Bacterial Resistance and Antisseptic Effects on the Eye Surface of Donor Corneas

Célia Ito
Universidade Federal de Goias
Carla Silva
Universidade Federal de Goias
Lilian Carla Carneiro (carlacarneirolilian@gmail.com)
Universidade Federal de Goias https://orcid.org/0000-0003-4067-1506
Mônica Barbosa
Universidade Federal de Goias
José Neto
Universidade Federal de Goias
Aline Messias
Universidade Federal de Goias
Ana Feitosa
Universidade Federal de Goias
Adailton Santos
Universidade Federal de Goias
Pedro Queiróz
Universidade Federal de Goias
janaina Monteiro
Universidade Federal de Goias
Andressa Santos
Universidade Federal de Goias
Marcos Avila
Universidade Federal de Goias

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BACTERIAL RESISTANCE AND ANTISSEPTIC EFFECTS ON THE EYE SURFACE OF DONOR CORNEAS

BACTERIAL RESISTANCE AND ANTISSEPTIC EFFECTS IN DONOR CORNEAS

Célia Regina Malveste Ito[1], Carla Afonso da Silva[2], Lilian Carla Carneiro[2], Mônica Santiago Barbosa[2], José Beniz Neto[1], Aline Cristine Magalhães Costa Messias[2], Ana Paula Pinto Feitosa[1], Adailton Pereira dos Santos[2], Pedro Henrique Pereira de Queiróz[2], Janaina Cotrim Monteiro[1], Andressa Liberal dos Santos[2], Marcos Pereira de Ávila[1].

1- Ophthalmology Reference Center, First Avenue, 355-447 number, Leste Universitário neighborhood, Goiânia city, Goiás State, Brazil. Zipcode: 74605-020.

2-Federal University of Goiás, 235 Street, without number, Universitário neighborhood, Goânia city, Goiás State, Brazil. Zipcode: 74605-050.

Correspondence author: Lilian Carla Carneiro, e-mail: carlacarneirolilian@gmail.com, telephone: 55 62 981837605. Address: odontology Faculty, First Avenue/ Fifth Avenue, Leste Universitário neighborhood, Goânia city, GO, Brazil. Zipcode: 74605-020.

Abstract

Background: The antiseptic solutions most used by Eye bank or in ophthalmologic surgeries are polyvinylpyrrolidone-iodine and gluconate of chlorhexidine. **Objetives:** The objective of this study was to evaluate the antiseptic effect in the reduction of ocular globe microbiota from corneal donors, as well as to analyze the susceptibility of the microbiota to gentamicin. **Methods:** Thirty pairs of corneas received antiseptics, with Povidone-iodine in the right eye and gluconate of chlorhexidine in the left eye, and for each time of action (5, 10 and 15 minutes), totalling three groups, and using 10 pairs of eyeballs to each group. Swabs were collected from the ocular surface prior to application of the solutions, after and at the time of preservation of the corneal tissue. After identification of the microbiota, an antibiogram test with gentamicin was performed. **Results:** Regarding the data obtained in the second group, after the use of antiseptics, there was a reduction of 40% in the total of gram positive bacteria and a reduction of 75.9% to gram negative bacteria, the results shows that in the second group, both antiseptics were more effective to Gram negative bacteria. The third group results show the residual effect of antiseptics and there was a reduction of 99.1% of all micro-organisms. To the antibiogram test, 88% of the microorganisms isolated, were sensitive to gentamicin. **Conclusion:** Was observed that using the tested concentration to each antissepetic, there are no action differences to descontamination between the two products, there was not differences between the action time too.

Key-words: eye bank, enucleation, povidone-iodine, gluconate of chlorhexidine, transplantation.

1. *Introduction*

Brazil is ranked second in absolute number of organ and tissue transplants in the world ranking and has a well-established public transplantation program, one of the largest in the world [1, 2].

Donated eyes are obtained from cadavers from non-sterile environments such as domicile, public thoroughfare, hospitals and morgues [3], and rigorous screening of the medical and social history, body and eye of the donor is required. For this, preventive strategies are used, such as exclusion of donors with septicemia or endocarditis, antiseptic preparation and decontamination of the donated tissue, as well as preservation in antibiotic containing medium. Some Ocular Tissue Bank (Eye bank) still perform a microbiological evaluation in order to certify the absence of microbial contamination before the distribution of corneal tissue or at the time of transplantation [4].

On the ocular surface, especially in the human conjunctiva is a resident microbiota that has a fairly uniform pattern, although slight variations of certain micro-organisms occur in some parts of the world. Among the bacteria, species such as coagulase negative *Staphylococcus* (SCN), *Streptococcus viridans* group, *Corynebacterium* spp. and *Moraxella* spp. [5].

