MEDICINA CLÍNICA Y SOCIAL
Med. clín. soc. 2024;8(2):222-231. e-ISSN: 2521-2281
MAYO-AGOSTO. https://doi.org/10.52379/mcs.v8i2.420

ORIGINAL

Comparative Study of Staphylococcus epidermidis Virulence Profiles in an animal Model of Endophthalmitis

Estudio comparativo de los perfiles de virulencia de Staphylococcus epidermidis en un modelo animal de endoftalmitis

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Received: 22/03/2024
Revised: 11/02/2024
Accepted: 13/05/2024

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Conflicts of interests

The authors declare that there is no conflict of interest.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

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ABSTRACT

Introduction: Gram-positive staphylococci are responsible for over 90% of cases of postoperative infectious endophthalmitis, with Staphylococcus epidermidis accounting for approximately 70% of isolated microorganisms. **Objective:** To evaluate the relationship between S. epidermidis virulence and severity of endophthalmitis in an animal model. **Methodology:** New Zealand albino rabbits were divided into two groups and administered 100 colony-forming units of S. epidermidis strains. In the virulent group, four rabbits received a multi-antibiotic resistant, mecA, ica, and atlE gene carrying S. epidermidis strain, from a patient´s conjuntival microbiota and three a biofilm-forming S. epidermidis ATCC 35984. In the non-virulent group, five rabbits were inoculated with a strain sensitive to all tested antibiotics and lacking mecA, ica, and atlE genes, also from a patient and three rabbits received the non-producer biofilm S. epidermidis ATCC 29122. Clinical and ultrasound examinations were conducted every three hours until endophthalmitis symptoms appeared, followed by daily clinical assessments. Histological evaluations were performed 15 days post-inoculation. **Results:** The less virulent group displayed milder inflammation and reduced intraocular damage in comparison to the more virulent group based on clinical and ultrasound observations. Nevertheless, histopathological analysis revealed similar inflammation in all groups, 15 days post-inoculation. **Discussion:** Less virulent S. epidermidis strains induced less severe inflammation as observed through clinical and ultrasound assessments. However, long-term histopathological assessments showed effects comparable to those seen with the more virulent strain.

Keywords: ocular microbiota; Staphylococcus epidermidis, animal model, virulence factors, endophthalmitis

RESUMEN

Introducción: Gram-positive staphylococci are responsible for over 90% of cases of postoperative infectious endophthalmitis, with Staphylococcus epidermidis accounting for approximately 70% of isolated microorganisms. **Objetivo:** Evualuar la relación entre la virulencia de S. epidermidis y la gravedad de la endoftalmitis en un modelo animal. **Metodología:** Se dividieron conejos albinos de Nueva Zelanda en dos grupos y se les administraron 100 unidades formadoras de colonias de cepas de S. epidermidis. En el grupo virulento, cuatro conejos recibieron una cepa multirresistente de S. epidermidis portadora de gen mecA, ica y atlE, de la microbiota conjuntival de un paciente y tres conejos la cepa S. epidermidis ATCC 35984 formadora de biopelículas. En el grupo no-virulento, se inocularon cinco conejos con una cepa sensible a todos los antibióticos probados y que carecía de los genes mecA, ica y atlE, también de un paciente y tres conejos recibieron la cepa S. epidermidis ATCC 29122 no productora de biopelícula. Se realizaron exámenes clínicos y ecográficos cada tres horas hasta la aparición de síntomas de endoftalmitis, seguido de evaluaciones clínicas diarias. Las evaluaciones histológicas se realizaron 15 días después de la inoculación. **Resultados:** El grupo menos virulento mostró una inflamación más leve y un daño intraocular reducido en comparación con el grupo más virulento según observaciones clínicas y ecográficas. Sin embargo, el análisis histopatológico reveló una inflamación similar en todos los grupos, 15 días después de la inoculación. **Discusión:** Las cepas de S. epidermidis menos virulentas indujeron una inflamación menos grave, como se observó mediante evaluaciones clínicas y ecográficas. Sin embargo, las evaluaciones histopatológicas a largo plazo mostraron efectos comparables a los observados con la cepa más virulenta.

Palabras clave: microbiota ocular; Staphylococcus epidermidis, modelo animal, factores de virulencia, endoftalmitis

How to cite this article: Samudio M, Abente S, Villalba V, Penniecook J, González E, Schinini A, et al. Comparative Study of Staphylococcus epidermidis Virulence Profiles in an animal Model of Endophthalmitis. Med. clín. soc. 2024;8(2):222-231

INTRODUCTION

Endophthalmitis is a condition characterized by significant inflammation in the fluids and tissues inside the eye, that often leads to severe visual impairment. More frequently it is caused by exogenous pathogens following ocular surgeries, intraocular medication administration, penetrating eye injuries, or as an extension of a corneal infection. More rarely, endogenous pathogens can also cause endophthalmitis (1).

