* 1/1	
Tobramycin						1/3

All resistances of the bacterial isolates of the conjunctiva that were detected via microbial culture.
(Description as for Table 39)

Table 40 shows all resistances of the bacterial isolates of the conjunctiva that were detected via microbial culture.

Among 7 detected *coagulase-negative Staphylococci* isolates, the table shows resistances/intermediate resistances to 10 different antibiotic agents.

As examples, 4 coagulase-negative Staphylococci isolates were tested for the susceptibility of Amoxicillin/Clavulanic acid and Ampicillin of which 1 isolate each showed resistance. Five isolates were tested for the susceptibility of Erythromycin of which 3 isolates showed resistances.

Among 5 detected *Staphylococcus aureus* isolates, the table shows resistance to 1 antibiotic agent. 5 isolates were tested for the susceptibility of Penicillin of which 2 isolates showed resistances.

One isolate of other Gram-positives was detected, was tested and showed resistance to Tetracyclin.

Regarding the conjunctiva, no Gram-positive bacillus was detected.

Among 2 detected *Gram-negative* bacilli, the table shows resistances to 7 different antibiotic agents. As examples, 2 isolates were tested for the susceptibility of Ampicillin of which both isolates showed resistances. One isolate was tested for the susceptibility of

Cefoxitin and 1 isolate was tested for the susceptibility of Tigecyclin of which both isolates showed intermediate resistance.

Among 4 detected *non-fermenters*, the table shows resistances to 2 different antibiotic agents. Three isolates were tested for the susceptibility of Aztreonam of which 2 isolates showed intermediate resistances. Three isolates were tested for the susceptibility of Tobramycin of which 1 isolate showed resistance.

3.5.3 Contact lens cases

Table 41: Contact lens cases – Resistances

Antibiotic agent	Coagulase-negative staphylococci (5)	Staphylococcus aureus (0)	Other Gram-positivi cocci (6)	Gram-positive bacilli (6)	Gram-negative bacilli (15)	Non-Fermenters (11)
Amikacin					1/1	
Amoxicillin/Clavulanic acid			1/1		6/6	
Ampicillin					12/12	
Ampicillin/Sulbactam			1/1		6/6	
Aztreonam					*1/1	*7/7
Caftazidim					4/5	
Cefepim					3/3	
Cefotaxim				5/5	4/6	
Cefoxitin					7/7	
Ceftriaxon					3/5	
Cefuroxim			1/1		5/6	
Ciprofloxacin					1/1	
Clindamycin	1/1		1/1			
Ertapenem					2/2	
Erythromycin	2/4		1/1			
Fosfomycin			1/1			
Gentamicin					2/2	
Imipenem			1/1			
Meropenem					2/2	
Oxacillin			1/1			
Penicillin				5/5		
Piperacillin/Tazobactam					2/5; *1/5	
Rifampecin			1/1			
Teicoplanin	1/4					
Tetracyclin			2/3			
Tobramycin					1/1	
Trimethoprim/Sulfonamide					2/2	

All resistances of the bacterial isolates of the contact lens cases that were detected via microbial culture.
(Description as for Table 39)

Table 41 shows all resistances of the bacterial isolates of the contact lens cases that were detected via microbial culture.

Among 5 detected *coagulase-neagtive Staphylococci* isolates, the table shows resistances to 3 different antibiotic agents. 1 coagulase-negative Staphylococcus isolate was tested for the susceptibility of Clindamycin and showed resistance.
4 isolates were tested for the susceptibility of Erythromycin of which 2 isolates showed resistances. 4 isolates were tested for the susceptibility of Teicoplanin of which 1 isolate showed resistance.
Regarding contact lens cases no *Staphylococcus aureus* isolate was detected.
Among 6 detected other *Gram-positive cocci* isolates, the table shows resistances to 10 different antibiotic agents. As examples, 1 isolate was tested for the susceptibility of Amoxicillin/Clavulanic acid and Ampicillin/Sulbactam and Cefuroxim and Clindamycin of which all tested isolates showed resistances. Three isolates were tested for the susceptibility of Tetracyclin of which 2 isolates showed resistances.
Among 6 detected *Gram-positive bacilli* isolates the table shows resistances to 2 different antibiotic agents.
Five isolates were tested for the susceptibility of Cefotaxim and Penicillin of which 5 isolates each showed resistances.
Among 15 detected *Gram-negative bacilli* isolates the table shows resistances/intermediate resistances to 18 different antibiotic agents.
As examples, 1 isolate was tested for the susceptibility of Amikacin and showed resistance, 6 isolates were tested for the susceptibility of Amoxicillin/Clavulanic acid of which all showed resistance, 12 isolates were tested for the susceptibility of Ampicillin of which all isolates showed resistances. 1 isolate was tested for the susceptibilitiy of Aztreonam and showed intermediate resistance, 5 isolates were tested for the susceptibilitiy of Piperacillin/Tazobactam of which 2 isolates showed resistances and 1 isolate showed intermediate resistance.
Among 11 *non-fermenters* the table shows resistance to Aztreonam. Seven isolates were tested and all of them showed intermediate resistances.

