# Experimentally-induced Endophthalmitis in a rabbit model with *Staphylococcus epidermidis* of two different virulence profiles

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# Abstract

**Purpose:**

To investigate the association between *S. epidermidis* virulence and severity of endophthalmitis in an animal model.

**Methodology:**

New Zealand albino rabbits were inoculated with 100 colony forming units (CFU) of *S. epidermidis* strains with different antibiotic-resistance and biofilm-producing profiles (virulent [Group 1] and no-virulent [Group 2] group). The virulent group comprised seven rabbits in total. Four rabbits were inoculated with a multi antibiotic resistant and *mec* A, *ica* and *atl*E genes carriers *S. epidermidis* isolated from the conjunctiva of patients undergoing cataract surgery, and three rabbits with *S. epidermidis* ATCC 35984 (biofilm-forming and antibiotic multi-resistance strain). In the non-virulent group (n = 8), five rabbits were inoculated with a strain sensitive to all tested antibiotics and non-carrier of the *mec* A, *ica* and *atl*E genes isolated from a patient, and three with *S. epidermidis* ATCC 29122 strain (none biofilm producer). Clinical and ultrasound examinations were performed in all animals every three hours until a sign of endophthalmitis was evident. The clinical course was evaluated every 24 hours thereafter. Fifteen days post inoculation the eyes were enucleated for histologic evaluations.

**Results:**

Rabbits inoculated with the less virulent strains showed less severe inflammation and damage to intraocular structures compared to those inoculated with more virulent strains by both clinical and ultrasound evaluation. However histopathologic results showed similar degree of inflammation 15 days after inoculation.

**Conclusions:**

*S. epidermidis* was able to induce endophthalmitis in all animals regardless of their virulence profile. While less virulent strains caused less severe inflammation and assessed clinically and by ultrasound examination, long-term effects in histopathologic evaluations were comparable with the more virulent strain.


# Introduction

Endophthalmitis is characterized by marked inflammation of intraocular fluids and tissues caused by exogenous - or more rarely endogenous pathogens - and often results in severe visual loss. Exogenous endophthalmitis may occur after ocular surgery, following intraocular administration of medication, penetrating eye trauma or as an extension of a corneal infection [1]. The rate of postoperative endophthalmitis following cataract surgery is between 0.056% and 1.3% and rises to 30% after open-globe trauma. The most frequent source of postoperative endophthalmitis is the patient's own bacterial flora of the conjunctiva and eyelid [2, 3].

The onset of intraocular inflammation after the introduction of microorganisms is variable and depends on their virulence, size of the inoculum, inflammatory reactions and the patient's immune status [4].

Gram-positive staphylococci are responsible for more than 90% of cases of postoperative infectious endophthalmitis. *S. epidermidis* represents 70% of the isolated microorganisms. Despite its relatively low virulence, once it penetrates the eye *S. epidermidis* can cause severe vision-threatening endophthalmitis [2].

Destruction of intraocular structures, which can result in complete vision loss, is due to toxins and enzymes released by the microorganism on the one hand and the host's immune system on the other [5]. There is evidence about the relationship between the ability of microorganisms such as *Staphylococcus aureus*, *Enterococcus faecalis* and *Bacillus cereus* to produce certain toxins and their virulence in endophthalmitis [6–9]. However, data on the effects of different strains of *S. epidermidis* are scarce.

Bacterial products contributing to biofilm formation are among the best-studied virulence determinants of *S. epidermidis* [10]. The genes responsible for the increased virulence of Staphylococci are ica (intercelular adhesin-operon – *ica* ADBC), of which icaA and *ica*D are responsible for the formation of biofilm and *atl*E gen, codifying a protein responsible for the primary adherence to surfaces [11–13].

In previous studies, the authors have determined biofilm production by phenotypic method in Coagulase Negative Staphilococci (CoNS) isolates [14] and a high frequency of genes encoding virulence factors for biofilm formation (*ica* and *atlE*) and the *mec*A gene by a multiplex PCR in coagulase-negative Staphylococcus isolates from ocular samples of patients undergoing cataract surgery [15, 16].

Endophthalmitis animal models provide a tool to understand the underlying mechanisms of the disease, depending on the source of infection and the type of pathogen involved. These models allow to examine various aspects of the pathogenesis and pathophysiology of bacterial endophthalmitis helping to promote the development of anti-inflammatory treatment strategies and evaluating the pharmacokinetics and efficacy of antibiotics. While no single animal model perfectly reproduces human pathology of bacterial endophthalmitis, researchers have successfully used these models to understand the infectious process and the host's response, and have provided new information on therapeutic options for the treatment of bacterial endophthalmitis [17].

