Revista Visa em debate sociedade, ciência & tecnologia

REVIEW
https://doi.org/10.22239/2317-269x.01853

Influence of donor microbiota and of graft storage on corneal transplantation

A influência da microbiota de doadores e do armazenamento do enxerto no transplante de córnea

Catiusca Reali[I*]
Fernando Pagnussato[II]
Mercedes Passos Geimba[I]

[I] Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brasil

[II] Banco de Multitecidos, Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, RS, Brasil

* **E-mail:** catiuscar@gmail.com

Received: Dec 30, 2020
Approved: Jul 21, 2021

ABSTRACT

Introduction: Corneal transplantation it is the main treatment for people who have corneal curvature or transparency disorders. In Brazil, there is no unified protocol on the means of preservation, storage time and antibiotics used. The concern is that pathogens are transferred to transplant recipients, causing eye infections after transplantation. **Objective:** Examine ocular microbiota of corneal donors, to verify a possible correlation with infections in recipients and thus assist in improving corneal storage methodologies and protocols. **Method:** Literature review conducted in PubMed, SciELO and the following websites: CAPES Journals, Anvisa, Brazilian Ministry of Health and ABTO, between 2018 and 2020. **Results:** Studies based on microorganism's cultivation show coagulase negative *Staphylococcus* in 30% to 100% of samples isolated from conjunctiva. In lesser quantities are *Streptococcus*, *Corynebacterium* and *Propionibacterium*. Gram-negative bacteria appear in much lower numbers, represented by the genera *Haemophilus*, *Neisseria*, *Pseudomonas*, *Enterobacter*, *Escherichia*, *Proteus* and *Acinetobacter*. On the other hand, results based on independent cultivation techniques bring *Pseudomonas* as the main colonizer of the conjunctiva. Also, they have a much greater diversity of colonizers, showing a potential field of study. The ocular surface may have a much greater diversity of species and potential pathogens than was expected. The main means of preservation used in Brazil contain the antimicrobials gentamicin and streptomycin in their composition; however, several studies have shown that bacteria present in the means of preservation are resistant to these antibiotics. Conclusions: These data point to the need for a reassessment of the efficiency of these means of preservation in decontaminating corneas for transplantation.

KEYWORDS: Microbiota; Corneal Transplantation; Mean of Preservation; Antibiotic Resistance

RESUMO

Introdução: O transplante de córneas é o principal tratamento para pessoas que apresentam distúrbios de curvatura ou transparência da córnea. No Brasil, não há protocolo unificado para meios de preservação, tempo de armazenamento e antibióticos utilizados. A preocupação é a de que patógenos possam ser transferidos aos receptores de transplantes. **Objetivo:** Realizar o levantamento da microbiota ocular de doadores de córneas a fim de verificar uma possível correlação com infecções em receptores e, dessa forma, auxiliar na melhoria de metodologias e protocolos de armazenamento de córneas. **Método:** Foi conduzido a partir de revisão da literatura, nas bases de dados PubMed, SciELO e nos portais: periódicos da CAPES, Anvisa, Ministério da Saúde e ABTO, entre 2018 e 2020. **Resultados:** Estudos baseados em cultivo de microrganismos trazem *Staphylococcus* coagulase negativa (CONS) de 30% a 100% das amostras isoladas de conjuntivas. Em menor quantidade estão *Streptococcus*, *Corynebacterium* e *Propionibacterium*. Bactérias Gram-negativas aparecem em número inferior, representadas pelos gêneros *Haemophilus*, *Neisseria*, *Pseudomonas*, *Enterobacter*, *Escherichia*, *Proteus* e *Acinetobacter*. Já as técnicas independentes de cultivo trazem *Pseudomonas* como a principal colonizadora da conjuntiva. Também apresentam uma diversidade maior de colonizadores, mostrando um potencial campo de estudos, no qual a superfície ocular pode ter uma diversidade muito maior de espécies e potenciais agentes patogênicos. Os principais meios de preservação utilizados no Brasil levam os antimicrobianos gentamicina e estreptomicina em sua composição, porém estudos têm mostrado que as bactérias presentes nos meios de preservação são resistentes a esses antibióticos. Conclusões: Os dados apontam para a necessidade de reavaliação da eficiência desses meios de preservação na descontaminação das córneas para transplante.

PALAVRAS-CHAVE: Microbiota; Transplante de Córnea; Meio de Preservação; Resistência a Antimicrobianos

INTRODUCTION

The year 2020 presented the healthcare industry with new challenges that had direct impacts on organ availability and donation services. On March 11, 2020, the World Health Organization (WHO) declared the SARS-CoV-2 pandemic. Because of the lack of directions and strategies from the federal government to support states and municipalities, in April 2021 Brazil reached the sad mark of 400,000 deaths, according to data from the Brazilian Ministry of Health[1]. This tragedy also led to a decrease in organ donation and transplantation in the country. The number of cornea transplants in the first half of 2020 decreased by 44.3% compared to the same period of the previous year[2].

The number of donors is insufficient when compared to the number of patients waiting for transplantation. A survey carried out in 2019 estimated an annual need for 18,765 cornea transplants in Brazil, but no more than 14,943 transplants were actually performed[3]. The pandemic made these numbers even worse because of the decrease in the number of donors. According to a survey by the Brazilian Organ Transplantation Society (ABTO), there were more than 12,000 active patients on the waiting list for cornea transplants in the first six months of 2020[2]. The possibility of using corneas from donors who had COVID-19 is still being discussed. Be that as it may, since there is a large number of infected people, the viability of corneas stocked from donors who were not infected with COVID-19 becomes even more important. Lost or non-viable tissues are a major concern because they become unavailable for transplantation. Microbiological contamination is one of the main reasons for non-viable corneas in eye banks[4]. Unlike most drugs used in ophthalmology, which are expected to gain efficacy as we learn more about them, antimicrobials tend to lose efficacy due to the increased resistance of microorganisms[5]. Learning more about the ocular microbiome is therefore of the utmost importance to enable us to identify the protective relationships of the commensal microbiota itself[6], understand the pathophysiology of various ophthalmic diseases[7], and identify pathogens that may be responsible for post-transplantation infections.

As we understand the complex relationship between the microorganisms that make up the ocular microbiota, we can devise more specific prevention or treatment plans. To prevent infections, antibiotics are used together with the preservation media used in storage protocols. They are added to the media to decontaminate the corneas, but these solutions have not been shown to be 100% efficient. In Brazil, the main antimicrobials used in preservation media are gentamicin and streptomycin[8], but studies have shown that several bacterial species are resistant to these antibiotics[9,10].

To date, there is no protocol standardizing either the use of preservation media, more efficient antimicrobials, temperature or storage time for corneas. Studies comparing these methods are necessary. The improvement of the methodology may result in fewer losses, as well as prevent the transmission of microorganisms from the donor or from the corneal preservation media to the recipients, a problem that can cause severe endophthalmitis and even loss of the eyeball.

This review article carried out a survey on the ocular microbiota of cornea donors to try to establish a potential correlation with post-transplantation infections as thus support future studies on methodologies and protocols for cornea storage in Brazil.