The incidence of postoperative endophthalmitis following cataract surgery ranges from 0.056% to 1.3%., which rises to 30% after open-globe trauma. Notably, the primary source of postoperative endophthalmitis is the patient's bacterial flora found in the conjunctiva and eyelid (2,3).

The onset of intraocular inflammation after microbial introduction can vary and depends on several factors, including the virulence of the microorganisms, the size of the initial infection, the patient's immune response, and the inflammatory reactions (4).

Gram-positive staphylococci are responsible for over 90% of cases of postoperative infectious endophthalmitis, with Staphylococcus epidermidis accounting for approximately 70% of isolated microorganisms. Despite its relatively low virulence, once inside the eye, S. epidermidis can lead to severe and vision-threatening endophthalmitis (2).

The destruction of intraocular structures, which can result in complete vision loss, occurs due to toxins and enzymes released by the microorganisms, as well as the response of the host's immune system (5). There is existing evidence linking the ability of microorganisms like Staphylococcus aureus, Enterococcus faecalis, and Bacillus cereus to produce specific toxins with their virulence in endophthalmitis (6-9). However, data on the effects of various strains of S. epidermidis in this context remain limited.

Bacterial products that play a significant role in biofilm formation are some of the most extensively studied virulence factors of Staphylococcus epidermidis (10). These virulence determinants include the genes responsible for enhancing the virulence of Staphylococci, namely ICA (intercellular adhesin-operon – ICA ADBC). Within this group, icaA and icaD are responsible for the formation of biofilms, while the atlE gene codes for a protein crucial in the initial adherence of bacteria to surfaces (11-13).

Previous research conducted by the authors involved assessing biofilm production through phenotypic methods in Coagulase-negative Staphylococci (CoNS) isolates (14). This work revealed a high frequency of genes associated with virulence factors related to biofilm formation (ica and atlE), as well as the mecA gene, identified through a multiplex PCR assay, in coagulase-negative Staphylococcus isolates obtained from ocular samples of patients undergoing cataract surgery (15,16).

Endophthalmitis animal models serve as valuable tools for unraveling the underlying mechanisms of this disease, depending on the infection source and the specific pathogen involved. These models enable researchers to explore various facets of the pathogenesis and pathophysiology of bacterial endophthalmitis, facilitating the development of anti-inflammatory treatment strategies and the evaluation of antibiotic pharmacokinetics and efficacy. While no single animal model can perfectly replicate the complexities of human bacterial endophthalmitis, researchers have successfully employed these models to gain insights into the infectious process, host responses, and potential therapeutic interventions (17).

However, data from studies using animal models to investigate the relationship between microbial virulence and the severity of endophthalmitis remain limited. For instance, Mino de Kaspar et al. demonstrated in the rabbit model that the severity of inflammation correlated with the resistance pattern of the S. epidermidis strain responsible for causing endophthalmitis (18). Resistant strains exhibited the ability to induce endophthalmitis in rabbits more rapidly and with greater severity than sensitive strains. Nonetheless, this study did not use molecular techniques to characterize the strains responsible for the infection.

To gain a deeper understanding of this phenomenon, our present study utilized the same animal model to assess whether mecA-positive (mecA+) and biofilm-producing S. epidermidis strains exhibit higher virulence compared to mecA-negative (mecA-) non-biofilm-producing strains. We evaluated clinical, ultrasound, and histopathological parameters to explore these differences comprehensively.

METHODS

S. epidermidis strains

S. epidermidis strains used in this study exhibited varying levels of antimicrobial susceptibility and virulence gene profiles, as detailed in Table 1. The S. epidermidis strains were initially cultivated on blood agar plates at 37°C for a period of 24 hours.

Subsequently, a single colony was selected from each strain and transferred into 10 ml of trypticase soy broth (TSB), where it underwent further incubation for an additional 24 hours at the same temperature, resulting in the creation of a stock solution. The bacterial organisms were then suspended in TSB to achieve an absorbance reading of 0.15 at 625 nm using a spectrophotometer, corresponding to a bacterial density of approximately 10^7 CFU/mL. To achieve the desired bacterial concentration for intravitreal injection, further dilution was carried out using sterile balanced salt solution. This final dilution was subsequently plated on trypticase soy agar to confirm the actual colony-forming units (CFU).

To ascertain the initial resistance pattern of each strain, antibiotic susceptibility testing was conducted using the Kirby-Bauer disc diffusion method. Mueller-Hinton agar (bioMérieux®, Stuttgart, Germany) was utilized for this purpose, following the guidelines established by the Clinical and Laboratory Standards Institute (CLSI).