3.6 Visual acuity

Table 42: Visual acuity at the beginning performed/not performed

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Not performed	34	34.0	34.0	34.0
	Performed	66	66.0	66.0	100.0
	Total	100	100.0	100.0	

Absolute numbers and percentages of patients in which visual acuity tests were performed/not performed at the beginning of therapy.

Table 42 shows that visual acuity tests at the beginning of therapy were *performed* in 66 patients (66%) out of 100 patients in total. Visual acuity tests at the beginning of therapy were *not performed* in 34 patients.

Table 43: Visual acuity at the beginning

		Frequency	Percent	Valid percent	Cumulative percent
Valid	NLP	1	1.0	1.5	1.5
	LP	3	3.0	4.5	6.1
	HM/CF	15	15.0	22.7	28.8
	1mVA	4	4.0	6.1	34.8
	0,05-0,49	24	24.0	36.4	71.2
	≥ 0,5	19	19.0	28.8	100.0
	Total	66	66.0	100.0	
Missing	System	34	34.0		
Total		100	100.0		

Visual acuities at the beginning of therapy in absolute numbers and percentages from those patients in which visual acuity tests were performed at the beginning of therapy. (Classified in NLP=no light perception, LP=light perception, HM/CF=hand motion/counting fingers, 1mVA=1m visual acuity, 0.05-0.49 and ≥ 0.5)

Table 43 shows that one patient (1.5%) showed no light perception, 3 patients (4.5%) showed light perception, 15 patients (22.7%) percepted hand motion or could count fingers, 4 patients (6.1%) showed 1m visual acuity, 24 patients (36.7) showed visual acuity of 0.05-0.49, 19 patients (28.8%) had a visual acuity greater than or equal to 0.5.

Table 44: Visual acuitiy at the end performed/not performed

		Frequency	Percent	Valid percent	Cumulative percent
Valid	Not performed	31	31.0	31.0	31.0
	Performed	69	69.0	69.0	100.0
	Total	100	100.0	100.0	

Absolute numbers and percentages of patients in which visual acuity tests were performed/not performed at the end of therapy.

Table 44 shows that visual acuity tests at the end of the therapy were *performed* in 69 patients (69%) out of 100 patients in totoal. Visual acuity tests at the end of therapy were *not performed* in 31 patients.

Table 45: Visual acuity at the end

		Frequency	Percent	Valid percent	Cumulative percent
Valid	NLP	3	3.0	4.3	4.3
	LP	3	3.0	4.3	8.7
	HM/CF	2	2.0	2.9	11.6
	1mVA	2	2.0	2.9	14.5
	0,05-0,49	25	25.0	36.2	50.7
	≥ 0,5	34	34.0	49.3	100.0
	Total	69	69.0	100.0	
Missing	System	31	31.0		
Total		100	100.0		

Visual acuities at the end of therapy in absolute numbers and percentages from those patients in which visual acuity tests were performed at the end of therapy. (Classified in NLP=no light perception, LP=light perception, HM/CF=hand motion/counting fingers, 1mVA=1m visual acuity, 0.05-0.49 and ≥ 0.5)

Table 45 shows that 3 patients (4.3%) showed no light perception, 3 patients (4.3%) showed light perception, 2 patients (2.9%) precepted hand motion or could count fingers, 2 patients (2.9%) showed 1m visual acuity, 25 patients (36.2%) showed visual acuity of 0.05-0.49, 34 patients (49.3%) had a visual acuity greater than or equal to 0.5.

3.7 Further Results

3.7.1 Gender

The study included 56 (56%) female and 44 (44%) male patients.

3.7.2 Distribution left/right eye

In total the study included 100 eyes, 50 (50%) right eyes and 50 (50%) left eyes.

3.7.3 Keratitis/Ulcer

100 patients were diagnosed with keratitis.
Among the 100 patients 27 patients (27%) were diagnosed with a corneal ulcer.