Donors who were hospitalized for a long time, under sedation, with muscle relaxant and maintained with mechanical ventilation, have the protection mechanisms altered, due to the blink reflex impairment and loss of muscle eyelid tone. These factors may result in incomplete palpebral closure, which allows a greater risk of corneal contamination, resulting in bacterial keratitis, mainly by *Pseudomonas aeruginosaa* [6]. Fungi contamination can also occur, with a prevalence of 3 to 28%, especially *Candida albicans*, *Saccharomyces cerevisae*, *Cryptococcus neoformans* and *Arpergillus flavus*, among others [7].

Among the antiseptic solutions most used by Eye bank or in ophthalmologic surgeries are polyvinylpyrrolidone-iodine and gluconate of chlorhexidine. Both are broad spectrum microbicides, with rapid action and depending on the concentration used, low corneal toxicity [8].

The Povidone-iodine target is the cytoplasmic membrane and its action to kill the micro-organism occurs in a few seconds, since the free iodine released will oxidize and ionize the vital molecules of the cell [9]. A 5 mg / mL solution, acting for two minutes, considerably reduces microbial contamination, without damage to the corneal tissue. However, in high concentrations, Povidone-iodine does not reduce conjunctival fornix contamination, with iodine being toxic when it penetrates the corneal layers and reaches stromal fibroblasts [10].

Gluconate of chlorhexidine, in turn, is a cationic bisbiguanide, which binds electrostatically to negatively charged surfaces, with specific and strong adsorption to the phosphate-containing compounds. Upon contact with the micro-organism, gluconate of chlorhexidine damages the outer layers of the cell wall, which makes the cell permeable and allows its entry into the cytoplasmic membrane. This cause loss of low molecular weight components, such as ions of potassium [11].

Depending on the concentration, the antiseptic gluconate of chlorhexidine acts differently in micro-organisms. At low concentrations, it acts as a microbiostatic agent, since only low molecular weight substances such as potassium and phosphate are lost, which is insufficient to damage the functioning of the cell. However, in high concentrations, gluconate of chlorhexidine precipitates the cytoplasm, which results in the death of the micro-organism [11].

After antisepsis the storage of ocular globes in preservation media is enriched with nutrients such as glucose, amino acids, minerals and vitamins, whose purpose is to protect cells from the cornea. The medium can have too the gentamicin and streptomicin antibiotics [12, 13], allowing prolonged storage. This medium is widely used by eye bank in Brazil, as well as an analogue, but without streptomycin [14].

The objective of this study was to evaluate the antiseptic effect in the reduction of ocular globe microbiota from corneal donors, prior to enucleation with 5% Povidone-iodine and 0.05% Gluconate of chlorhexidine at different application times, as well as to analyze the susceptibility of the microbiota alone to gentamicin.

2. *Methods*

The research was submitted to the Ethics Committee of the Hospital das Clínicas of the Federal University of Goiás (HC / UFG), under the number CAAE: 58444316.3.0000.5078, and was approved. Eyeballs were collected from 30 donors from September 2016 to July 2017. All had the donation term signed by a first or second degree relative, as well as a witness.

After the authorization of the family members, the entire process followed the Procedures Manual Standards of the eye bank, and the evaluation of the donor's medical history was carried out by means of medical records, exams, anamnesis by the coroner and epidemiological interviews with the family. We also documented the location, time of death and time of enucleation of the eyeball. Donors with signs or suspicions of infection were excluded from the study.

Prior to face and ocular surface antisepsis, a swab soaked in 0.9% saline solution was rubbed throughout the conjunctival fornix of the right eye and immediately transferred to a tube containing Brain Heart Infusion (BHI) broth, duly identified with the data of the donor. The same procedure was then performed on the left eye.

After this first harvest, the anti-sepsis of the donor's face and eyelids was performed, according to the medical standards of the eye bank and it is essential to change gloves. Cleaning was performed with 0.9% saline irrigation, above the eyelids, to remove any impurity. Then, with the aid of sterile gauze, the 10% topical povidone iodine was applied, always in the same direction. To do so, the eyes were closed tightly and the procedure performed very carefully, so that there was no penetration of the antiseptic on the ocular surface.

The eyes were then opened and the ocular surface was cleaned by irrigating 10 mL of 0.9% saline solution. After the excess liquid was withdrawn with a sterile gauze and with a sterile glove, the antiseptics were applied to the ocular surface.

On the ocular surface of the right eye were applied 5 mL of 5% Povidone-iodine diluted solution and in the left eye 5 mL of 0.05% dilute Gluconate of chlorhexidine solution. After 5 minutes of antiseptic application, a new cleaning with 10 mL of 0.9% saline was performed. Then a swab was passed back into the conjunctival fornix and transferred to a tube containing BHI broth, duly identified with the donor data.

This procedure was also carried out in 10 and 15 minutes, with 10 samples in each group, totaling 30 donors. After the second harvest, enucleation of the ocular globes was performed, which were sent in a moist chamber to the eye bank.