The presence of genes encoding biofilm formation (ica and atlE) and gene encoding methicillin resistance (mecA) were detected by a multiplex PCR as described elsewhere (1).

TABLE 1. ANTIBIOTIC SENSITIVITY AND VIRULENCE GENE FACTOR PROFILE OF EXPERIMENTAL *S. EPIDERMIDIS* STRAINS

Antibiotic tested	Virulent Strain Group Isolated from a patient[a]	ATCC[b]	Less Virulent Strain Group 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

Animals

A group of 15 New Zealand white albino rabbits, each weighing between 2.0 and 2.5 kg, were selected for this study. These rabbits were individually housed in cages within a controlled environment, following a 12-hour light-dark cycle, and had unrestricted access to food and water. These accommodations were provided at the animal care facility of the Instituto de Investigaciones en Ciencias de la Salud at the National University of Asunción in Paraguay.

The well-being of the rabbits was carefully maintained, in compliance with the Guiding Principles for the Care and Use of Animals. The research team consisted of experts from various fields, including ophthalmologists, a veterinarian, a microbiologist, and a pathologist.

Inoculation

The fifteen rabbits were divided into two groups. In Group 1, seven rabbits were inoculated with highly virulent strains of *S. epidermidis*. Among them, four rabbits received a strain of *S. epidermidis* that demonstrated resistance to more than three antibiotics. This strain carried the *mec*A, *ica*, and *atl*E genes, and it was originally isolated from a patient who had undergone cataract surgery. The remaining three rabbits in Group 1 were inoculated with a multi-antibiotic resistant *S. epidermidis* strain, ATCC 35984 (Microbiologics, USA), known for its biofilm-forming capabilities. In Group 2, eight rabbits were inoculated with less virulent strains of *S. epidermidis*. Five of these rabbits received a strain isolated from a patient who had undergone cataract surgery.

This strain was sensitive to all tested antibiotics and did not carry the *mec*A, *ica*, and *atl*E genes. The remaining three rabbits in Group 2 were inoculated with a non-biofilm-forming *S. epidermidis* strain, ATCC 29122 (Microbiologics, USA).

All fifteen rabbits underwent intramuscular anesthesia through the administration of ketamine hydrochloride (35 mg/kg body weight) and lidocaine hydrochloride (5 mg/kg body weight). Before to bacterial inoculation,

additional topical anesthesia was applied using 0.5% proparacaine hydrochloride eye drops. Under anesthesia, a paracentesis was created on the right eye, allowing for the aspiration of 0.1 ml of aqueous humor from the anterior chamber using a 30-gauge needle attached to a tuberculin syringe. Subsequently, 0.1 ml of the *S. epidermidis* suspension, containing 100 CFU, was introduced into the vitreous cavity of one eye in each rabbit via the pars plana, approximately 2 mm posterior to the limbus, using a 30-gauge needle on a tuberculin syringe. The other eye remained untreated and served as the control.

Slit-lamp biomicroscopy and indirect ophthalmoscopy

Following the inoculation procedure, slit-lamp biomicroscopy and indirect ophthalmoscopy were conducted at three-hour intervals until the emergence of endophthalmitis symptoms, and subsequently, at 24-hour intervals. Before to each examination, 1% tropicamide and 2.5% phenylephrine eye drops were applied to dilate the pupils. The severity of ocular inflammation was assessed based on anterior chamber reaction and fundus reflex, utilizing the Peyman et al. model as a reference (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

Ultrasound evaluation

After the application of topical anesthesia using 0.5% proparacaine hydrochloride eye drops into the conjunctival sac, a trans-palpebral ultrasound examination using a 10 MHz transducer was performed. To ensure accurate imaging, an abundant amount of methylcellulose gel was applied over the eyelid to prevent the formation 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.

Animal sacrifice

On the 15th day post-injection, the animals were humanely euthanized in a CO_2 chamber. Subsequently, 0.1 ml of vitreous humor was aspirated using a 30-gauge needle attached to a tuberculin syringe, and the eyes were enucleated and fixed in a 10% formalin solution in phosphate-buffered saline for histopathological analysis.

Histopathological evaluation

For histopathological analysis, the eyes were embedded in paraffin, sectioned, and stained using standard protocols, including hematoxylin-eosin, periodic acid-Schiff, and Gram stain. Sections were carefully examined and assessed by an investigator who was unaware of the treatment group identities. A modified defined classification scheme was used to quantify the level of inflammation in various ocular structures, including the cornea, iris, vitreous base, ciliary body, and retina (Table 2). The assessment of retinal inflammation was performed at three distinct locations: the central retina, approximately 20° paracentral retina, and near the ora serrata. Evaluation

of the retina was conducted on both the nasal and temporal sides to prevent any false readings due to localized swelling.