3.7.4 Referral

53 patients (53%) attended the University Eye Hospital Graz as a referral from resident ophthalmologists.
8 Patients (8%) attended as referral from a general practitioner, 32 patients (32%) attended as outpatients without referral, 1 patient (1%) attendet as medical consultation, 1 patient (1%) attended as referral from the Department of Rheumatology from the University Hospital of Graz, 1 patient (1%) attended as referral from the Outpatient Clinic for Inner Medicine from the Hospital of St. John of God located in Graz,
1 patient (1%) attended as referral from the Department of Ophthalmology from the Regional Health Insurance of Graz (Österreichische Gesundheitskasse),
Three patients (3%) were referred from State Hospitals located in Styria (2 patients (2%) from the State Hospital Hochsteiermark, 1 patient (1%) from the State Hospital Fürstenfeld)

3.7.5 Contact lens wearer (yes/no) + material (soft/rigid)

51 patients (100%) were contact lens wearers, of which 39 patients (76.47%) wore soft contact lenses, and 3 patients (5.88%) wore rigid contact lenses.
For 9 patients (17.65%) contact lens wear could be noted without additional information about the material or replacement schedule.
Among the 39 patients who wore soft contact lenses, 24 pairs of contact lenses (61.54%) were monthly disposable, 1 pair of contact lenses (2.56%) was disposable after 2 weeks duration of wear, 6 pairs of contact lenses (15.39%) were daily disposable and 8 pairs of contact lenses (20.51%) had an unknown replacement schedule.

Among the 3 patients, who wore rigid contact lenses, all 3 pairs of contact lenses (100%) had an unknown replacement schedule.

3.7.6 Proven Acanthamoeba via LPCB stainings/PCR/Culture - Contact lenses/Trauma

12 patients showed positive LPCB staining results and/or positive PCR test results and/or positive culture results for Acanthamoeba cysts or trophozoites. Among these 12 patients, 11 patients (91.67%) were contact lens wearers.
Among the 11 contact lens wearers (100%), 5 patients (45.45%) wore soft contact lenses, 1 patient (9,1%) wore rigid contact lenses and 5 patients (45.45%) wore contact lenses without additional information about the material.

3 patients (25%) out of the 12 patients with positive test results for Acanthamoeba cysts or trophozoites had an ocular trauma.

3.7.7 Proven fungus via fungal culture/PAS/PCR - Contact lenses/Trauma

25 patients showed positive fungal culture results and/or positive PAS staining results and/or positive PCR test results for fungus. Among these 25 patients, 16 patients (64%) were contact lens wearers.
Among the 16 contact lens wearers (100%), 11 patients (68.75%) wore soft contact lenses, no patient (0%) wore rigid contact lenses and 5 patients (3125%) wore contact lenses without additional information about the material.

2 patients (8%) out of the 25 patients with positive test results for fungus had an ocular trauma.

3.7.8 Trauma + type of trauma

Out of 100 patients 8 patients (8%) suffered an ocular trauma, 92 patients (92%) did not.
Among the 8 patients (8%) with an ocular trauma, 1 patient (125%) reported injury while inserting a contact lens, 1 patient (12.5%) reported injury due to a foreign body after washing his car, 1 patient (12.5%) reported injury due to a foreign body and contact lens wear overnight, 1 patient (12.5%) reported injury due to a a foreign body without further information, 1 patient (12.5%) reported injury while working with an angle grinder

(12.5%), 1 patient (12.5%) reported injury due to his finger, 1 patient (12.5%) reported injury due to a branch while gardening.

3.7.9 Eye related surgical interventions before infection

Out of 100 patients, 9 (9%) patients underwent a surgical intervention within a few months before their first consultation, 91 patients (91%) did not.
Among these 9 patients (100%), 6 patients (66.6%) underwent a surgical intervention within one week before their first consultation. 2 patients (222%) underwent a corneal cross-linking treatment because of keratoconus, 2 patients (22.2%) underwent a LASIK surgery, 1 patient (11.1%) underwent a surgical treatment due to pterygium 1 patient (11.1%) underwent a trabeculectomy.
3 patients (33.3%) underwent a surgical intervention within a period of 4 to 6 months before their first consultation. 1 patient (11.1%) underwent surgical intervention due to ptosis, 1 patient (11.1%) underwent a surgical treatment due to cataract, 1 patient (11.1%) underwent a YAG laser iridectomy.

3.7.10 Hypopyon

Out of 100 patients 17 patients (17%) showed a hypopyon at the first presentation or during the course of the disease.

3.7.11 Local therapy before medical attendance at the clinic

Before attending the clinic, 54 patients (54%) received a local therapy in the form of eye drops. In 38 cases (38%) patients received antibiotics. In 8 cases (8%) patients received antiviral agents and in one case (1%) a patient received antifungal therapy. Moreover, some patients received topical corticosteroids, anesthetics, mydriatic agents, moisturizing eye drops and cyclosporin.
56 patients (56%) did not receive a topical therapy in the form of eye drops, before attending the clinic.

3.7.12 In-patient stay

Out of 100 patients 38 patients (38%) were admitted as inpatients.
62 patients (62%) were treated as outpatients.

3.7.13 Local treatment - Antibiotics

Out of 100 patients 96 patients (96%) received local antibiotic treatment.