After biomicroscopic evaluation of the eyeball and cornea, the tissue preservation phase and the last sample harvest were initiated. In the laminar flow, the eyeball was scraped with a 11 scalpel blade, to remove any remnants of conjunctiva and foreign body, as well as cleaning of the corneal-scleral flap. Subsequently, 0.9% saline was irrigated over the entire surface of the corneal tissue. A swab was then rubbed across the corneal surface and horn-scleral flap and immediately transferred to tube containing BHI broth. The cornea was then preserved in a preservation medium containing gentamicin. After this procedure, the corneas were kept in quarantine for at least 24 hours until the donor serological tests were performed. Subsequently, the corneas were again evaluated for release or discard.

The tubes containing the samples were immediately sent to the Laboratory of Anaerobes, Phenotyping and Molecular Biology (LAFEBIM) of the Institute of Tropical Pathology and Public Health of the Federal University of Goiás, for processing. A 0.1 mL aliquot of each tube was seeded on nutrient Agar, which was incubated in aerobic at 37°C for 24 hours for bacterial countinag. The tubes containing the samples were also incubated under the same conditions. After the tubes were

141 homogenized and a 0.1 mL aliquot of the broth was seeded in the MacConkey agar medium, Saline
142 Mannitol agar, base agar supplemented with 5% horse defibrinated blood, which were incubated
143 under the same conditions. A 0.1 mL aliquot of the broth was also seeded on Sabouraud agar and
144 kept at room temperature for seven days.

145 Afterwards, external ocular diagnostic microbiology tests were performed to identify the
146 micro-organisms, as well as antibiotic testing with gentamicin. The option of using gentamicin in
147 the antibiogram test is justified by the fact that the preservation medium used in the eye bank
148 participant of the present study only contains this antimicrobial. To compare proportions, the Chi-
149 square test or Fisher's exact test, when applicable, was used; to compare continuous variables, the
150 T-test was used; and to evaluate the reduction in the number of colonies, the paired McNemar Test
151 was used. The level of statistical significance of 5% ($p < 0.05$) was considered for all tests.

152

153 **3. *Results***

154 Microbiological samples were collected from 60 eyes, which corresponded to 30 donors of
155 corneas, 63% were male. Donor age had a median of 54 years, with a minimum of 29 and a
156 maximum of 78 years (Table 1). The mean temperature at the time of collection had a median of
157 29°C (minimum 24°C, maximum 32°C), but the lowest temperature was observed in the group of
158 donors who received 15-minute antiseptic treatment, whose median was 25.5°C. This lower
159 temperature had a statistically significant analysis, with $p = 0.011$, for a better antiseptic effect.

160 The interval between deaths and enucleation of the eyeballs was 5 minutes and 32 seconds,
161 with a minimum of 1 minute and a maximum of 7 minutes. When analyzing the influence of
162 collection time on the number of isolated micro-organisms, it can be noticed a lower contamination
163 of the ocular surface in the group of individuals with less time of interval between deaths and
164 enucleation of the eyeball. Donors who had this shortest time focused on the antiseptic treatment

group for 15 minutes, which influenced the potentiation of antisepsis at this time, and was statistically significant, with a value of 0.004, as shown in Table 1.

The most frequent causes of death were acute myocardial infarction (AMI), represented by 53.4% of the total, followed by stroke 16.7% and 13.4% died due to traumatic brain injury, 6.7% died of acute respiratory failure and another 6.7% of pulmonary thromboembolism, finally had a group of 3% who died of intestinal cancer. In the present study, the mortis causes had no correlation with the number of micro-organisms isolated from the ocular surface microbiota and with the times of action of the antiseptics.

The corneas were processed and evaluated following the protocols of quality control and serological screening. Of the 60 corneas preserved, 72% were classified as optic and released for transplantation; 5% released as tectonic tissue, two of which were discarded by expiration date; 20% considered inappropriate due to reactive serology, with prevalence for the anti-HBc marker and 3% discarded due to failure in processing, the surgical instruments were not properly sterilized due to autoclave malfunction.

A 100% microbial growth was observed in all samples collected prior to application of the antiseptics, which corresponds to the ocular microbiota of the donor after death. A total of 353 CFU (Colony Forming Unity) were isolated from bacteria and fungi. Gram-positive bacteria predominated, 70%, followed by gram-negative bacteria, 28.3% and fungi 1.7 %.

Among the gram positive bacteria, the genus *Staphylococcus* was the majority 81.8%, followed by catalase negative cocci 9.7%. As regards Gram negative bacteria, 77% of enterobacteria and no fermenting Gram-negative rods represented 14% of the isolated colonies.

Fungi were isolated from four donors (1.7%), and in two, *C. albicans* (1.13%), was identified in both eyes; in one donor there were *Aspergillus* spp. and in another, *Penicillium* spp. It is important to note that the fungi were only present in the first group (data not shown).