Data of studies in animal models examining the association of virulence of microorganisms and severity of endophthalmitis are limited. Mino de Kaspar et al. showed in an animal model that the severity of the inflammation is related to the resistance pattern of the of *S. epidermidis* strain causing endophthalmitis [18]. Resistant strains were shown to be capable of causing endophthalmitis in rabbits in a shorter time and with greater severity than sensitive ones. However, in this study no molecular techniques have been used to establish the characteristics of the strains causing the infection.

In order to establish a better understanding of the phenomenon, the present study was conducted using the same animal model to determine if (*mec*A + gene) and biofilm producing *S. epidermidis* strains are more virulent than sensitive strains (*mec*A gene-) non-biofilm producers by evaluating clinical, ultrasound and histopathological parameters.

## Materials And Methods

New Zealand white albino rabbits (weighing 2.0–2.5 kg) with free access to food and water were used. The animals were housed in separate cages under a 12-h light/dark cycle at the animal care facility of the *Instituto de Investigaciones en Ciencias de la Salud* of the National University of Asunción, Paraguay. The rabbits were maintained in accordance with the Guiding Principles in the Care and Use of Animals. The research team was composed by ophthalmologists, one veterinarian, microbiologist and pathologist.

After approval of the institutional review board and the local ethics committee, 15 New Zealand albino rabbits and different *S. epidermidis* strains were used for this experimental study. *S. epidermidis* with different antimicrobial susceptibility and virulence gene profiles were used (Table 1). Seven rabbits were inoculated with virulent strains of *S. epidermidis* [Group 1]. Of these, four rabbits received a *S. epidermidis* strain resistant to more than three antibiotics and carriers of the *mec*A, *ica* and *atl*E genes isolated from a patient undergoing cataract surgery; and three rabbits a biofilm-forming multi antibiotic resistant *S. epidermidis* ATCC 35984 (Microbiologics, USA) strain. Eight rabbits were inoculated with less virulent strains of *S. epidermidis* [Group 2]. Of these five rabbits received a strain isolated from a patient undergoing cataract surgery, sensitive to all tested antibiotics and non-carrier of *mec*A, *ica* and *atl*E genes, and three rabbits a non-biofilm- forming ATCC 29122 *S. epidermidis* (Microbiologics, USA) strain.

Table 1
Antibiotic sensitivity and virulence gene factor profile of experimental *S. epidermidis* strains

| | Virulent Strain Group | | Less Virulent Strain Group | |
|---|---|---|---|---|
| **Antibiotic tested** | **Isolated from a patient[a]** | **ATCC[b]** | **Isolated from a patient[c]** | **ATCC[d]** |
| Penicillin | R | R | S | R |
| Gentamicin | S | R | S | S |
| Chloramphenicol | S | S | S | S |
| Tetracycline | S | S | S | R |
| Ciprofloxacin | S | S | S | S |
| Erythromycin | R | R | S | S |
| Moxifloxacin | S | S | S | S |
| Clindamycin | S | R | S | S |
| Tobramycin | R | I | S | S |
| Trimethoprim Sulfamethoxazole | S | R | S | S |
| Cefoxitin | R | R | S | S |
| **Virulence genes** | *mecA*, *ica*, *atl*E | *mecA*, *ica* | none | none |
| S: Sensible I: Intermediate, R: Resistant *mecA*: methicillin resistance gene, *ica* and *atlE*: biofilm-forming genes | | | | |
| a. *S. epidermidis mecA*, *ica*, *atl*E carrier, isolated from conjunctiva of a patient undergoing cataract surgery | | | | |
| b. *S. epidermidis* ATCC 35984 biofilm-forming strain. | | | | |
| c. *S. epidermidis mecA*, *ica*, *atl*E non-carrier, isolated from conjunctiva of a patient undergoing cataract surgery | | | | |
| d. *S. epidermidis* ATCC 29122 non-biofilm forming strain | | | | |

*S. epidermidis* strains were incubated on blood agar at 37°C for 24 hours. From each strain, a colony was transferred from the 24-hour blood agar plate to 10 ml of trypticase soy broth (TSB) and incubated for another 24 hours at 37°C (stock solution). The organism was resuspended in the TSB to an absorbance of 0.15 on a spectrophotometer at 625 nm, which would give a density of bacteria at $10^7$ CFU/mL. ?Further dilution in sterile balanced salt solution was made to obtain the desired concentration of bacteria for intravitreal injection. The final dilution was replated on trypticase soy agar to confirm the actual CFU.

Antibiotic susceptibility testing using the Kirby-Bauer disc diffusion technique was done on Mueller-Hinton agar (bioMérieux®, Stuttgart, Germany) as established by the CLSI (Clinical and Laboratory Standards Institute) to confirm the initial resistance pattern for each strain.