Commonly used antibiotic agents included Aminoglycosides (Gentax = Gentamycin, Refobacin = Gentamycinsulfat), Fluorochinolones (Floxal = Ofloxacin, Ofloxa vision sine = Ofloxacin, Vigamox = Moxifloxacin), Fusidic acid (Fucithalmic), Chloramphenicol (Halomycetin), Glycopeptides (Vancomycin), Makrolides (Azyter = Azithromycin).

3.7.14 Local treatment - Antifungal agents

Out of 100 patients 30 patients (30%) received antifungal local treatment.
Commonly used antifungal agents included Voriconazole, Natamycin and Amphotericin B.

3.7.15 Local treatment - Acanthamoeba

Out of 100 patients 11 patients (11%) received local treatment against Acanthamoeba. 89 patients (89%) did not receive any treatment against Acanthamoeba.
Commonly used agents against Acanthamoeba included propamidine isethionate (Brolene), polyhexanide (Lavanid) and chlorhexidine.

3.7.16 Local treatment - Corticosteroids

Out of 100 patients 66 patients (66%) received local corticosteroids like dexamethasone, prednisolone or betamethasone. 34 patients (34%) did not receive local corticosteroids.

3.7.17 Systemic therapy

Out of 100 patients 51 patients (51%) received a sytemic therapy. Among systemic therapies antibiotics, antiviral agents and pain killers were used.
9 patients (9%) received a systemic antiviral therapy.

3.7.18 Surgical interventions in the context of treatment

In 29 cases (29%) a surgical intervention was necessary within the context of treatment. Among the surgical interventions anterior chamber punctures/washouts of anterior chambers were performed in 8 cases (8%), in 5 cases (5%) intravitreal/intracameral injections, in 4 cases (4%) amniotic membrane transplants, in 7 cases (7%) penetrating keratoplasties, in 1 case (1%) entropion surgery, in 1 case (1%) refractive surgery, in 1 case (1%) evisceration was performed. In 2 cases (2%) the surgical interventions were not described.

4 Discussion

The cornea is essential for visual perception of the environment, as it is the entry port for light. Its functional integrity is important to ensure clear imaging on the retina. Diseases of the eye, such as infectious keratitis, in which the cornea becomes damaged and light transmission is disrupted, can result in decreased visual acuity or even visual loss/blindness. The damage to the eye can be temporary or irreversible and can cause a high level of suffering for patients.
Knowledge of the pathogens responsible for infectious keratitis and corneal ulcer and their resistances to antibiotics and antifungals is the basis for adequate therapeutic measures in the treatment of these diseases. The germ spectrum of keratitis or corneal ulcer is also characteristic for different geographical locations.
With the help of the germs presented in this study and their resistance patterns, conclusions can be drawn about dominant germs and possibly dangerous resistances. Therapy approaches can be critically questioned and adapted if necessary.

In this retrospective study, the germ spectrum of infectious keratitis or corneal ulcers for the care area of the University Eye Hospital Graz (parts of Styria, Burgenland and Carinthia) is presented. 100 eyes from 100 patients (56% female, in average 49±21 years old, from 16 to 93 years), who were treated for infectious keratitis or corneal ulcer in 2018, were included in the study. In 27 cases an ulcer was diagnosed.
51 patients (100%) were contact lens wearers, of which 39 patients (76.47%) wore soft contact lenses, and 3 patients (5.88%) wore rigid contact lenses.

The germ spectrum is presented for the three categories cornea, conjunctiva and contact lens cases.
For the purpose of clarity, different representations of the isolates were chosen.
On the one hand microbial isolates were grouped into categories like *coagulase-negative Staphylococcus*, *Staphylococcus aureus*, *other Gram-positive cocci*, *Gram-positive bacilli*, *Gram-negative bacilli*, *non-fermenting bacteria* and fungi. Beside the categories, each single microbial isolate of the cornea, conjunctiva and contact lens cases and their related frequencies were represented for a closer look.
Furthermore, in our study we summarized the resistances to several antibiotic agents of our bacterial isolates. For this purpose, we also chose the categories *coagulase-negative*

Staphylococcus, *Staphylococcus aureus*, *other Gram-positive cocci*, *Gram-positive bacilli*, *Gram-negative bacilli* and *non-fermenting bacteria.*

4.1 Distribution of pathogens

In conclusion, Bacteria were detected in *100 patients*, *fungi in 25 patients*, *Acanthamoeba in 12 patients*, and *viruses in 8 patients*.

In most patients, not all methods performed to detect a particular pathogen were positive or negative. This can be well illustrated by the example of the methods for the detection of fungi (PAS staining, fungal culture, fungal PCR). In 23 cases PAS stainings were positive, in 2 cases fungal cultures and in 2 cases fungal PCR were positive. (Figure 1, page 32)

Since the positive test results of the different methods in some patients overlap or in some patients only one of the detection methods is positive, fungi were detected in a total of 25 patients. The same applies to the detection of bacteria and Acanthamoeba. For the detection of viruses, only PCR was used for pathogen detection.