A highly significant difference of the untreated stage for the antiseptic stage was observed at the CFUs evaluation stage from first to second collect of each group. In the right eye, which was used Povidone-iodine, the *p* was 0.014 (SD 592.3-82.4), while in the left eye, with the use of gluconate of chlorhexidine, the *p* was 0.002 (SD 247.5-22.4), which proves a significant reduction of the ocular microbiota of the corpse with the use of antiseptics. To the third collect none bacteria growed (Table 2).

The most of the collections, 73%, were performed in donors at the Medicalegal Death Investigation Service (MDIS) and 27% were hospitalized in Goiânia city / Goiás State hospitals, whose hospitalization period was on average, eight days, with minimum time of three and maximum of 20 days. When analyzing the distribution of the microbiota in relation to the origin of the donor, there was no statistically significant difference ($p > 0.05$), which shows that the collect place did not influence the composition of the ocular microbiota (Table 3).

Regarding the data obtained with both the use of antiseptics, in the second group, after antisepsis, there was a reduction of 39.5% in the total Gram-positive bacteria, and 76.5% in the Gram negative, there being no significant statistical difference ($p = 0.494$), which shows that the bacterial elimination capacity of antiseptics was similar for both groups. It is observed that both antiseptics were more effective for the Gram-negative, with statistically significant difference ($p < 0.001$), than for Gram-positive, with no statistically significant difference ($p = 0.183$) (Table 4).

In the third group, there was a reduction of 99.1%, due to the residual action of the antiseptics between the second group, which comprised the enucleation process and the third, preservation of the corneal tissue, which was 2 hours and 11 minutes (SD = 39), with variation between 1 hour and 18 minutes and 3 hours and 35 minutes of all micro-organisms, except for the growth of 0.9% to *Staphylococcus* spp. in a donor at the time of application of 5 minutes in both antiseptics. For *Staphylococcus* spp., it was possible to observe that both Povidone-iodine and gluconate of chlorhexidine were more effective with 15 minutes, with a reduction rate of 31.6%,

214 respectively. As for the enterobacteria, there was a reduction of 70.6% in the action time of 5
215 minutes for both antiseptics (Figure 1).

216 When comparing the efficacy of the antiseptics tested, there was a reduction in the number
217 of contaminants, both with the use of Povidone-iodine and with the use of gluconate of
218 chlorhexidine, with no significant statist difference (p>0.05). However, when comparing,
219 separately, the two largest groups of isolates, *Staphylococcus* spp. and enterobacteria, it was
220 possible to observe differences between the times of action of antiseptics, with reduction of
221 contaminated donors.

222 Regarding the antimicrobial action of gentamicin, of the 335 CFUs, antibiotic test against
223 gentamicin was performed in 305 samples and 88% were sensitive to the antibiotic and 12%
224 resistant. Among the resistances, the Gram-positive bacteria are highlighted, as shown in figure 2.

225 The corneas used in this study were processed and evaluated following the protocols of
226 quality control and serological screening. Between the 60 corneas preserved, 72% were classified as
227 optic and released for transplantation and 28 % were considered inappropriate and were discarded
228 (Figure 3).

229

230 **4. *Discussion***

231 Of the 30 corneal donors in this study, 63.3% were males, a percentage that is similar to
232 other studies, which reported a rate of 62.2% and 61%, respectively [15, 16], of corneal donor men
233 and the age of the donors had a median of 54 years, data very similar to those described em others
234 articles [17,18], and found 55 and 55.57 years of age, respectively.

235 The variables sex and age in this study did not interfere in the results found in relation to the
236 times of 5, 10 and 15 minutes of action of the antiseptics for the reduction of the ocular surface
237 microbiota, with p values found, p <0.999 for sex and the p <3.41 for age, are not statistically
238 significant.

The overall mean time between death and interval between deaths and enucleation from the eye was 5 minutes, which is in line with the Eye Bank Association of America (EBAA) recommendation, which is up to six to minimize metabolic changes, which can alter endothelial cells and microbiological contamination [19].

The cause of death was another variable of the present study and had the highest prevalence death due to acute myocardial infarction (data not shown). In the study by Araújo and Scarpi [20], the highest index (26%) of corneal donor deaths was also due to cardiovascular diseases. This high prevalence of causa mortis can be explained by the fact that cardiovascular diseases are the main cause of death in the world. Data from the World Health Organization in 2012 revealed that 17.5 million people worldwide died from cardiovascular diseases, which represents 31%, mainly risk factors such as tobacco use, unhealthy diets, obesity, sedentary lifestyle and harmful use of alcohol [21].

Staphylococcus spp. were the most prevalent (81.8%) among the isolates and some researches have demonstrated that 63.8% of the strains found were *Staphylococcus* spp. were the most isolated micro-organisms of the conjunctiva [22], eyelids and tears, and are part of the microbiota of the eyes of living people, being a percentage of 20% to 80% isolated from the conjuctiva and 30% to 100% isolated from the eyelid[23]. These micro-organims, although considered to be of low virulence may be carried into the cornea preservation medium and subsequently transferred to the recipient, which may result in corneal transplant endophthalmitis.