Fifteen New Zealand albino rabbits (1.20 to 3.70 kg) were anesthetized by intramuscular injection of ketamine hydrochloride (35 mg/kg body weight) and lidocaine hydrochloride (5 mg/kg body weight). Additional topical anesthesia with 0.5% proparacaine hydrochloride eye drops was applied before inoculation of the bacterial solution. Under anesthesia, a paracentesis was created on the right eye, and 0.1 ml of aqueous humor was aspirated from the anterior chamber using a 30-gauge needle on a tuberculin syringe. Afterwards, 0.1 ml of the *S. epidermidis* suspension containing 100 CFU was inoculated into the vitreous cavity of one eye of each rabbit via pars plana approximately 2 mm posterior to the limbus with a 30-gauge needle on a tuberculin syringe. The other eye remained untreated and served as a control.

After inoculation, slit-lamp biomicroscopy and indirect ophthalmoscopy were performed every three hours until signs of endophthalmitis appeared, and every 24 hours thereafter. Before each examination topical 1% tropicamide and 2.5% phenylephrine eye drops was applied to dilate the pupil. Anterior chamber reaction and fundus reflex were graded for severity of ocular inflammation using the model proposed by Peyman et al. (Table 2) [19].

Table 2
Endophthalmitis severity grading scale and histopathologic grading of eyes infected with *Staphylococcus epidermidis*

| **Endophthalmitis severity** | **0** | **1** | **2** | **3** |
|---|---|---|---|---|
| **Conjunctiva** | Normal | Mild edema | Edema, mild hyperemia, slight exudate | Edema, marked hyperemia, heavy exudate |
| **Cornea** | Clear | Focal edema | Diffuse edema | Opaque |
| **Iris** | Normal | Mild hyperemia | Marked hyperemia | Marked hyperemia, synechiae, irregular pupil |
| **Vitreous** | Clear | Areas of vitreous haze, some fundus details visible, good red reflection with "haze" | Moderate vitreous haze, no fundus details visible, partial red reflex | No red reflex |
| **Anatomic Structure** | | | | |
| **Cornea** | Normal | Partial-thickness infiltration | Segmental full-thickness infiltration | Total full-thickness infiltration |
| **Anterior chamber** | Normal | Partially filled with fibrin without infiltrate | Partially filled with fibrin with infiltrate | Completely filled with infiltrate |
| **Vitreous** | Clear | Inflammatory cells without focal abscess | Partially filled with abscess of infiltrate | Completely filled with infiltrate |
| **Retina** | Normal | Partially infiltrated | Totally infiltrated and partially necrotic, normal retina | Complete necrosis of all retinal layers |

After application of topical anesthesia (proparacaine hydrochloride 0.5% eye drops) in the conjunctival sac, trans-palpebral ultrasound with a 10 MHz transducer was performed. Abundant amounts of methylcellulose gel were applied over the eyelid to avoid interposition of air bubbles between the transducer and the skin surface. Ocular and orbital examination was carried out systematically starting with a parasagittal plane through the center of the eye. From this initial plane, angling the transducer to the right and left, the sweep was made from the innermost part to the outer part of the organ studied. Then, the axial plane was explored, also through the center of the cornea and the vitreous chamber and angling the transducer from the top to the bottom, until the entire globe was observed.

Animals were sacrificed on day 15 post injection in a $CO_2$-chamber. Afterwards, 0.1 ml of vitreous humor was aspirated with a 30-gauge needle on a tuberculin syringe and the eyes were enucleated and fixed in 10% formalin in phosphate buffered saline for histopathologic analysis.

Eyes were embedded in paraffin, sectioned, and stained with hematoxylin-eosin, periodic acid-Schiff, and Gram stain according to standard protocols. Sections were examined and scored by an investigator masked to the identity of the treatment group.

A modified defined classification scheme was used to quantify the degree of inflammation of the cornea, iris, vitreous base, ciliary body, and retina (Table 2). Assessment of inflammation focused on the retina at three different locations: the central retina, approximately 20° paracentral retina, and near the ora serrata. The retina was evaluated on both the nasal and temporal sides to avoid a false reading due to localized swelling.

The histopathological study graded presence or absence of acute inflammation and classified the degree of involvement in "no inflammation", "mild", "moderate", and "severe inflammation". The anatomical structures evaluated were cornea, iris, ciliary body, choroid, vitreous and retina. According to the findings, scores were noted. The score for the anatomic structure is observed in Table 2 with the following scores: Zero: for uncompromised structures; One: for light involvement; two: for moderate involvement; three: for serious involvement. The possible total score in histopathological quantification was 12.

## Results

Fifteen rabbits inoculated with *S. epidermidis* strains with two profiles of antibiotic susceptibility and virulence factors were studied. Tables 3 and 4 show the clinical scores of study eyes after intravitreal injection of *Staphylococcus epidermidis* strains.