METHOD

This work was done based on a review of the literature available in the following databases: PubMed, Scientific Electronic Library Online (SciELO), Portal of Journals of Brazil's Higher Education Improvement Coordination (CAPES), Portal of Brazil's Health Surveillance Agency (Anvisa), Brazil's Ministry of Health and the Brazilian Organ Transplantation Society (ABTO), for the period between 2018 and 2020. The first three portals mentioned above were used to search for local and international scientific articles using the following keywords: "cornea transplant", "corneal storage media", "corneal preservation", "eye banking", "ocular microbiome", "eye infections". To search for data related to COVID-19, since there was scarce literature at the time of the research, in addition to the previous portals, searches were conducted on Google Scholar using the keywords "SARS-CoV-2 corneal infection" and "COVID-19". Anvisa, Ministry of Health and ABTO portals were used to collect free data, published by the Brazilian government and ABTO. To select the articles, first we used criteria like interest in the topic and publication date, based on the titles. After reading the titles of the articles, we noticed some of them appeared in more than one database and others did not fulfill the criteria of this study. After this screening, the bibliography used in the study was evaluated. We assessed whether it was up to date, the design of the experiments and the relevance of the topic. Articles published in Portuguese, Spanish and English were considered. We then read the abstracts of the selected articles, and those that did not relate to the purpose of this study were excluded. Finally, 87 articles were used to inform this work, seven of which from PubMed, four from SciELO, 68 selected from searches in the CAPES Portal and eight from Google Scholar. We read these articles in full to garner the information contained in this work.

RESULTS AND DISCUSSION

Cornea transplants in Brazil

The cornea is the interface between the eye and the external environment. In addition to its protective function, it is the main refractive surface of the eye[11]. When its quality is impaired, several vision disorders can occur, including complete vision loss. Among the leading causes of blindness are cataract (51%), glaucoma (8%), age-related macular degeneration (5%), childhood blindness and corneal opacity (4%), uncorrected refractive errors and trachoma (3%), diabetic retinopathy (1%) and undetermined causes (21%)[12]. Research shows that corneal diseases are the main causes of reversible blindness in the world[12,13], and cornea transplantation is an important treatment to recover vision.

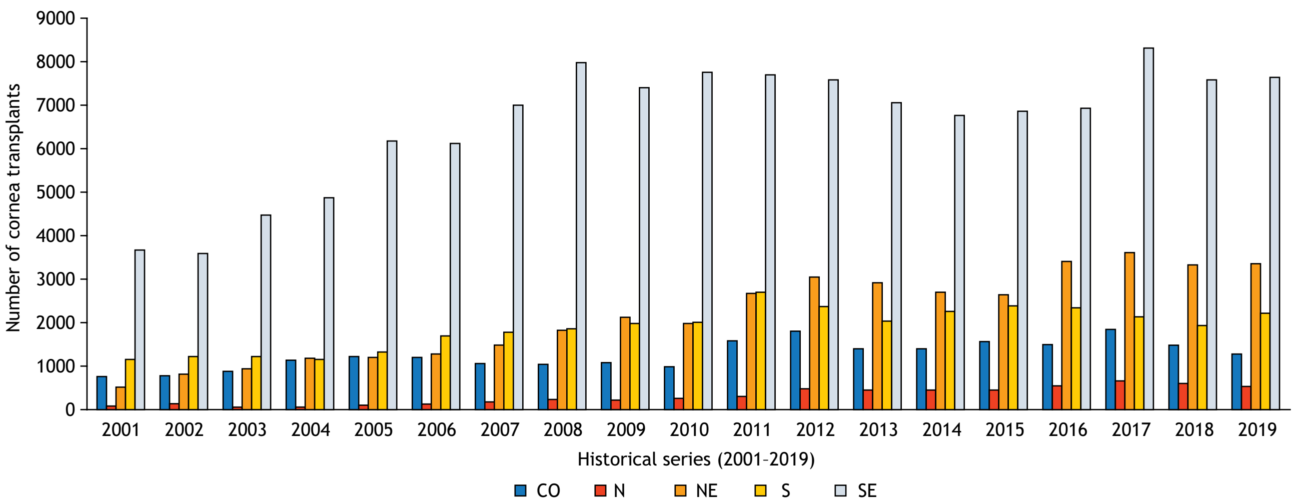
The Brazilian Unified Health System (SUS) is responsible for coordinating the system of transplants, which must be made available to the entire population of the country at no cost. In other words, there must be universal access to transplants and treatment. The National Transplantation Policy is regulated by Law n. 9.434, of February 4, 1997, and Law n. 10.211, of March 23, 2001. Transplants can be performed by public or private institutions, but the procedure must be free of charge, as provided by the law.

The Ministry of Health publishes data on the total number of transplants performed in the country. However, although the SUS coordinates the transplant system, it does not provide a centralized database for follow-up after the procedures. Therefore, estimating the number of successful cornea transplants can be particularly difficult.

A recent publication by ABTO revealed that 3,963 cornea transplants were performed from January to June 2020, just over half of the transplants performed in the same period in 2019, 7,112[2]. The complete historical series of ABTO[3], with data from 2012 to 2019, shows the Southeast region with the highest number of cornea transplants in 2019, 7,558, with highlights to the state of São Paulo (69.4 % of the transplants). In the same year, the South region performed 2,209 cornea transplants, of which 32% were done in the state of Rio Grande do Sul (Figure 1).

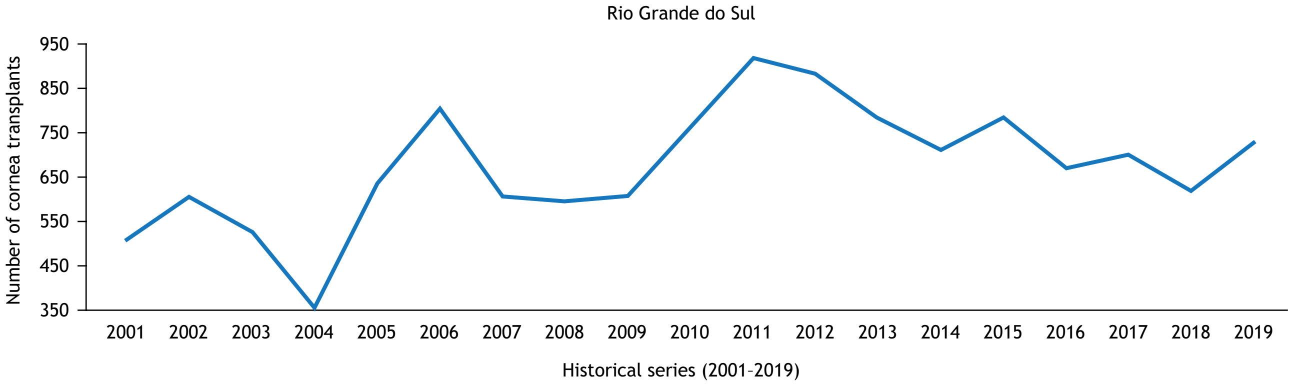
Rio Grande do Sul had the highest number of cornea transplants performed between 2011 and 2012: 918 and 882, respectively (Figure 2). Since then, it has not again reached the number of transplants performed in the mentioned years. In 2019, 727 transplants were performed in the state.