Despite the low incidence, Gram-negative bacteria and fungi were also isolated. Studyes [9, 20], in their studies found a rate of 41%/45.14%, respectively, of Gram negative bacteria, given slightly above this study; the high incidence of Gram-negative bacteria in cadaver eyes is due to the fact that donor eyes to be enucleated, often after the autopsy. This fact also occurred in the present study, with a longer period between death and enucleation in these donors [9].

263 The fungi were isolated from three donors who were hospitalized and one from the MDIS.
264 In all, the incidence was 1.7%, being two *Candida albicans*. Although fungi are not considered to
265 belong to the microbiota of the ocular surface, they have been isolated at a rate of 28% in the eyes
266 of healthy people [23]. Researchs [3] reported a fungal isolation rate of 3.81% in donor eyes, a
267 percentage higher than that found in the present study, which may be due to the lower number of
268 donors. In a previous study [24], authors described a rate of 0.5% of *C. albicans*, similar to the
269 percentage found in this study, which was 0.6% of the total.

270 Study [25] reported that it is important to observe the risk factors that lead to the
271 contamination of corneas donated with fungi. This contamination may occur due to diseases of the
272 ocular surface or to the permanence in environments conducive to their growth [7]. For example,
273 patients who have been hospitalized for many days with respirators are more prone to
274 contamination ocular by fungi [26]. Another factor that can contribute is the type of environment,
275 which can be hot and humid [7]. This explains the observed in this research, where two of the three
276 donors who presented fungi were hospitalized for a long period and with respirator use.

277 The third individual, although not hospitalized, was a chronic renal patient, who underwent
278 weekly hemodialysis, with constant contact with the hospital environment, which may justify the
279 finding. It should be noted that of this patient submitted to hemodialysis, the bacterium
280 *Sphingomonas* spp. was also isolated, according with researchers [27], it is an environmental
281 bacterium that contaminates water, being of importance in the hospital environment, for infecting
282 patients on hemodialysis or peritoneal dialysis.

283 In the present study, the significant reduction of the total microbiota of the ocular surface of
284 donors of corneas observed for both Povidone-iodine and gluconate of chlorhexidine occurred due
285 to the broad spectrum of action that these antiseptics presented 53.6% decontamination. Some
286 researchs [28], showed a 36% reduction rate in the ocular surface microbiota, when performing
287 antisepsis with 5% Povidone-iodine solution for 2 minutes. With this procedure, the authors

emphasized that the reduction of the amount of microbial contaminants on the surface is significant, but does not totally eliminate the risk of contamination.

Although the literature states that gluconate of chlorhexidine has difficulty acting on Gram-negative bacteria, the result obtained in this research for gluconate of chlorhexidine was similar to that of Povidone-iodine for this bacterial group and even higher than that obtained for Gram-positive. This is probably due to the lower number of Gram-negative isolates when compared to Gram-positive ones.

When evaluating the results obtained for *Staphylococcus* spp. and enterobacteria, with respect to antiseptic residence times and their effect on donor eye decontamination, although not statistically significant, both Povidone-iodine and gluconate of chlorhexidine were more effective in 15 minutes time for *Staphylococcus* spp., the effect of gluconate of chlorhexidine being higher than that of Povidone-iodine. For the enterobacteria both were effective from 5 minutes. However, when the third group was performed, on average 2 minutes and 11 seconds after enucleation the rate of reductionof the microbiota was above 99.1% for the two antiseptics.

Some authors [29] used 5% Povidone-iodine solution for 5 minutes in scarified corneas and found a significant reduction rate of 24.7% to 4.3%, but concluded that this concentration and this time of action of ododopovidone significantly decreases the contamination of the corneal epithelium but does not completely sterilize the cornea and occasionally leaves a sufficient number of bacteria on the ocular surface to contaminate the preservation medium.

A research [9], in their antisepsis protocol, tested three different protocols, the first being gentamicin at 0.4%, the second gentamicin at 0.4% with Povidone-iodine at 1%, and the third amicacin at 4 % with 5% Povidone-iodine. In all treatments the ocular globes were immersed for 3 minutes and the reduction of the microbiota was Gram-positive of 38.6%, 27.6% and 10.8% respectively, and for Gram-negative, 10.2%, 18, 8% and 19.8%, respectively.

When comparing the results presented in the literature with those obtained in the present study, it is noted that the residual time of Povidone-iodine and gluconate of chlorhexidine was the fundamental factor for the high rate of decontamination found, close to 100%. The next result of this study was that, when the authors [30] combined gluconate of chlorhexidine with Povidone-iodine, obtaining reduction rate of 98.6%. The difference in methodologies was that, in addition to the combined use of Povidone-iodine and gluconate of chlorhexidine, the preservation of corneal tissue occurred minutes after immersion in antiseptics. Already in this research, the antiseptics were used separately, irrigated before enucleation, within the established action time of each step. Next, the eyeballs were placed in a humid chamber and sent to eye bank for evaluation, processing and preservation, with an average duration of 2 minutes and 11 seconds.