It is also possible that mixed infections occurred in some patients of our study.

For our summery of pathogen distribution, we considered only the test results of the cornea, because regarding the conjunctiva only detection of bacteria was performed. Contact lens cases are used as a complementary diagnostic tool.

This distribution of causative organisms for infectious corneal disease shows a clear trend and resembles the data from the spanish study from Tena et al. so far that bacterial keratitis (culture proven) was detected the most with 64.6%. In this study bacterial keratitis is followed by viral keratits with 3.4% and fungal keratitis with 1%. Protozoan keratitis unfortunately was not considered in the study. (25)

4.2 Cornea – Laboratory diagnostic methods – positive/negative - performed/not performed

Regarding to *Figure 1 on page 32* that shows the positive test results of all laboratory diagnostic methods that were applied for the investigation of the cornea, it can be seen that a clear majority in the frequency of detected bacteria (Gram: 99, microbial culture: 71), followed by detected fungi (PAS: 23; fungal culture: 2; PCR: 2), detected Acanthamoeba cysts or trophozoites (LPCB: 11; Culture/PCR: 6) and finally detected HSV (Virus- PCR: 8) was observed.

The following percentages refer to the valid percentages from the tables.
In comparison of Gram staining results and microbial culture results for the detection of bacteria, 100% of the Gram stainings (Table 2, page 33) and 71% of the microbial cultures were positive (Table 4, page 34).
In comparison of PAS staining results, fungal culture results and PCR results for the detection of fungi, 23.5% of the PAS stainings (Table 9, page 39), 2.1% of the fungal cultures (Table 11, page 40) and 50% of the fungal PCR test results (Table 13, page 41) were positive.
In comparison of the LPCB staining results and the PCR test results for the detection of Acanthamoeba, 19.3% positive LPCB stainings (Table 15, page 42) and 46.2% of positive PCR test results (Table 17, page 43).
Virus PCR showed positive test results in 26.7% of the cases (Table 19, page 44).

At this point it is important to mention the significantly different frequencies of performed tests. Gram stainings were performed in 99 cases (Table 1, page 32), microbial cultures were performed in 100 cases (Table 3, page 34), PAS stainings were performed in 98 cases (Table 8, page 39), fungal cultures were performed in 96 cases (Table 10, page 40), fungal PCR was performed in 4 cases (Table 12, page 41), LPCB stainings were performed in 57 cases (Table 14, page 42), Acanthamoeba-PCR/Culture was performed in 13 cases (Table 16, page 43) and Virus- PCR was performed in 30 cases (Table 18, page 44).
The frequencies of performed tests differ because tests are adapted to the clinical appearance of the patients.

4.3 Conjunctiva – Laboratory diagnostic methods – positive/negative - performed/not performed

Regarding the conjunctiva, only microbial cultures were performed. Therefore, a comparison of laboratory diagnostic methods cannot be performed.
Neverthelless it stands out that regarding the conjunctiva, the proportion of positive microbial cultures with 27.4% (Table 21, page 45) is significantly smaller compared to the positive microbial cultures of the cornea with 71% (Table 4, page 34) and compared to the positive microbial cultures of the contact lens caes with 95.5% of positive microbial cultures (Table 28, page 51).

4.4 Contact lens cases – Laboratory diagnostic methods – positive/negative - performed/not performed

Regarding to Figure 4 on page 49 that shows the positive test results of all laboratory diagnostic methods that were applied for the investigation of contact lens cases, *a clear majority in the frequency of detected bacteria (Gram:20, microbial culture:21), followed by detected fungi (PAS: 14; fungal culture: 4) and detected Acanthamoeba cysts or trophozoites (LPCB:2) can be seen.*
This distribution of organisms detected in contact lens cases via microbial culture resembles the trend of the test results of the cornea. (Figure 1, page 32)

The following percentages refer to the valid percentages from the tables.
*The comparison of Gram staining results and microbial culture results for the detection of bacteria shows that 95.2% of the Gram stainings (Ta*ble 26, page 50) and 95.5% of the microbial cultures (Table 28, page 51) were positive.
In comparison of PAS staining results and fungal cultures that were applied for the investigation of contact lens cases for the detection of fungi, *70% of the PAS stainings (Table 33, page 56) and 17.4% of the fungal culture test results (Table 35, page 57) were positive. Compared to the cornea, only* 23.5% of the PAS stainings (Table 9, page 39) and only 2.1% of the fungal cultures were positive (Table 11, page 40).
LPCB stainings, regarding contact lens cases, were positive in 10% of the cases (Table 37, page 58), whereas 19.3% of the corneal LPCB stainings were positive (Table 15, page 42).