Although cornea transplants are the most common type of tissue transplantation in the world[14], the demand for donations remains unmet, especially because of the insufficient number of donors[15]. ABTO's numbers attract attention. In the first half of 2020, only 31.56% of all potential organ donors in Rio Grande do Sul actually became donors. In other words, out of 301 potential donors, only 110 became effective donors and, of these, 95 actually had their organs transplanted. There are 12,234 active patients on the waiting list for cornea donations, and the numbers vary greatly by state. In São Paulo, there are 2,984 patients on the waiting list, in Rio Grande do Sul, this number drops to 201. According to the Ministry of Health, one of the main reasons for the low number of effective donations is family refusal (43%), identified



Source: Prepared by the authors, 2020, based on data released by the Ministry of Health (2001 to 2017) and the Brazilian Organ Transplantation Society (2012 to 2019).

Figure 1. Historical series of the number of cornea transplants performed in Brazil between 2001 and 2019, in the following regions: Center-West (CO); North (N); Northeast (NE); South (S) and Southeast (SE).



Source: Prepared by the authors, 2020, based on data released by the Ministry of Health (2001 to 2017) and the Brazilian Organ Transplantation Society (2012 to 2019).

Figure 2. Historical series of the number of cornea transplants performed in Rio Grande do Sul, between 2001 and 2019.

in interviews with family members. However, in 31% of cases, family members are not even interviewed. The reasons for not interviewing them have not been disclosed by the Ministry of Health, nor have any data detailing the types of donation.

Because of the insufficient number of cornea donors and long waiting lists, eye banks play a fundamental role not only in harvesting but also in providing good storage and conservation conditions for the subsequent transplants[16]. Studies indicate that, even after consent for donation, a large number of corneas are discarded and transplantation does not occur. In a study carried out in the Brazilian state of Paraná between 2011 and 2015, 45.6% of all donated corneas were discarded for various reasons. In Brazil, this number reached 29.5%[17]. Some of the main reasons for disposal are positive serology and poor viability and quality of the cornea, and, to a lesser extent, contamination of the ocular tissue.

Human ocular surface microbiota

The normal microbiota is defined as the species of microorganisms found in the majority of individuals in a particular location[18]. The conjunctiva, as well as eyelid margins and tears, have a much smaller amount of microbial species when compared to other mucous membranes like the surface of the oral mucosa. Although in smaller numbers, the ocular surface is continuously exposed to the environment and to different species of microorganisms[19]. Any imbalance in the normal microbiota or the insertion of transitory species can result in the onset of diseases[20].

The ocular surface microbiota can be affected by several factors: environment, diseases, antibiotics[21,22], among others. The dry eye syndrome is frequently associated with inflammatory conditions of the ocular surface[23], since the lubrication enabled by tears, which contain antimicrobial compounds[24], limits the number of species of microorganisms in the ocular mucosa.

Studies based on culture-dependent methods have shown a predominance of Gram-positive bacteria in the human conjunctiva, especially coagulase-negative *Staphylococcus* (CoNS), isolated in 20% to 80% of conjunctiva swabs and in 30% to 100% of eyelid swabs; as well as the genera of *Streptococcus*, *Corynebacterium* and *Propionibacterium* (*P. acnes*)[18,19,22,25,26,27,28] and, to a lesser extent, *S. aureus* sp., *Bacillus* sp., *Enterococcus* sp., *Lactobacillus* sp.[19]. Also, albeit in smaller numbers, there can be Gram-negative bacteria like *Haemophilus* sp., *Neisseria* sp.[22], *Pseudomonas aeruginosa*, *Enterobacter* sp., *Escherichia coli*, *Proteus* sp. and *Acinetobacter* sp.[19,29,30,31,32], as well as some groups of fungi[19]. In the eyelids and/or tears, CoNS can be found more frequently, as well as *Propionibacterium* sp., *Corynebacterium* sp., *S. aureus*, *Micrococcus* sp. and *Streptococcus* sp. Again, in smaller numbers, there can be Gram-negative bacteria like *Moraxella* sp., *Pseudomonas* sp., *Neisseria* sp. and *Proteus* sp.[19].

In recent years, there has been an increase in the use of culture-independent identification methods, like polymerase chain reaction (PCR) and sequencing using the 16S rRNA gene. With this new approach, other genera are being identified as possible colonizers of the ocular conjunctiva[20]. Among the genera found by researchers via independent cultivation techniques and not mentioned above are: *Millisia, Anaerococcus, Finegoldia, Simonsiella, Veillonella*[20], *Bradyrhizobium, Brevundimonas, Aquabacterium, Sphingomonas, Streptophyta* and *Methylobacterium*[33].

In the same study, Dong et al.[28] presented 12 bacterial genera found in the conjunctival microbiome, ordered by frequency: *Pseudomonas* sp. (20%), *Propionibacterium* (20%), *Bradyrhizobium* (16%), *Corynebacteria* (15%), *Acinetobacter* (12%), *Brevundomonas* (5%), *Staphylococcus* (4%), *Aquabacterium* (2%), *Sphingomonas* (1%), and *Streptococcus* (1%). Other studies mention the *Pseudomonas* genus as the most abundant microorganisms in the conjunctiva, via independent cultivation techniques. Lee et al.[23] found *Pseudomonas*, *Elizabethkingia*, *Corynebacterium*, *Staphylococcus*, *Delftia*, *Propionibacterium* and *Streptococcus* accounting for 63.6% of the sequences. Again, *Pseudomonas* sp. was the most abundant sequence in the studies by Zhou et al.[34], followed by *Bradyrhizobium* sp. and *Acinetobacter* sp.

The frequency of species identified by culture-independent methods in these studies is surprising insofar as the *Pseudomonas* genus appears in a high frequency, but CoNS no longer does, as it did in results found by cultivation-dependent techniques. Gram-negative bacteria are the least abundant by isolation and identification of bacterial genera by culture-dependent methods. Different sites of the eyes have different microbiota. *Pseudomonas* sp. was more frequent on the eyelids and conjunctiva, but it is not as frequently found on the ocular surface[35].

The culture-dependent analysis methodology eventually favors the identification of microorganisms with faster growth and less fastidiousness[33,36,37]. The culture media, as well as the growth conditions, make a selection of species, which does not occur in independent cultivation methods. It is estimated that only 1% of microorganisms are cultivable by routine techniques[38]. In addition to the methodology, environmental conditions, seasonality and age group may be important factors to warrant the differences in the data[21,22,34]. In the studies we assessed, there was no statistical difference in the ocular microbiota between males and females, but there is a difference between age groups[28,33].

The introduction of culture-independent techniques and metagenomic analysis have shown an ocular microbiota that is more similar to that of skin, and not to that of other mucous membranes, like mouth and throat[39]. Studies infer that the ocular microbiome may have developed as a result of physical interactions between the skin at the edges of eyelids or fingers with the ocular surface[31,40], as well as particles of dust, water, pollen etc. It is important to determine which microorganisms are transient and possible causes of disturbance in the composition of the microbiome and which are commensals and contribute to the maintenance of this balance[20].

Microbiota related to eye infections

Although it is known that normal microbiota helps protect the eyes, some species found in the conjunctiva contribute to the onset of infectious and autoimmune diseases of the eyes like keratitis, conjunctivitis, endophthalmitis, and dry eye syndrome[19]. For this reason, knowing and distinguishing commensal microbiota from transient microbiota is important. Despite the increase in studies using more sensitive techniques, there is no consensus on how many phylotypes actually contribute to the ocular microbial balance and make up the normal microbiota, nor do we know the exact relationship between the addition or exclusion of species[20].