The observed growth of *Staphylococcus* spp. in the third group from a single donor, can be attributed to an extensive epithelial defect in both eyes. Authors [31] aim that the damaged corneal epithelial tissue can retain micro-organisms in crypts and thereby protect them from the action of antiseptics during irrigation.

Regarding the antibiogram test, 88% were sensitive to gentamicin, with the highest resistance rate found for the group of Gram positive bacteria (7%). Others research [10, 32], found an 82% sensitivity to the same antibiotic and 86.4%, respectively, which was similar to that detected in this study. This sensitivity rate is still of concern, because if there is adequate antisepsis of the ocular tissues prior to preservation, micro-organisms resistant to the antibiotic contained in the preservation medium may remain in the corneal tissue at the time of transplantation, resulting in an endophthalmitis in the recipient of the cornea. This fact was observed in a study [33] a rate of 56.8% of cases of endophthalmitis after corneal transplantation, where the isolated micro-organisms were both in the corneal-scleral flap donor tissue, and in the eye of the recipient. In 2004, studies [34] described a much lower rate, 16% of contaminated corneal buds, of which only 1.5% caused infection in the recipient, which resulted in 1.27% of ulcer and 0.22% endophthalmitis.

Two groups of researchers [10], found that the gentamicin is the most effective antibiotic for the decontamination of donor eyes before enucleation and corneas preserved for transplantation, being the most used in the composition of commercial preservation media. For a research group [35], it is extremely important to detect trends of microbial resistance to the antibiotics that are used in most of the presently used corneal preservation solutions.

5. Conclusion

In the present study, after the quarantine period and new reassessment of the preserved corneas, no vial presented turbidity and alteration in the color of the preservation medium, as indicative of pH change and possible contamination. In the evaluation of the tissue traceability protocols sent to the transplantation centers, there was no report of the surgeons indicating the presence of infection in the eyes of the cornea receptors, nor of the cornea that presented bacterial growth in the third group, emphasizing that the micro-organisms found in these corneas were sensitive to gentamicin.

Thus, after evaluating the results obtained, it is believed that the use of strict antiseptic procedures, from removal of the eyeball to preservation, guarantees the safety of a corneal tissue to be used in transplants, which will reduce the risks of disorders after surgery.

There was no statistically significant difference between the action of Povidone-iodine and gluconate of chlorhexidine in the reduction of the micro-organisms of the ocular surface of the cornea donors, both of which were effective. The time between removal of the eyeball and the preservation of the cornea allows the residual action of the antiseptics, which increases the decontamination power. Although some strains resistant to gentamicin have been found, the antibiotic-containing cornea preservation medium complements tissue decontamination procedures and provides greater storage safety.

5. Declarations

Ethical approval and consent to participate: Declaration The manuscript does not contain animal experiments and the manuscript does not contain studies in humans

Consent for publication: The authors declare consent for publication

Data and Material Availability: The authors declare data and material availability. All data generated or analyzed during this study is included in this published article.

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Authors' Contributions:

CRMI: responsible for developing the experiments and writing of the article

CAS: responsible for help with the writing of the article

LCC: responsible for help with developing the experiments and writing of the article

MSB: responsible for help with the writing of the article

JBN: responsible for Eyes Bank and help with the writing of the article

ACMCM: responsible for help with the experiments

APPF: responsible for help with the samples collections

APS: responsible for help with the experiments

PHPQ: responsible for help with developing the experiments

JCM: responsible for help with the samples collections

ALS: responsible for help with developing the experiments

MPÁ: responsible by article supervision and help with the writing the article.

All authors read and approved final manuscript

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465

466 **Figures legends**

467 **Figure 1:** Legend: 1.RE: right eye; 2.LE: left eye; 3.min: minutes.