Table 3
Clinical grading of experimental eyes after intravitreal injection of *Staphylococcus epidermidis* strains

| Rabbit codes | Conjunctiva | | | | | Cornea | | | | | Iris | | | | | Vitreous | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T1 | T2 | T3 | T4 | T5 | T1 | T2 | T3 | T4 | T5 | T1 | T2 | T3 | T4 | T5 |
| G1 | | | | | | | | | | | | | | | | | | | | |
| 1 | 1 | 1 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 2 | 2 |
| 3 | 1 | 2 | 2 | 2 | 2 | 0 | 0 | 1 | 2 | 2 | 0 | 3 | 3 | 3 | **3** | 0 | 0 | 1 | 3 | 3 |
| 8 | 2 | 2 | 2 | 2 | 3 | 0 | 2 | 2 | 2 | 2 | 0 | 1 | 1 | 1 | 2 | 0 | 2 | 3 | 3 | 3 |
| 9 | 2 | 2 | 3 | 3 | 3 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 2 | 2 | 1 | 0 | 0 | 1 | 1 | 2 |
| 13 | 1 | 1 | 1 | 1 | 2 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 2 | 2 |
| 14 | 1 | 1 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 3 | 0 | 0 | 0 | 2 | 2 |
| 15 | 1 | 1 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 2 | 2 |
| G2 | | | | | | | | | | | | | | | | | | | | |
| 2 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 |
| 4 | 1 | 2 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | **0** | 0 | 3 | 3 | 3 | **3** | 0 | 0 | 0 | 1 | **1** |
| 5 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 2 |
| 6 | 1 | 1 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 2 |
| 7 | 2 | 3 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 0 | 0 | 1 | 1 | 2 | 0 | 2 | 2 | 2 | 2 |
| 10 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| 11 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 |
| 12 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 |

G1: Virulent strain (rabbits 1, 3, 8, 9: inoculated with a strain isolated from a patient; rabbits 13, 14, 15: ATCC35984). G2: Non-virulent strain (rabbits 2, 4, 5, 6, 7: inoculated with *S. epidermidis* from a patient ocular microbiota, 10, 11 y 12: inoculated with *S. epidermidis* ATCC29122). T0 = baseline; T1 = 8 h post inoculation; T2 = 16h post inoculation; T3 = 24h post inoculation; T4 = 32h post inoculation; T5 = 72h post inoculation.

Table 4
Total clinical scores of experimental eyes after intravitreal injection of *Staphylococcus epidermidis* strains

| Group | Rabbit code | T0 | T1 | T2 | T3 | T4 | T5 |
|---|---|---|---|---|---|---|---|
| Virulent strain | 1 | 0 | 1 | 1 | 1 | 5 | 6 |
| | 3 | 0 | 1 | 4 | 7 | 10 | 10 |
| | 8 | 0 | 2 | 7 | 8 | 8 | 10 |
| | 9 | 0 | 2 | 2 | 7 | 7 | 6 |
| | 13 | 0 | 1 | 1 | 1 | 5 | 7 |
| | 14 | 0 | 1 | 1 | 3 | 5 | 8 |
| | 15 | 0 | 1 | 1 | 3 | 5 | 6 |
| | Mean | 0.0 | 1.3 | 2.4 | 4.3 | 6.4 | 7.6 |
| Non-virulent strain | 2 | 0 | 0 | 0 | 0 | 3 | 3 |
| | 4 | 0 | 1 | 5 | 5 | 6 | 6 |
| | 5 | 0 | 1 | 1 | 2 | 3 | 4 |
| | 6 | 0 | 1 | 1 | 2 | 2 | 7 |
| | 7 | 0 | 3 | 7 | 7 | 7 | 8 |
| | 10 | 0 | 0 | 0 | 0 | 2 | 5 |
| | 11 | 0 | 0 | 1 | 1 | 3 | 4 |
| | 12 | 0 | 0 | 0 | 0 | 3 | 4 |
| | Mean | 0.0 | 0.8 | 1.9 | 2.1 | 3.6 | 5.1 |

Virulent strain (isolated from a patient: rabbits 1, 3, 8, 9, ATCC35984:13, 14, 15). Non-virulent strain (rabbits 2, 4, 5, 6, 7: inoculated with *S. epidermidis* from a patient ocular microbiota, 10, 11 y 12: inoculated with *S. epidermidis* ATCC29122). T0 = baseline; T1 = 8 h post inoculation; T2 = 16h post inoculation; T3 = 24h post inoculation; T4 = 32h post inoculation; T5 = 72h post inoculation.

All rabbits inoculated with the virulent strains [Group 1] developed endophthalmitis, with vitritis observed 8 hours after inoculation, beginning with mild vitreous opacities, becoming severe by the end of the evaluation. In all three rabbits inoculated with the biofilm-producing *S. epidermidis* ATCC 35984 strain, moderate vitreous opacity was observed 24h post-inoculation, with no further changes until the end of the evaluation. Two rabbits were classified as grade 3 and 4 rabbits were classified as grade 2 in the clinical scores.