Among 51 contact lens wearers, 23 contact lens cases were available. *Gram stainings of the contact lens cases were performed in 21 cases (Table 25, page 50), microbial cultures were performed in 22 cases (Table 27, page 51), PAS stainings were performed in 20 cases (Table 32, page 56), fungal cultures were performed in 23 cases (Table 34, page 57) and LPCB stainings were performed in 20 cases (Table 36, page 58).*
The frequencies of performed tests differ because tests are adapted on the clinical appearance of the patients. More then half contact lenses/contact lens cases were not available for further diagnostics, because in some cases lenses were discarded or lost by the patients before attending the clinic.

4.5 Comparison of the bacterial isolates of the cornea, conjunctiva and contact lens cases

Regarding the cornea, in total 100 microbial cultures were performed (Table 3, page 34), 71 of them showed positive test results (Table 4, page 34). In total 84 bacterial isolates were detected (Table 5, page 35; Table 7, page 38). 84 bacterial isolates were detected per 71 positive microbial cultures (Ratio of detected isolates to positive microbial cultures: 84/71=1.18).

Regarding the conjunctiva, in total 62 microbial cultures were performed (Table 20, page 45), 17 of them showed positive test results (Table 21, page 45). In total 19 bacterial isolates were detected (Table 22, page 46; Table 24, page 48). 19 bacterial isolates were detected per 17 posive microbial cultures (19/17=1.11).

Regarding the contact lens cases, in total 22 microbial cultures were performed (Table 27, page 51), 21 of them showed positive test results (Table 28, page 51). In total 45 microbial isolates were detected (Table 29, page 52; Table 31, page 55). 43 of them represented bacterial isolates, 2 of them represented fungal isolates. Only the microbial cultures of the contact lens cases showed fungal isolates. 45 microbial isolates were detected per 21 positive microbial cultures (45/21=2.14). The ratio of detected isolates to positive microbial cultures (45/21=2.14) and the special feature of fungal growth, could indicate that contact lens cases show better conditions for microbial growth than the cornea or conjunctiva.

The prevalence of the categorized bacterial isolates of the cornea (Figure 2, Table 5, page 35), conjunctiva (Figure3, Table 22, page 46) and contact lens cases (Figure 5, Table 29, page 52) are compared.

Regarding the cornea and conjunctiva, coagulase-negative Staphylococci have the largest proportion with 40.5% and 36.8%. Regarding contact lens cases, coagulase-negative Staphylococci only had a proportion of 11.1% but Gram-negative bacilli represented the largest proportion of detected isolates with 33.3%.

The second largest proportion of corneal isolates represented Gram-positive bacilli with 34.5%. This category was not represented at all by conjunctival isolates, instead coagulase-positive Staphylococcus aureus with 26.3% was represented second most. Regarding contact lens cases, non-fermenters were represented second most with a proportion of 24.4%.

The third largest category, regarding the cornea and the conjunctiva represented non-fermenters with 9.5% and 21.1%. Like mentioned above, non-fermenters made up a proportion of 24.4% regarding contact lens cases as second most representative. Interestingly to see is that all categories but fungi are represented by corneal isolates. On the other hand, beside fungi, Gram-positive bacilli are not represented at all by conjunctival isolates, which could indicate a lower diversity compared to the corneal isolates. Another category is missing regarding contact lens cases. Staphylococcus aureus, as an important causative for bacterial keratits is not occurring at all, on the other hand the category fungi is represented with 4.4% as a unique feature.

In comparison of the prevalence of individual bacterial isolates from the cornea (*Table 7, page 38)* conjunctiva (*Table 24, page 48)* and contact lens cases (*Table 31, page 55)* some commonalities and differences stand out.
Concerning the cornea (*Table 7, page 38*), Propionibacterium acnes was detected most frequently with 28.6% of isolates, followed by Staphylococcus epidermidis with 26.2% and Pseudomonas aeruginosa with 9.5%.
Concerning the conjunctiva (*Table 24, page 48)*, Staphylococcus aureus was detected most frequently with 26.3% that had a share of only 3.6% regarding the cornea (*Table 7, page 38*) and was not represented at all by contact lens cases isolates (*Table 31, page 55*).
Second most Pseudomonas aeruginosa and Staphylococcus epidermidis were detected as conjunctival isolates with a share of 21.1% each (*Table 24, page 48)*. Pseudomonas aeruginosa had a share of 9.5% regarding the cornea (*Table 7, page 38*) and 15.6% regarding contact lens cases (*Table 31, page 55*). Staphylococcus epidermidis had a share of 26.2% regarding the cornea (*Table 7, page 38)* and 8.9% regarding contact lens cases (*Table 31, page 55)*.
Regarding contact lens cases, Pseudomonas aeruginosa was detected most with 15.6%, followed by Bacillus cereus with 13.3% and Staphylococcus epidermidis and Klebsiella oxytoca with 8.9% each (*Table 31, page 55)*.