468

Figures

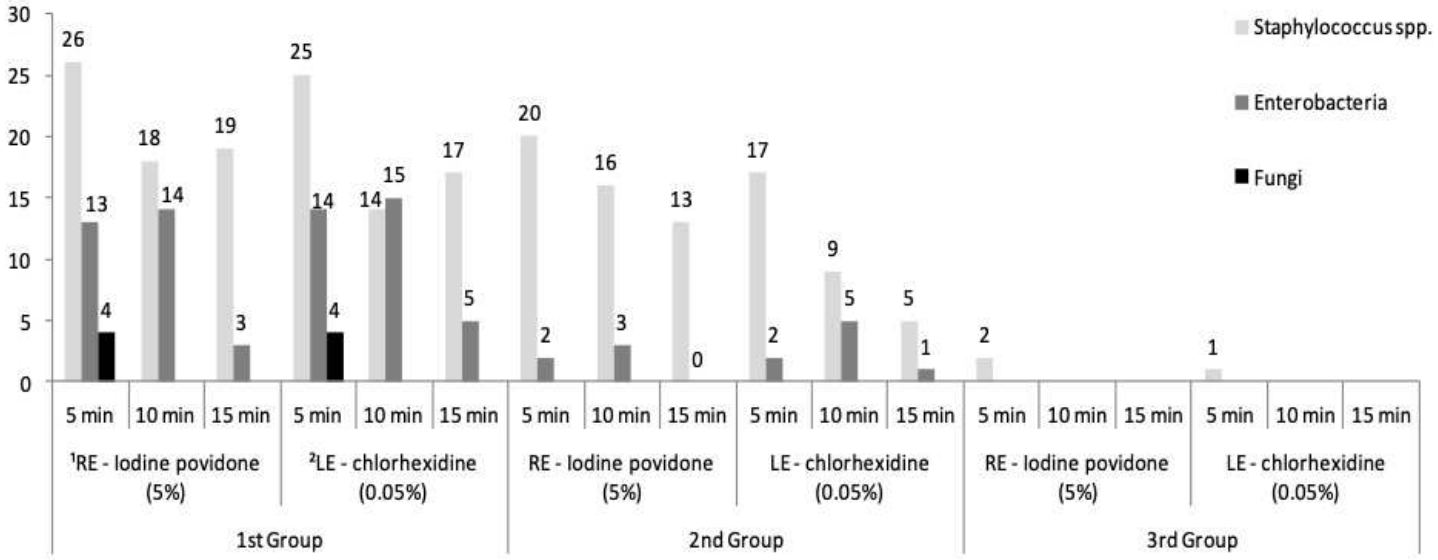

Figure 1

Number of contaminants isolated in the first, second, third groups by type of microorganisms, demonstrating the reduction of the microbiota after application of the antiseptics. 1.RE: right eye; 2.LE: left eye.