In the non-virulent group [Group 2], one rabbit developed mild vitritis 24 hours post-inoculation, while two rabbits had mild vitritis observed 8 hours post-inoculation, which increased to moderate by the end of the evaluation. In one rabbit, moderate vitritis started 8 hours post-inoculation. In two of the three rabbits inoculated with a strain of *S. epidermidis* ATCC 29122 a slight vitreous opacity was observed 24 h post-inoculation, without any further changes.

The severity clinical scores were higher in the animals inoculated with the most virulent strains of *S. epidermidis* than in those rabbits inoculated with the less virulent strains (Table 4). Seventy two hours post-inoculation average score was significantly higher in the virulent group (p value = 0.029) Fig. 1.

Ultrasound scans were performed prior to inoculation and afterwards until signs of endophthalmitis had been clinically verified. Initial ultrasound scans were normal in both groups, with clear vitreous and no inflammatory signs (Fig. 2A and C). In the follow-up scans, in the virulent group [Group 1] two rabbits had vitreous condensations with a moderate inflammatory appearance (Fig. 2B), two mild to moderate inflammatory vitreous condensations, two moderate inflammatory vitreous condensations and one rabbit had vitreous condensations with an appearance of severe inflammation (Fig. 2D). In the non-virulent strains [Group 2], four rabbits showed mild to moderate inflammatory vitreous condensations, and in four rabbits were seen mild inflammatory vitreous condensations.

Histopathological examination revealed different degrees of inflammatory infiltration of the vitreous and retina, predominantly by polymorphonuclear leucocytes in both groups of rabbits (Table 5).

Table 5
Histopathologic grading of experimental eyes after intravitreal injection of the different groups of *Staphylococcus epidermidis*

| Strain | Code | Cornea | Anterior chamber | Vitreous | Retina | Total | Mean score |
|---|---|---|---|---|---|---|---|
| Virulent | 1 | 0 | 0 | 2 | 2 | 4 | Isolated from a patient: 4,0 |
| | 3 | 0 | 0 | 1 | 1 | 2 | |
| | 8 | 0 | 0 | 2 | 2 | 4 | |
| | 9 | 0 | 0 | 2 | 1 | 3 | |
| | 13 | 0 | 0 | 3 | 3 | 6 | ATCC: 5,3 |
| | 14 | 0 | 0 | 2 | 2 | 4 | |
| | 15 | 0 | 0 | 3 | 3 | 6 | |
| Non virulent | 2 | 0 | 0 | 0 | 0 | 0 | Isolated from a patient: 2,8 |
| | 4 | 0 | 0 | 1 | 1 | 2 | |
| | 5 | 0 | 0 | 2 | 2 | 4 | |
| | 6 | 0 | 0 | 2 | 1 | 3 | |
| | 7 | 0 | 0 | 3 | 2 | 5 | |
| | 10 | 0 | 0 | 2 | 2 | 4 | ATCC: 3,3 |
| | 11 | 0 | 0 | 2 | 2 | 4 | |
| | 12 | 0 | 0 | 1 | 1 | 2 | |

Virulent strain (rabbits 1, 3, 8, 9: isolated from a patient; 13, 14, 15: ATCC). Non-virulent strain (rabbits 2, 4, 5, 6, 7: isolated from a patient; 10, 11, 12: ATCC). No significant difference (*p* value = 0.175)

Rabbits inoculated with the most virulent *S. epidermidis* strain presented on average a higher histopathologic score, indicating greater presence of tissue inflammation than the group with the less virulent strain (Table 5).

Some of the rabbits inoculated with more virulent strains of *S. epidermidis* showed abundant purulent exudative material consisting of a large number of polymorphonuclear leukocytes (Fig. 3) especially in the posterior part of the vitreous. Likewise, retinal detachment with subretinal polymorphonuclear inflammatory infiltrate was observed. The other ocular structures remained preserved. In the histological sections of the eyes of one of the rabbits inoculated with the less virulent strain of *S. epidermidis*, no purulent exudative changes or alteration of the ocular structures were observed (Fig. 3).

## Discussion

Coagulase-negative staphylococci, including *S. epidermidis*, are responsible for about 70% of all cases of postoperative endophthalmitis. However, the pathobiology of CoNS endophthalmitis is limited to epidemiological and clinical case studies with few experimental studies performed to date.

The present study evaluates the clinical outcome of *Staphylococcus epidermidis*-induced endophthalmitis in rabbits in relation to the antibiotic resistance and virulence-gene factor profiles of the infecting strain. *S. epidermidis* strains are often multidrug resistant and its association with biofilm formation makes treatment of these infections difficult. The ability to form biofilms is regarded as the major pathogenic factor [20].