We did not find any comparable data for the German-speaking countries in the literature. In a review fromTena et al. from spain, Gram-positive bacteria were detected the most with 87.1% via microbial culture. (25)
If we summarize the Gram-positive categories of corneal isolates of our study that were detected via microbial culture (Coagulase-negative staphylococci 40.5%, Staphylococcus

aureus 3.6%, other Gram-positive cocci 4,8%, Gram-positive bacilli 34.5%) we get a share of 83.4% Gram-positive bacteria (*Table 5, page 35*) that also make up the biggest proportion. (25)
In the cited study, among Gram-positives, coagulase-negative Staphylococci made up the biggest proportion with 30.3% compared to our study with 40.5% (*Table 5, page 35*), in which coagulase-negative Staphylocooci made up the biggest proportion as well.
12.7% of Gram-negative isolates were detected in the study from Tena et al.. In our study 16.6% of Gram-negative isolates were detected (Gram-negative bacilli 7.1%, non-fermenters 9.5%) (*Table 5, page 35*).
In the review from Tena et al. Staphylococcus strains accounted for the largest share with 30.3% compared to our study with 44.1% (Coagulase-negative staphylococci 40.5%, Staphylococcus aureus 3.6%) (*Table 5, page 35*).
In our study Propionibacterium acnes with 28.6%, Staphylococcus epidermidis with 26.2% and Pseudomonas aeruginosa with 9,5% represented the three most detected corneal isolates (*Table 7, page 38*). In the review from Tena et al. Propionibacterium spp. with 20.7% made up the biggest proportion of bacterial isolates as well, followed by Corynebactrerium spp. with 10.4% (in our study: 1.2%), Stapylococcus aureus and Streptococcus spp. with each 9.9% (in our study: 3.6% and 2.4%), Pseudomonas aeruginosa with 5.8% (in our study: 9.5%) and Bacillus spp. with 3.3% (in our study: 3.6%). (25) (*Table 7, page 38*)

Regarding *Table 38* that compares the isolates of the cornea, conjunctiva and contact lens cases, it can be seen that only in two cases, the isolates matched in all of the three categories like cornea, conjunctiva and contact lens cases. Hence, the importance of conducting pathogen identification for all three categories, to avoid the risk of missing a causative pathogen, seems to be indicated.
For the case that the patient does not wear contact lenses, *Table 38* might indicate the importance of conducting both corneal culture and conjunctival culture. On the other hand, in our study, conjunctival cultures with 72.6% often have negative microbial culture results (Table 21, page 45), why the benefit of additional conjunctival microbial culture might be questionable.

4.6 Resistances

In our tables that describe the resistances (Table 39, page 61; Table 40, page 64; Table 41, page 65) we listed a large scale of antibiotic agents. Not all of the tested agents are commonly used for the treatment of infectious keratitis. Hence, we here chose some representatives that can be used for the treatment of infectious keratitis.

Cornea:

Regarding Aminoglycosides, 5 out of 22 tested coagulase-negative isolates showed resistances to Gentamycin.

Regarding Fluorochinolones, 5 out of 22 tested coagulase-negative isolates showed resistances to Moxifloxacin, 5 out of 22 tested coagulase-negative isolates showed resistances to Levofloxacin and 1 out of 1 tested Gram-positive bacillus showed resistance to Ciprofloxacin.

Six out of 23 tested coagulase-negative Staphylococci isolates showed resistances to Fusidic acid.

Conjunctiva:

Regarding Aminiglycosides, 1 out of 4 tested coagulase-negative Stapphylocccus isolate showed resistance to Gentamicin, 1 out of 3 tested non-fermenters showed resistance to Tobramycin.

Regarding Fluorochinolones, 1 out of 1 tested Gram-negative bacillus showed resistance to Moxifloxacin.

Contact lens cases:

Regarding Aminoglycosides, 2 out of 2 tested Gram-negative bacilli isolates showed resistance to Gentamicin, 1 out of 1 tested Gram-negative bacillus showed resistance to Tobramycin.

Regarding Fluorochinolones, 1 out of 1 tested Gram-negative bacillus showed resistance to Ciprofloxacin.

4.7 Visual acuity

The assessment of visual acuity at first presentation and at the end of the therapy is important for the evaluation of the clinical course, the severity of the infection or the

healing process of the disease and can also serve as an indication for the success or failure of therapy.