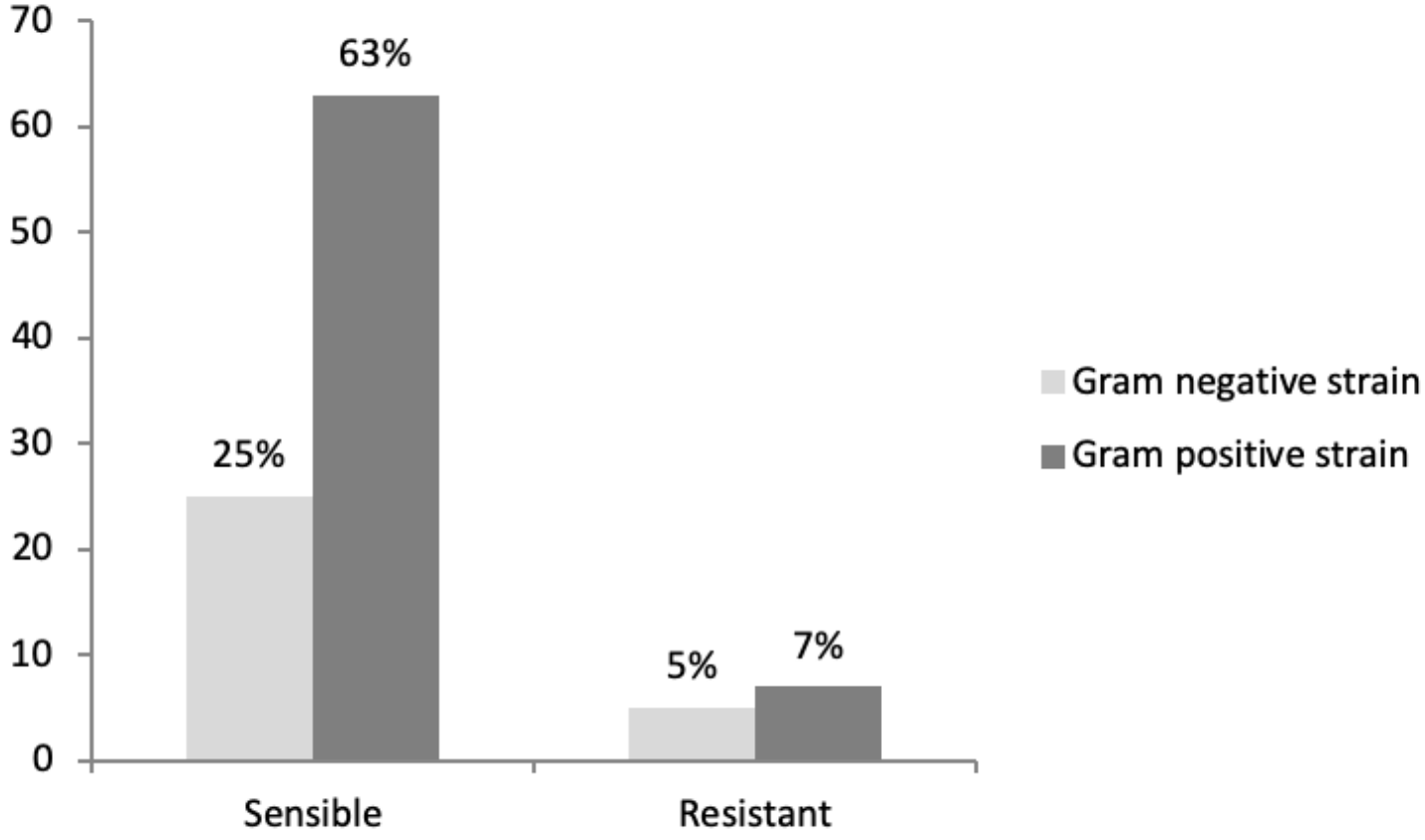

Figure 2

Percentage of gram-positive and gram negative bacterial strains sensitive and resistant to gentamicin, isolated from the ocular surface of donors of cornea.

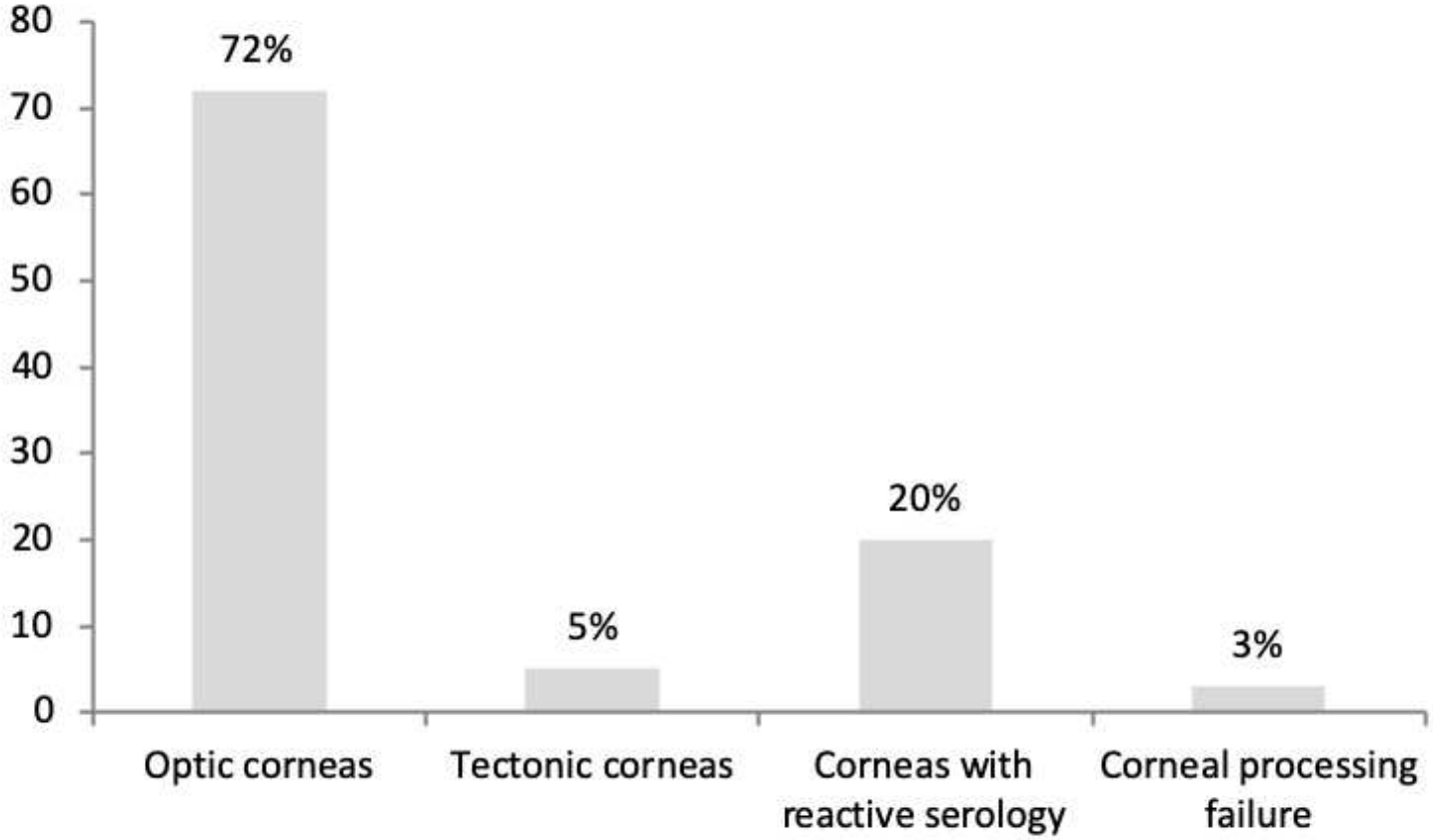

Figure 3

Classification of corneas for use in transplants.