Up to 82% of postsurgical endophthalmitis in patients who reversed cataracts may be caused by the ocular microbiota[41]. Bacteria are the main causative agents of endophthalmitis and Gram-positive pathogens are responsible for 60% to 80% of acute infections. CoNS is the most commonly isolated microorganism[6,42,43] and, to a lesser extent, Gram-positive and *P. acnes*[43] can also be found. In studies that evaluated 55 cases of endophthalmitis after cornea transplants, 44 were of bacterial origin with proven culture, and the same microorganism was isolated from the recipient and the donor in 56.8% of cases[44]. These data underscore the importance of understanding and monitoring the distribution of microorganisms and ocular infections and their resistance to antimicrobial agents in order to better adapt pre, peri and postoperative treatments[6]. The increased incidence of fungal infections after cornea transplants also suggests the need to review material selection methods[45].

Gram-negative bacteria are very common on the ocular surface of people who wear contact lenses[46], and the *Pseudomonas* genus appears among the most abundant bacteria[47]. The same occurs on the surface microbiota of healthy individuals in analyses that used independent cultivation techniques. Other bacterial genera, including Gram-positive bacteria, are also reported to be found in the microbiome of contact lens wearers, namely: *Methylobacterium*, *Lactobacillus* and *Acinetobacter* and, to a lesser extent, *Haemophilus*, *Streptococcus*, *Staphylococcus* and *Corynebacterium* spp[47]. It is known that the risk of infections in contact lens wearers is increased by the lack of contact with the tear film and antimicrobial proteins present in it, but also by mechanical friction on the ocular surface, which is now known to be non-sterile—to the contrary, it is home to a large number of microorganisms.

In a study evaluating Gram or positive cultures in corneal tissue donors related to cases of post-transplant infection, of 46 positive results, 42 isolates were by bacteria, of which two by *Candida*, one by *Acanthamoeba* and one by nonspecific budding yeast. Among the bacterial species, 11 were caused by CoNS, eight by *P. acne* and, in smaller numbers, *Enterococcus faecalis*, *E. coli*, *Peptostreptococcus anaerobius*, *P. aeruginosa*, *Streptococcus viridans*. However, correlating the infection in the cornea recipient was only possible for *Candida*[48]. The results found by the authors corroborate other aforementioned studies in which there was a prevalence of these species in cultivation-dependent methods. However, the limitations implied by the methodology must be taken into account. The study has also shown ten positive cultures, with only Gram stain results. The genera or species were not identified, and correlating the agents with subsequent infections was not possible. Other case studies also reported transmission of *Candida* sp. from donor to host[45,49,50].

Farrel et al.[9] evaluated 446 corneal tissues, and 14.1% of the growths in culture were positive. The most abundant species were *Streptococcus* (41%), *Propionibacterium* (23%) and *Staphylococcus* (22%), most of which resistant to gentamicin[9]. Although the article did not relate the species found to endophthalmitis in recipients, the authors inferred that knowing the pathogens can inform the selection of antibiotics for use in surgery and also in the postoperative period, as well as the right preservation media in eye banks, since most of the species found were resistant to gentamicin.

The studies that relate the infectious agents found in donors to endophthalmitis in recipients were all conducted with techniques that use the cultivation of microorganisms. Most studies do not find a correlation between donor and recipient, which corroborates the data we already mentioned[51,52]. However, isolates from eye samples are not necessarily the causative agents of eye infections, since several bacteria can occur on the ocular surface simultaneously[53]. Cultivation-dependent techniques, although less accurate, are still the most widely used due to the low cost of the analysis and the ease of obtaining results, since more robust techniques depend on sequencing and bioinformatics analyses.

The presence of pathogens in a culture increases the probability of endophthalmitis by up to 1%, whereas for fungi it could reach 1.23%. The early identification of the infectious agent could enable early and more aggressive treatments, which could in turn prevent further damage or consequences, like the need for a new surgery[48]. We should also bear in mind that the most commonly used techniques could be underestimating the species found[7], therefore the infectious agents may not appear in the assessment of the contaminants. The difference between the results achieved by conventional culture and molecular biology tests could affect clinical results[53].

Despite the large presence of bacteria as pathogens, the high rate of infections by the new SARS-CoV-2 is also important in this discussion. The numbers for September 2020 stand out: there had been almost 30 million cases of COVID-19 and almost a million deaths worldwide[54]. Currently, corneas affected by SARS-CoV-2 are discarded due to their high transmissibility and potential presence in the conjunctiva, which may cause infection in the recipients[55]. Some studies indicate a low frequency of worrisome implications for corneal tissue donation, but some cases reported in China and Singapore have shown patients with ocular symptoms like conjunctivitis, especially in patients with severe systemic disease[56,57,58,59], and that could be detected in tears or conjunctival secretions. However, Ang et al.[60] and Desautels et al.[55] argued that the ocular symptoms may have been caused by the ventilators used in the treatment.

There is currently no evidence that SARS-CoV-2 can infect deeper corneal tissues in patients with COVID-19, but studies are still based on observations in animals and *in vitro*[61]. Moreover, they cannot yet infer that there is a considerable viral load in the corneal stroma and endothelium, which could cause significant risks to recipients[62,63]. Although Xia et al.[59] suggested that SARS-CoV-2 could be transmitted through the eyes, the study was based on a very small number of observations. A recent study published in August 2020 assessed five corneal donors for viral expression. Corneal and conjunctival epithelia express ACE2, DC-SIGN/DC-SIGNR and TMPRSS2, which suggests that the ocular surface is a potential route for SARS-CoV-2 transmission and that the risk of viral transmission with cornea transplantation cannot be ruled out due to the presence of ACE2 in the corneal epithelium and endothelium[64]. *A priori*, the suspicion was that the virus settled superficially, so local sterilization with povidone-iodine solution after excision from the donor[65,66] would be possible. However, recent studies have shown contrary results. There is a clear need for further studies to infer whether or not COVID-19 positive donor corneas can be used.

Corneal selection and preservation media

In Brazil, Resolution n. 67, of September 30, 2008, from Anvisa, regulates contraindications in the use of donated corneas, such as death from sepsis, HIV seropositive, acute viral hepatitis, positive diagnosis for rabies and other diseases associated with microorganisms, so as to prevent the transmission of pathogens to recipients. Even in donors whose cause of death or history is not contraindicated for corneal donation, the presence of microorganisms in enucleated eyes is inevitable. Therefore, assessing the material is very important so that the microorganisms are not taken from the graft into the receiving eye. For contamination control purposes, the enucleated material is immersed in eye drops and, afterward, the material is stored in solutions containing antibiotics. Studies that analyzed the conjunctiva of donors have shown a contamination rate of 40% to 100% before any treatment and preservation of the cornea[25]. Nevertheless, even after this treatment, the corneas did not become sterile and could still carry some type of microorganism.

In Brazil, the storage temperature, as well as the time, are determined by Anvisa in Resolution n. 67/2008, which indicates storage in refrigerators with temperature control from 2ºC to 8ºC. This methodology is the most widely adopted in the world and the only used in North America[67]. It is relatively efficient and easy to perform in eye banks. Most European eye banks use corneal storage temperatures from 31ºC to 37ºC. This temperature could offer advantages when compared to storage at 4ºC, since microorganisms are metabolically more active at higher temperatures. It could increase the efficiency of antimicrobials and also the storage time due to the viability of endothelial cells[4,67].