The following percentages refer to the valid percentages from the tables.
When comparing the visual acuity at the beginning (Table 43, page 67) and at the end of the therapy (Table 45, page 68) it is noticeable that the severe visual loss (NLP) more than doubled from 1 patient (1.5%) to 3 patients (4.3%) during the course of the disease. However, a significant improvement in visual acuity predominated, as the incidence of HM/CF decreased by almost 20% from 22.7% percent to 2.9%. At the same time, there is an increase in visual acuity of ≥ 0.5 from 28.8% to 49.3%, an increase of more than 20%. When comparing the Other visual acuity classifications, minor changes are noted (LP: 4.5% vs. 4.3%; 1mVA: 6.1% vs. 2.9%; 0.05-0.49: 36.4% vs. 36.2%).
Visual acuity tests at the beginning of therapy were performed in 66 cases (Table 42, page 67) and visual acuity tests at the end of therapy were performed in 69 cases (Table 44, page 68). In 57 patients, visual acuity tests were performed both at the beginning of therapy and at the end of the therapy. Therefore, a direct comparison of the visual acuities is possible in these 57 patients. In the remaining cases, visual acuity tests were performed only at the beginning of therapy or only at the end of therapy.

4.8 Gender

The study included 56% female and 44% male patients with infectious keratitis/corneal ulcer. 12% more men than women were affected by the diagnosis.

4.9 Distribution of affected eyes

It could be assumed that there would be a difference in the distribution of affected eyes, for example in connection with a higher quantitiy of right-handed people in the population, but interestingly the distribution with 50% right eyes and 50% left eyes is entirely balanced.

4.10 Ulceration

Ulceration is a serious clinical expression of infectious keratitis. It can lead to perforation and indicates the danger of serious visual impairment. (166)
In 27 cases an ulcer was diagnosed. In a possible next step, it would be interesting to investigate the causing pathogens, that lead to ulceration and to proove a possible correlation between clinical expression and other characteristics like contact lenses, trauma or surgeries.

4.11 Contact lenses - Trauma/ Eye surgery

Contact lenses, especially soft contact lenses, trauma and eye surgery are important risk factors for infectious keratitis. (27-29)
In a study, by Yildiz et al., conducted in 2012, 44% of over 500 patients with infectious keratitis were contact lenses related. (30)
In our study even of 51 patients (51%) were contact lens wearers.
39 patients (76.47%) wore soft contact lenses and 3 patients (5.88%) wore rigid contact lenses. In 9 cases no additional information about the material or replacement schedule was available.
In a review by Bourcier et al., up to 21% of the patients suffered an ocular trauma. (36)
In our study, 8 patients (8%) suffured an ocular trauma, 9 patients underwent an eye related surgery before the infection. Overall, 68 patients (68%) exhibited one of the three risk factors described above.

4.12 Hypopyon

17 patients (17%) showed a hypopyon at the first presentation or during the course of the disease. The accumulation of white blood cells in the anterior chamber can indicate an intraocular inflammation. (167)

4.13 Local therapy before medical attendance at the clinic

Before attending the clinic 54 patients (54%) received a local therapy in the form of eye drops. Among the pharmaceutical agents, there were antibiotic agents, antiviral agents, antifungal agents, corticosteroids, anesthetics, mydriatic agents, moisturizing eye drops and cyclosporin.

4.14 Proven Acanthamoeba via LPCB stainings/PCR/Culture – Contact lenses/Trauma

The high number of patients who were contact lens wearers or had an ocular trauma among the patients with Acanthamoeba keratitis or fungal keratitis, confirms the assumption that especially contact lenses and corneal trauma belong to the main risk factors for Acanthamoeba keratitis and fungal keratitis. (136)
12 patients showed positive test results for Acanthamoeba cysts or trophozoites. It is interesting to see that 11 out of these 12 patients (91.67%) were contact lens wearers. Among the 11 contact lens wearers (100%) 5 patients (45.45%) wore soft contact lenses, 1 patient (9.1%) wore rigid contact lenses and 5 patients (45.45%) wore contact lenses without additional information about the material.
Furthermore, 3 out of 12 patients of the patients (25%) who showed positive test results for Acanthamoeba cysts or trophozoites, had an ocular trauma.

25 patients showed positive test results for fungal components. 16 out of these 25 patients (64%) were contact lens wearers.
Among the 16 contact lens wearers (100%), 11 patients (68.75%) wore soft contact lenses, no patient (0%) wore rigid contact lenses and 5 patients (31.25%) wore contact lenses without additional information about the material.
2 out of 25 patients (8%), who showed positive test results for fungal components had an ocular trauma.

As a further step, other correlations could be checked or highlighted, such as the distribution of germs in contact lens wearers compared to patients who do not wear contact lenses.

It would also be interesting to repeat the study in a few years to see how the germ distribution and resistances develop.

With anticipation, we look forward for researchers of other German-speaking countries to provide similar data in order to be able to compare them to our data.

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