Biofilm production and implicated genes, especially *ica*, have been suggested as markers for CoNS strains with clinical significance. Some authors reported that *S. epidermidis* isolated from patients with infections have higher capacity of producing biofilm

compared to *S. epidermidis* isolated from normal biota [21, 22]. However, a large percentage of commensal methicillin-resistant *Staphylococcus epidermidis* (MRSE) isolates recovered from nares produced biofilm and are *ica*-AD, and *atl*E genes carriers [23]. In agreement with Araujo et al. [24] and other studies [24–26] we have shown a high percentage of the ocular microbiota of patients undergoing cataract surgery had biofilm encoding genes [16, 17]. The high percentage of CoNS isolates - obtained from non-infected eyes with and without prophylactic treatment - with genes involved in virulence highlight the potential danger of the CoNS ocular microbiota in ocular infectious.

A total of 15 rabbits were inoculated by intravitreal injection with *S. epidermidis* strains harboring different antibiotic resistance and biofilm forming genes. The animals were monitored every three hours by clinical examination and ultrasound until signs of endophthalmitis was evident. In addition, histopathological assessment was carried out at day 15 post-inoculation.

Bacterial invasion of the body causes two pathological processes, which include toxic effects of bacterial virulence factors on the host and host defense [27]. Many studies have shown that bacteria can secrete a variety of toxic substances and that bacterial cell wall components have toxic effects on intraocular structures [28]. A clinical feature of infectious endophthalmitis is an inflammatory exudate which is apparent at an early stage. In the study, all animals developed some degree of infectious endophthalmitis. The clinical score was higher in those animals inoculated with the virulent strain [Group 1] and the time-interval between inoculation and clinical signs of endophthalmitis was shorter. These results are in agreement with the study carried out by Kaspar et al. [18], where experimental endophthalmitis induced by fully susceptible coagulase-negative staphylococci resulted in a clinically distinct milder inflammatory response in the early stage compared to endophthalmitis resulting from partially resistant or multiresistant bacteria.

Another clinical feature of infectious endophthalmitis is inflammatory cell infiltration. Neutrophils play a key role in both bacterial clearance and inflammation in bacterial endophthalmitis. In the present study, the histopathological results showed a greater inflammatory reaction in the vitreous and retina, correlating with the clinical and ultrasound findings in rabbits from the virulent group, however the total histologic score was not significantly different between the two groups. The lack of significant difference in the histological results might be explained by the fact that the time of animal sacrifice was 15 days after inoculation as compared to 5 days carried out in the study by Kaspar et al [18].

Meredith et al. [29] evaluated the development of endophthalmitis depending to the concentration of the inoculum and found that the initial inflammatory clinical signs were positively correlated the size of the inoculum. The results of the present study show that *S. epidermidis* was able to cause endophthalmitis in rabbit eyes at a relatively low inoculum resulting in significant inflammation and retinal tissue damage after a short time-interval of less than 12 hours following bacterial inoculation. These findings highlight the need for appropriate preventive measures in the surgical setting and stress the importance of diligent povidone-iodine prophylaxis before cataract surgery. Many studies report that the greatest reduction of the conjunctival bacterial load, which is used as a surrogate parameter for the risk of postoperative endophthalmitis, is achieved by povidone-iodine and not by topical antibiotics [30–32]. Furthermore, povidone-iodine has been shown to reduce the actual rate of postoperative endophthalmitis in a prospective trial by Speaker and Menikoff [33]. Other studies report a decline of the rate of infectious endophthalmitis after intraocular surgery and intravitreal injections following the introduction of standardized preoperative prophylaxis protocols including a flush-irrigation of the conjunctival sac with povidone-iodine [34, 35]. Ophthalmologists must be vigilant of early signs of endophthalmitis.

In patients undergoing intraocular surgery, the identification of multiresistant and virulent genes CoNS carriers in the conjunctiva and eyelid by a suitable rapid molecular method should be developed and included among the routine laboratory testing. In those patients carrying these type of bacteria a more drastic protocol to ensure elimination of these bacteria should be applied in order to reduce even more the incidence of endophthalmitis rate after intraocular surgeries, especially in the presence of intra surgery complications, such as rupture of the posterior capsule posterior – during cataract surgery.