The storage time of the organs determines the choice of the methodology. For longer periods, up to 48 days, the recommended temperature is 31ºC to 37ºC; for shorter periods, up to 14 days, the recommended temperature is 4ºC[68]. In most parts of the world, shorter storage periods are more frequent since there is a constant need for donations, that is, the demand is greater than the amount of corneas available for donation. This would explain the frequent use of storage temperatures at 4ºC. The interesting thing about longer periods of storage is the possibility of conducting further diagnostic tests for pathogens, possible causes of infections, before performing the transplant[69]. However, one of the problems of storing corneas for longer periods could be potential changes in the composition of the storage medium[70].

A study carried out in New Zealand comparing corneal preservation media has shown Optisol-GS with an average ideal storage time of 3.5 days[71], contrary to information from manufacturers, for whom the recommended use time is up to 14 days. The medium-term preservation media used in Brazil are Optisol-GS

(Bausch & Lomb, USA) and Eusol-C (Al.Chi.Mia, Italy), the corneal endothelial preservation time, for both, is of 14 days, and 64% of eye banks in the country use preservation solutions with gentamicin and streptomycin[8]. In Europe, the antimicrobials used together with storage solutions are more frequently penicillin, streptomycin and amphotericin B[67].

The use of streptomycin together with gentamicin to increase the antimicrobial spectrum against contaminating bacteria and reduce the possibility of endophthalmitis was introduced in Optisol GS. In Brazil, a problem with using Optisol GS or Optisol G—which has only gentamicin in its formula—would be the storage temperature adopted in the country, which is 4ºC. Gentamicin and streptomycin have a decrease in their activity at 4ºC, when compared to 37ºC. Additionally, corneas would have to be left in stock solution at room temperature for three hours before refrigeration[43]. Also, before performing the surgical procedure, the cornea would have to sit at room temperature for about one hour[72]. Nonetheless, there are reports that, after the introduction of Optisol GS there has been a 77% decrease in endophthalmitis caused by bacteria compared to fungi, and a 3.4-fold increase in endophthalmitis caused by fungi when the cornea was stored for more than four days[73].

Gentamicin is a widely used antibiotic for corneal preservation media all over the world[10], but a study from 1991 showed that gentamicin-resistant bacteria were already found in stored corneas, although they were not always related to post-transplant infections[9]. Even though the proven number of postoperative infections is low, Eastlund[10] suggested that these data are understudied, since patients with postoperative infections do not always return to the hospital or to the professional who performed the transplantation. Another challenge is to isolate the microorganism found in the donor's cornea to compare it with the microorganism that caused the post-transplantation infection. This follow-up is done in only a few cases, so these data could also be underestimated. The contraindications for the use of corneas from patients with death from sepsis, mechanical ventilation or other illness, listed by Resolution n. 67/2008, intend precisely to prevent the transmission of pathogens with the tissue, as has already been proven by various case studies[73,74,75,76,77,78].

The *Streptococcus, Propionibacterium* and *Staphylococcus* genera, as well as diphtheroids that are resistant to gentamicin, are commonly found in preservation media[10]. In a study on the culture of corneas in preservation media with gentamicin and streptomycin, growth of microorganisms was reported in 72.5% of the samples[25], which shows the inefficiency of the preservation media in terms of corneal decontamination. The same study showed that, of 76 isolates, 81.6% were Gram-positive bacteria, in the following order of frequency: CoNS in 44.8% of cases, *Corynebacterium* sp. in 19.7%, *S. aureus* in 15.8%, and *Bacillus* sp. in 1.3%. We should keep in mind that CoNS is the most commonly isolated microorganism in endophthalmitis[71,79]. Another study on gentamicin-only preservation media found that 81% of the isolates were *Streptococcus*, 60% were *Propionibacterium* and 71% were *Staphylococcus* resistant to this antibiotic. The same study showed that all isolates were sensitive to vancomycin[9]. Similarly, a case study reported by Khokhar et al.[80] indicated that the vancomycin-resistant species of *Alcaligenes faecalis* was responsible for infection in the transplanted cornea[80].

A study carried out at Hospital de Clínicas de Porto Alegre, Brazil, between 2001 and 2003, analyzed positivity in corneal-scleral donor halos preserved in Optisol GS. Of 63 halos, 11 showed positive cultures and, of these, four were by *Staphylococcus epidermidis*, one by *S. aureus*, one by *Serratia* sp. and one by *P. aeruginosa*, all resistant to gentamicin[52]. Baer et al.[81], as early as 1988, found *S. viridans* resistant to this antibiotic and reported three cases of endophthalmitis caused by this pathogen[81]. The same occurred in a study published by Fong et al.[82], in which staphylococci, streptococci and fungi were not eliminated. For Broniek et al.[83], gentamicin was not effective in inhibiting bacterial replication during corneal storage, in a study performed with bacterial culture in corneas stored in Eusol-C medium.

The storage time of the corneas proves to be a determining factor in increasing the risk of contamination[73]. According to some studies, the risk of contamination increases after five days of storage[84,85], although preservation media like Optisol-GS keep corneas with viable endothelial cells for up to 14 days at 4ºC[86]. The recommendation of the Eye Bank Association of America (EBAA) is that the enucleation of the eyeball be performed preferably within the first six hours after death. The ideal recommendation for reimplantation is that it be performed within four days after enucleation. Studies comparing different combinations of antibiotics and antifungals are necessary if we want more effectiveness in the decontamination of corneas for donation. Cases of antimicrobial-resistant bacteria are becoming increasingly common, including in communities where corneas for donation are usually harvested. The increase in antimicrobial resistance is a challenge, not only in the treatment of diseases caused by microorganisms, but also in the decontamination of organs for donation.

CONCLUSIONS

Studies based on culture-independent techniques show that there is a much greater diversity of bacteria colonizing the eyeball than previously thought. The most commonly found bacteria are of the *Pseudomonas* genus and not CoNS, as suggested by the results of studies based on growth in culture media. The antibiotics of culture media used in Brazil—gentamicin and streptomycin—are controversial, since many bacterial species isolated from preservation media and post-transplant infections are resistant to gentamicin and some are resistant to both gentamicin and streptomycin. There is a need to review the antimicrobials used in preservation media, either considering their efficiency or the target groups to be addressed, since studies indicate the presence of a large amount of Gram-negative bacteria in the ocular biome, which can lead to the development of a unified protocol for use in Brazil.

REFERENCES

1. Ministério da Saúde (BR). Painel coronavírus Brasil. Brasília: Ministério da Saúde; 2020[acesso 12 set 2020]. Disponível em: https://covid.saude.gov.br/
2. Associação Brasileira de Transplante de Órgãos – ABTO. Registro brasileiro de transplante: dados numéricos da doação de órgãos e transplantes realizados por estado e instituição no período: janeiro/junho 2020. São Paulo: Associação Brasileira de Transplante de Órgãos; 2020[acesso 12 set 2020]. Disponível em: https://site.abto.org.br/wp-content/uploads/2020/08/rbt-1sem-final-leitura.pdf
3. Associação Brasileira de Transplante de Órgãos – ABTO. Registro brasileiro de transplante: dimensionamento dos transplantes no Brasil e em cada estado (2012-2019). São Paulo: Associação Brasileira de Transplante de Órgãos; 2019[acesso 12 set 2020]. Disponível em: http://www.abto.org.br/abtov03/Upload/file/RBT/2019/RBT-2019-leitura.pdf
4. Gruenert AK, Rosenbaum K, Geerling G, Fuchsluger TA. The influence of donor factors on corneal organ culture contamination. Acta Ophthalmol. 2017;95(7):733-40. http://doi.org/10.1111/aos.13402
5. Ta CN, Chang RT, Singh K, Egbert PR, Shriver EM, Blumenkranz MS et al. Antibiotic resistance patterns of ocular bacterial flora. Ophthalmology. 2003;110(10):1946-51. http://doi.org/10.1016/s0161-6420(03)00735-8
6. Grzybowski A, Brona P, Kim SJ. Microbial flora and resistance in ophthalmology: a review. Graefes Arch Clin Exp Ophthalmol. 2017;255(5):851-62. https://doi.org/10.1007/s00417-017-3608-y
7. Petrillo F, Pignataro D, Lavano MA, Santella B, Folliero V, Zannella C et al. Current evidence on the ocular surface microbiota and related diseases. Microorganisms. 2020;8(7):1-13. https://doi.org/10.3390/microorganisms8071033
8. Pereira MLM, Santos AMC, Passos MC. Análise comparativa entre os bancos de olhos brasileiros: da preservação à distribuição da córnea doada. Rev Bras Oftalmol. 2002;61(3):169-72.
9. Farrell PL, Fan JT, Smith RE, Trousdale MD. Donor cornea bacterial contamination. Cornea. 1991;10(5):381-6. https://doi.org/10.1097/00003226-199109000-00004
10. Eastlund T. Bacterial infection transmitted by human tissue allograft transplantation. Cell Tissue Bank. 2006;7(3):147-66. https://doi.org/10.1007/s10561-006-0003-z
11. Niederkorn JY. Cornea: window to ocular immunology. Curr Immunol Rev. 2011;7(3):328-35. https://doi.org/10.2174/157339511796196593
12. Pascolini D, Mariotti SP. Global estimates of visual impairment: 2010. Br J Ophthalmol. 2012;96(5):614-8. https://doi.org/10.1136/bjophthalmol-2011-300539
13. Cruz GKP, Azevedo IC, Carvalho DPSRP, Vitor AF, Santos VEP, Ferreira Júnior MA. Clinical and epidemiological aspects of cornea transplant patients of a reference hospital. Rev Latino-Am Enfermagem. 2017;25:1-9. https://doi.org/10.1590/1518-8345.1537.2897
14. Robaei D, Watson S. Corneal blindness: a global problem. Clin Experiment Ophthalmol. 2014;42(3):213-4. https://doi.org/10.1111/ceo.12330
15. Gain P, Jullienne R, He Z, Aldossary M, Acquart S, Cognasse F et al. Global survey of corneal transplantation and eye banking. JAMA Ophthalmol. 2016;134(2):167-73. https://doi.org/10.1001/jamaophthalmol.2015.4776
16. Santos CG, Pacini KM, Adán CBD, Sato EH. Motivos do descarte de córneas captadas pelo banco de olhos do Hospital São Paulo em dois anos. Rev Bras Oftalmol. 2010;69(1):18-22. https://doi.org/10.1590/S0034-72802010000100004
17. Freitas RA, Dell'Agnolo CM, Melo WA, Andrade L, Pimentel RR, Pelloso SM et al. Do donated corneas become transplanted corneas? The causes of discard in southern Brazil. Cornea. 2019;38(4):419-25. https://doi.org/10.1097/ICO.0000000000001856
18. Kugadas A, Gadjeva M. Impact of microbiome on ocular health. Ocul Surf. 2016;14(3):3420-9. https://doi.org/10.1016/j.jtos.2016.04.004
19. Willcox MDP. Characterization of the normal microbiota of the ocular surface. Exp Eye Res. 2013;117:99-105. https://doi.org/10.1016/j.exer.2013.06.003
20. Huang Y, Yang B, Li W. Defining the normal core microbiome of conjunctival microbial communities. Clin Microbiol Infect. 2016;22(7):7-12. https://doi.org/10.1016/j.cmi.2016.04.008
21. Zegans ME, Van Gelder RN. Considerations in understanding the ocular surface microbiome. Am J Ophthalmol. 2014;158(3):420-2. https://doi.org/10.1016/j.ajo.2014.06.014
22. Miller D, Iovieno A. The role of microbial flora on the ocular surface. Curr Opin Allergy Clin Immunol. 2009;9(5):466-70. doi:10.1097/ACI.0b013e3283303e1b
23. Lee SH, Oh DH, Jung JY, Kim JC, Jeon CO. Comparative ocular microbial communities in humans with and without blepharitis. Invest Ophthalmol Vis Sci. 2012;53(9):5585-93. https://doi.org/10.1167/iovs.12-9922
24. McDermott AM. Antimicrobial compounds in tears. Exp Eye Res. 2013;117:53-61. https://doi.org/10.1016/j.exer.2013.07.014
25. Araújo MEXS, Scarpi MJ. Microbiota bacteriana da conjuntiva de doadores de córnea. Arq Bras Oftalmol. 2004;67(6):927-33. https://doi.org/10.1590/S0004-27492004000600016
26. Hamelin J, Fromin N, Tarnawski S, Teyssier-cuvelle S, Aragno M. nifH gene diversity in the bacterial community associated with the rhizosphere of *Molinia coerulea*, an oligonitrophilic perennial grass. Environ Microbiol. 2002;4(8):477-81. https://doi.org/10.1046/j.1462-2920.2002.00319.x
27. Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S. Agricultural sustainability and intensive production practices. Nature. 2002;418(6898):671-7. https://doi.org/10.1038/nature01014