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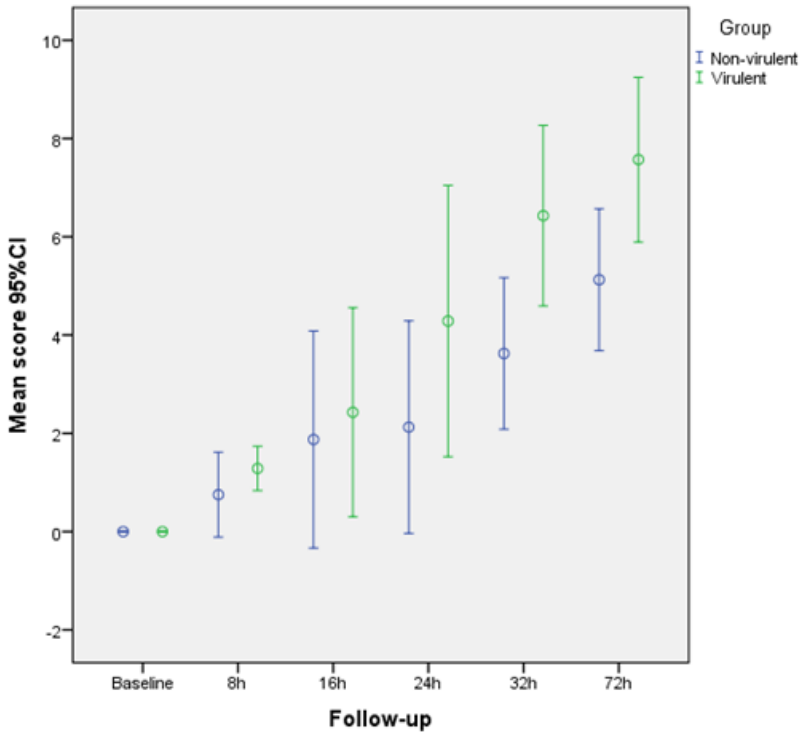
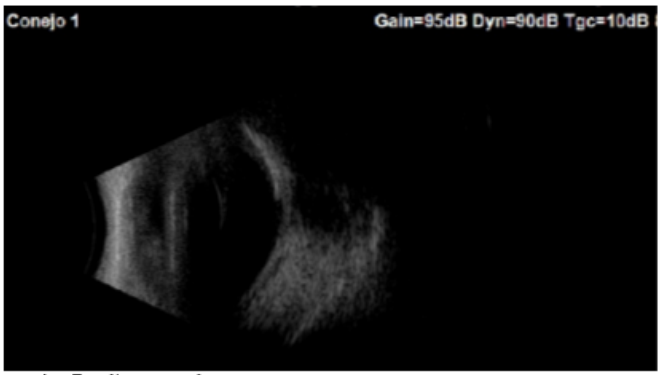
## Figures

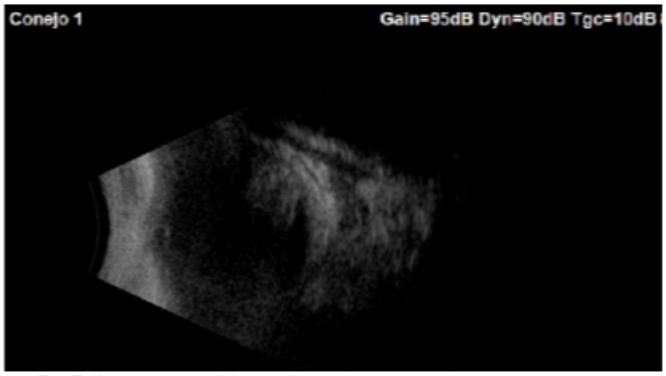



## Figure 1

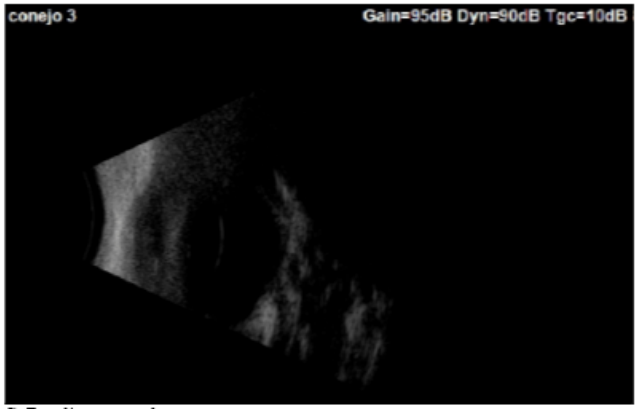
Post-inoculation follow-up. Comparison of the total clinical scores of the eyes of rabbits inoculated with virulent and non-virulent strains of *S. epidermidis*. Average score was significantly higher in the virulent group at 32 h (*p* value = 0,014) and 72 h post inoculation (*p* value = 0.019)