28. Doan T, Akileswaran L, Andersen D, Johnson B, Ko N, Shrestha A et al. Paucibacterial microbiome and resident DNA virome of the healthy conjunctiva. Invest Ophthalmol Vis Sci. 2016;57(13):5116-26. https://doi.org/10.1167/iovs.16-19803
29. Campos MS, Silva LQC, Rehder JR, Lee MB, O'Brien T, McDonnell PJ. Anaerobic flora of the conjunctival sac in patients with AIDS and with anophthalmia compared with normal eyes. Acta Ophthalmol (Copenh). 2009;72(2):241-5. https://doi.org/10.1111/j.1755-3768.1994.tb05023.x
30. Elander TR, Goldberg MA, Salinger CL, Tan JR, Levy B, Abbott RL. Microbial changes in the ocular environment with contact lens wear. CLAO J. 1992;18(1):53-5.
31. Graham JE, Moore JE, Jiru X, Moore JE, Goodall EA, Dooley JSG et al. Ocular pathogen or commensal: a PCR-based study of surface bacterial flora in normal and dry eyes. Investig Opthalmology Vis Sci. 2007;48(12):5616-23. https://doi.org/10.1167/iovs.07-0588
32. Hsu HY, Lind JT, Tseng L, Miller D. Ocular flora and their antibiotic resistance patterns in the midwest: a prospective study of patients undergoing cataract surgery. Am J Ophthalmol. 2013;155(1):36-44. https://doi.org/10.1016/j.ajo.2012.06.024
33. Dong Q, Brulc JM, Iovieno A, Bates B, Garoutte A, Miller D et al. Diversity of bacteria at healthy human conjunctiva. Investig Opthalmology Vis Sci. 2011;52(8):5408-13. https://doi.org/10.1167/iovs.10-6939
34. Zhou Y, Holland MJ, Makalo P, Joof H, Roberts CH, Mabey DCW et al. The conjunctival microbiome in health and trachomatous disease: a case control study. Genome Med. 2014;6(11):1-10. https://doi.org/10.1186/s13073-014-0099-x
35. Ozkan J, Willcox M, Wemheuer B, Wilcsek G, Coroneo M, Thomas T. Biogeography of the human ocular microbiota. Ocul Surf. 2019;17(1):111-8. https://doi.org/10.1016/j.jtos.2018.11.005
36. Woo PCY, Lau SKP, Teng JLL, Tse H, Yuen KY. Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. Clin Microbiol Infect. 2008;14(10):908-34. https://doi.org/10.1111/j.1469-0691.2008.02070.x
37. Burns DG, Camakaris HM, Janssen PH, Dyall-Smith ML. Combined use of cultivation-dependent and cultivation-independent methods indicates that members of most haloarchaeal groups in an Australian crystallizer pond are cultivable. Appl Environ Microbiol. 2004;70(9):5258-65. https://doi.org/10.1111/j.1469-0691.2008.02070.x
38. Amann RI, Ludwig W, Schleifer KH. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. Microbiol Rev. 1995;59(1):143-69. https://doi.org/10.1128/mr.59.1.143-169.1995
39. Grice EA, Kong HH, Renaud G, Young AC, Bouffard GG, Blakesley RW et al. A diversity profile of the human skin microbiota. Genome Res. 2008;18(7):1043-50. https://doi.org/10.1101/gr.075549.107
40. Berry M, Harris A, Lumb R, Powell K. Commensal ocular bacteria degrade mucins. Br J Ophthalmol. 2002;86(12):1412-6. https://doi.org/10.1136/bjo.86.12.1412
41. Speaker MG, Milch FA, Shah MK, Eisner W, Kreiswirth BN. Role of external bacterial flora in the pathogenesis of acute postoperative endophthalmitis. Ophthalmology. 1991;98(5):639-50. https://doi.org/10.1016/s0161-6420(91)32239-5
42. Gray ND, Miskin IP, Kornilova O, Curtis TP, Head IM. Occurrence and activity of archaea in aerated activated sludge wastewater treatment plants. Environ Microbiol. 2002;4(3):158-68. https://doi.org/10.1186/1471-2180-12-140
43. Durand ML. Endophthalmitis. Clin Microbiol Infect. 2013;19(3):227-34. https://doi.org/10.1111/1469-0691.12118
44. Kloess PM, Stulting RD, Waring GO, Wilson LA. Bacterial and fungal endophthalmitis after penetrating keratoplasty. Am J Ophthalmol. 1993;115(3):309-16. https://doi.org/10.1016/s0002-9394(14)73580-9
45. Tsui E, Fogel E, Hansen K, Talbot EA, Tammer R, Fogel J et al. *Candida* interface infections after descemet stripping automated endothelial keratoplasty. Cornea. 2016;35(4):456-64. https://doi.org/10.1097/ico.0000000000000778
46. Boost M, Cho P, Wang Z. Disturbing the balance: effect of contact lens use on the ocular proteome and microbiome. Clin Exp Optom. 2017;100(5):459-72. https://doi.org/10.1111/cxo.12582
47. Zhang H, Zhao F, Hutchinson DS, Sun W, Ajami NJ, Lai S et al. Conjunctival microbiome changes associated with soft contact lens and orthokeratology lens wearing. Investig Opthalmology Vis Sci. 2017;58(1):128-36. https://doi.org/10.1167/iovs.16-20231
48. Garg S, Said B, Farid M, Steinert RF. Prevalence of positive microbiology results from donor cornea tissue in different methods of corneal transplantation. Cornea. 2013;32(2):137-40. https://doi.org/10.1097/ICO.0b013e3182542368
49. Villarrubia A, Cano-Ortiz A. *Candida* keratitis after descemet stripping with automated endothelial keratoplasty. Eur J Ophthalmol. 2014;24(6):964-7. https://doi.org/10.5301/ejo.5000499
50. Koenig SB, Wirostko WJ, Fish RI, Covert DJ. *Candida* keratitis after descemet stripping and automated endothelial keratoplasty. Cornea. 2009;28(4):471-3. https://doi.org/10.1097/ICO.0b013e31818ad9bc
51. Gomes JAP, Dana MR, Dua HS, Goren MB, Laibson PR, Cohen EJ. Positive donor rim culture in penetrating keratoplasty. Cornea. 1995;14(5):457-62.
52. Borowsky CM, Wallau AD, Reetz A, Kwitko S, Rymer S, Locatelli CI. Contaminação de halos doadores córneo-esclerais em ceratoplastia penetrante no Hospital de Clínicas de Porto Alegre. Arq Bras Oftalmol. 2008;71(1):13-7. https://doi.org/10.1590/S0004-27492008000100003

53. Eguchi H, Hotta F, Kuwahara T, Imaohji H, Miyazaki C, Hirose M et al. Diagnostic approach to ocular infections using various techniques from conventional culture to next-generation sequencing analysis. Cornea. 2017;36(Supl.1):S46-S52. https://doi.org/10.1097/ICO.0000000000001338
54. World Helth Organization – WHO. WHO coronavirus disease (COVID-19) dashboard. Geneva: World Helth Organization; 2020[acesso 12 set 2020]. Disponível em: https://covid19.who.int/
55. Desautels JD, Moshirfar M, Martheswaran T, Shmunes KM, Ronquillo YC. Risks posed to corneal transplant recipients by COVID-19-affected donors. Ophthalmol Ther. 2020;9(3):371-9. https://doi.org/10.1007/s40123-020-00254-w
56. Wu P, Duan F, Luo C, Liu Q, Qu X, Liang L et al. Characteristics of ocular findings of patients with coronavirus disease 2019 (COVID-19) in Hubei Province, China. JAMA Ophthalmol. 2020;138(5):575-8. https://doi.org/10.1001/jamaophthalmol.2020.1291
57. Guan W, Ni Z, Hu Y, Liang W, Ou C, He J et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. 2020;382(18):1708-20. https://doi.org/10.1056/NEJMoa2002032
58. Seah IYJ, Anderson DE, Kang AEZ, Wang L, Rao P, Young BE et al. Assessing viral shedding and infectivity of tears in coronavirus disease 2019 (COVID-19) patients. Ophthalmology. 2020;127(7):977-9. https://doi.org/10.1016/j.ophtha.2020.03.026
59. Xia J, Tong J, Liu M, Shen Y, Guo D. Evaluation of coronavirus in tears and conjunctival secretions of patients with SARS-CoV-2 infection. J Med Virol. 2020;92(6):589-94. https://doi.org/10.1002/jmv.25725
60. Ang M, Moriyama A, Colby K, Sutton G, Liang L, Sharma N et al. Corneal transplantation in the aftermath of the COVID-19 pandemic: an international perspective. Br J Ophthalmol. 2020;104(11):1477-81. https://doi.org/10.1136/bjophthalmol-2020-317013
61. Belser JA, Rota PA, Tumpey TM. Ocular tropism of respiratory viruses. Microbiol Mol Biol Rev. 2013;77(1):144-56. https://doi.org/10.1128/MMBR.00058-12
62. Bayyoud T, Iftner A, Iftner T, Bartz-Schmidt KU, Rohrbach JM, Ueffing M et al. Absence of severe acute respiratory syndrome-coronavirus-2 RNA in human corneal tissues. Cornea. 2020;40(3):342-7. https://doi.org/10.1097/ICO.0000000000002479
63. Chaurasia S, Sharma N, Das S. COVID-19 and eye banking. Indian J Ophthalmol. 2020;68(6):1215-6. https://doi.org/10.4103/ijo.IJO_1033_20
64. Roehrich H, Yuan C, Hou JH. Immunohistochemical study of SARS-CoV-2 viral entry factors in the cornea and ocular surface. Cornea. 2020;39(12):1556-62. https://doi.org/10.1097/ICO.0000000000002509
65. Kampf G, Todt D, Pfaender S, Steinmann E. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. J Hosp Infect. 2020;104(3):246-51. https://doi.org/10.1016/j.jhin.2020.01.022
66. Eye Bank Association of America – EBAA. Procedures manual. Washington: Eye Bank Association of America; 2019[acesso 12 set 2020]. Disponível em: https://restoresight.org/wp-content/uploads/2019/11/EBAA-ProcMan-October-2019.pdf
67. Armitage WJ. Preservation of human cornea. Transfus Med Hemotherapy. 2011;38(2):143-7. https://doi.org/10.1159/000326632
68. Jeng BH. Preserving the cornea: corneal storage media. Curr Opin Ophthalmol. 2006;17(4):332-7. https://doi.org/10.1097/01.icu.0000233950.63853.88
69. Frueh BE, Böhnke M. Prospective, randomized clinical evaluation of optisol vs organ culture corneal storage media. Arch Ophthalmol. 2000;118(6):757-60. https://doi.org/10.1001/archopht.118.6.757
70. Engelmann K, Ventura AS, Drexler D, Staude HJ. A sensitive method for testing the quality of organ culture media and of individual medium components in a cornea bank. Graefes Arch Clin Exp Ophthalmol. 1998;236(4):312-9. https://doi.org/10.1007/s004170050084
71. Patel HY, Brookes NH, Moffatt L, Sherwin T, Ormonde S, Clover GM et al. The New Zealand national eye bank study 1991-2003. Cornea. 2005;24(5):576-82. https://doi.org/10.1097/01.ico.0000155035.55751.b2
72. Lass JH, Gordon JF, Sugar A, Norden RA, Reinhart WJ, Meyer RF et al. Optisol containing streptomycin. Am J Ophthalmol. 1993;116(4):503-4. https://doi.org/10.1016/S0002-9394(14)71413-8
73. Hassan SS, Wilhelmus KR. Eye-banking risk factors for fungal endophthalmitis compared with bacterial endophthalmitis after corneal transplantation. Am J Ophthalmol. 2005;139(4):685-90. https://doi.org/10.1016/j.ajo.2004.12.016
74. Gandhi SS, Lamberts DW, Perry HD. Donor to host transmission of disease via corneal transplantation. Surv Ophthalmol. 1981;25(5):306-10. https://doi.org/10.1016/0039-6257(81)90156-9
75. Leveille AS, McMullan FD, Cavanagh HD. Endophthalmitis following penetrating keratoplasty. Ophthalmology. 1983;90(1):38-9. https://doi.org/10.1016/S0161-6420(83)34601-7
76. Seedor JA, Stulting RD, Epstein RJ, Nay RE, Dreizen NG, Waring 3rd GO et al. Survival of corneal grafts from donors supported by mechanical ventilation. Ophthalmology. 1987;94(2):101-8. https://doi.org/10.1016/S0161-6420(87)33490-6
77. Spelsberg H, Reinhard T, Sengler U, Daeubener W, Sundmacher R. Organ-cultured corneal grafts from septic donors: a retrospective study. Eye (Lond). 2002;16(5):622-7. https://doi.org/10.1038/sj.eye.6700145
78. Robert PY, Camezind P, Drouet M, Ploy MC, Adenis JP. Internal and external contamination of donor corneas before in situ excision: bacterial risk factors in 93 donors. Graefes Arch Clin Exp Ophthalmol. 2002;240(4):265-70. https://doi.org/10.1007/s004170100322

79. Ciulla TA, Starr MB, Masket S. Bacterial endophthalmitis prophylaxis for cataract surgery. Ophthalmology. 2002;109(1):13-24. https://doi.org/10.1016/S0161-6420(01)00899-5

80. Khokhar DS, Sethi HS, Kumar H, Sudan R, Sharma N, Nayak N. Postkeratoplasty endophthalmitis by *Alcaligenes faecalis*: a case report. Cornea. 2002;21(2):232-3. https://doi.org/10.1097/00003226-200203000-00024

81. Baer JC, Verinder S, Nirankari MD, Dean S, Glaros MD. Streptococcal endophthalmitis from contaminated donor corneas after keratoplasty. Arch Ophthalmol. 1988;106(4):517-20. https://doi.org/10.1001/archopht.1988.01060130563037

82. Fong LP, Gladstone D, Casey TA. Corneo-scleral rim cultures: donor contamination a case of fungal endophthalmitis transmitted by K-Sol stored cornea. Eye (Lond). 1988;2(Pt.6):670-6. https://doi.org/10.1038/eye.1988.123

83. Broniek G, Langwińska-Wośko E, Sybilska M, Szaflik J, Szaflik JP, Wróblewska M. Prevalence of bacteria and fungi in samples of cornea preservation fluid. Arch Med Sci. 2018;14(3):541-6. https://doi.org/10.5114/aoms.2016.58927

84. Pels E, Vrensen GF. Microbial decontamination of human donor eyes with povidone-iodine: penetration, toxicity, and effectiveness. Br J Ophthalmol. 1999;83(9):1019-26. https://doi.org/10.1136/bjo.83.9.1019

85. Antonios SR, Cameron JA, Badr IA, Habash NR, Cotter JB. Contamination of donor cornea: postpenetrating keratoplasty endophthalmitis. Cornea. 1991;10(3):217-20. https://doi.org/10.1097/00003226-199105000-00006

86. Kanavi MR, Javadi MA, Chamani T, Fahim P, Javadi F. Comparing quantitative and qualitative indices of the donated corneas maintained in Optisol-GS with those kept in Eusol-C. Cell Tissue Bank. 2015;16(2):243-7. https://doi.org/10.1007/s10561-014-9466-5

Authors' Contribution

Reali C – Conception, planning (study design), data acquisition, analysis, and interpretation, writing of the manuscript. Pagnussato F, Geimba MP – Conception, planning (study design), data acquisition, analysis and interpretation. All authors approved the final draft of the manuscript.

Disclosures

The authors report that there is no potential conflict of interest with peers and institutions, nor political or financial conflicts in this study.

"Attribution-NonCommercial: CC BY-NC" License. With this license you may access, download, copy, print, share, reuse and distribute the articles, provided that for non-commercial use and with the citation of the source, conferring the proper credits of authorship and mention to Visa em Debate. In such cases, no permission is required by the authors or publishers.