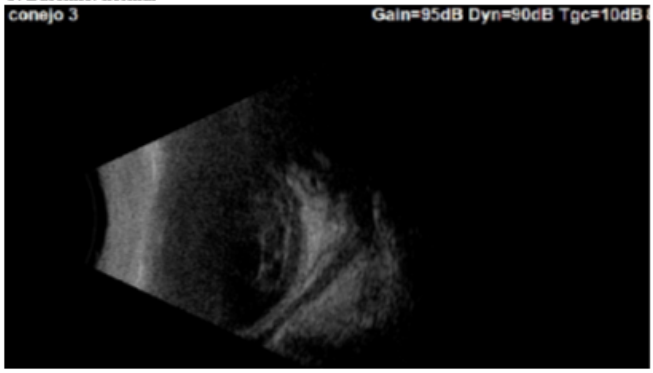

A. Baseline: normal




B. Follow-up scan: mild to moderate vitreous opacities




C. Baseline: normal




D. follow-up scan: vitreous opacities of severe density.

Figure 2

Sonographic results of experimental eyes after intravitreal injection of virulent and non-virulent strains of *Staphylococcus epidermidis.* Initial ultrasound scans were normal in both groups, with clear vitreous and no inflammatory signs (A and C). In the follow-up scans, in the virulent group [Group 1] two rabbits had vitreous condensations with a mild to moderate inflammatory appearance (B), and one rabbit had vitreous condensations with an appearance of severe inflammation (D).

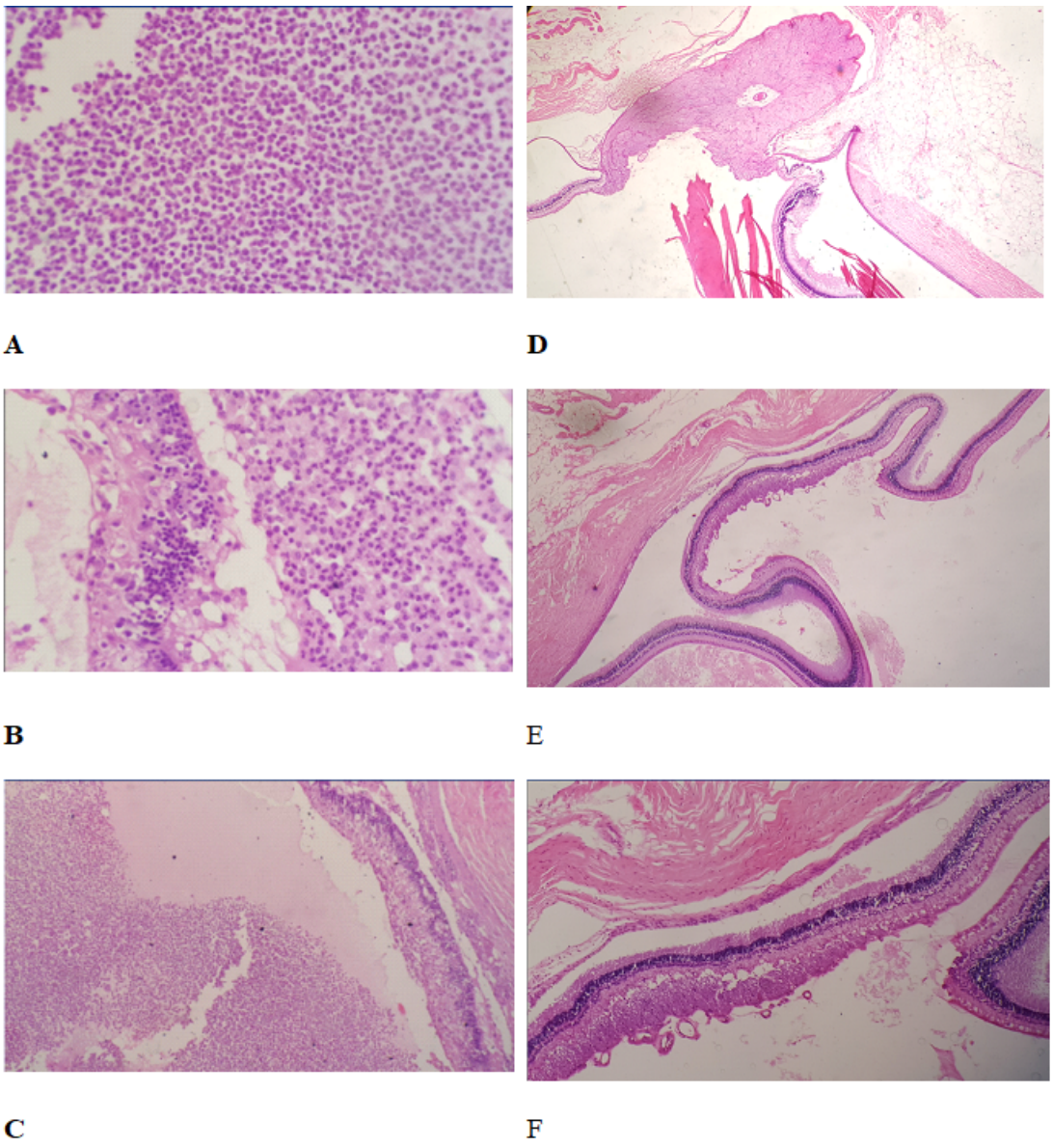



Figure 3

Histopathologic evaluation of the eyes of rabbits inoculated with more virulent (left A-C) and less virulent (right D-F) strains of *S. epidermidis*