The histopathological evaluation involved categorizing the presence or absence of acute inflammation and classifying its degree into four categories: "no inflammation," "mild," "moderate," and "severe inflammation" for various anatomical structures such as the cornea, iris, ciliary body, choroid, vitreous, and retina. Scores were assigned based on the findings, as detailed in Table 2, with zero indicating uncompromised structures, one for mild involvement, two for moderate involvement, and three for severe involvement. The total possible score in the histopathological quantification was 12.

Data analysis

Results were presented as means ± SD. Intergroup comparisons were performed using Kruskal Wallis test or Mann Whitney test. A p–value less than 0.05 was considered statistically significant.

RESULTS

Fifteen rabbits inoculated with *S. epidermidis* strains with two profiles of antibiotic susceptibility and virulence factors were studied. The clinical scores of the study eyes following intravitreal injection of these *Staphylococcus epidermidis* strains are presented in Table 3 and Table 4.

In the virulent strain-inoculated group (Group 1), all rabbits developed endophthalmitis, characterized by vitritis observed within 8 hours post-inoculation. This vitritis initially manifested as mild vitreous opacities, which progressed to severe by the end of the evaluation. Among the three rabbits inoculated with the biofilm-producing *S. epidermidis* ATCC 35984 strain, moderate vitreous opacity was observed 24 hours post-inoculation, with no significant changes thereafter. Clinical scores classified two rabbits as grade 3 and the remaining 4 as grade 2.

In contrast, the non-virulent group (Group 2) exhibited a milder response. One rabbit developed mild vitritis 24 hours post-inoculation, while two rabbits showed mild vitritis 8 hours post-inoculation, which escalated to moderate by the end of the evaluation. In one rabbit, moderate vitritis was evident 8 hours post-inoculation. Among the three rabbits inoculated with a strain of *S. epidermidis* ATCC 29122, a slight vitreous opacity was noted 24 hours post-inoculation, with no significant changes subsequently.

Clinical scores revealed higher severity in animals inoculated with the most virulent S. epidermidis strains compared to those inoculated with the less virulent strains (Table 4). At the 72-hour post-inoculation mark, the average score was significantly higher in the virulent group (p-value = 0.029) as depicted in Figure 1. Average score was significantly higher in the virulent group at 32 h (*p* value = 0,014) and 72 h post inoculation (Mann Whitney test *p* value = 0.019)

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

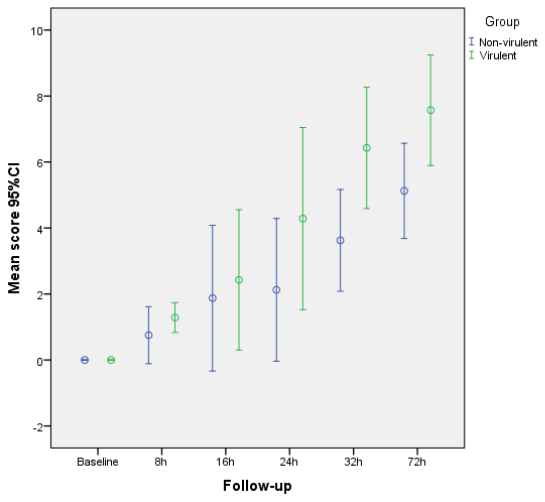
G1: Virulent strain (rabbits 1, 3, 8, 9: inoculated with a strain isolated from a patient; rabbits 13, 14, 15: ATCC35984). G2: Non-virulent strain (rabbits 2, 4, 5, 6, 7: inoculated with *S. epidermidis* from a patient ocular microbiota, 10, 11 y 12: inoculated with *S. epidermidis* ATCC29122). T0 = 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

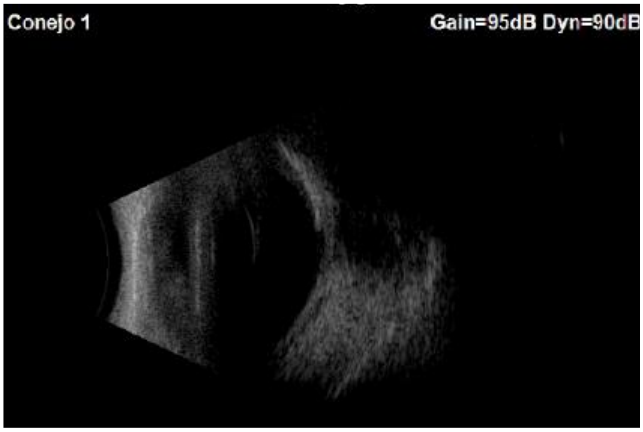
Virulent strain (isolated from a patient: rabbits 1, 3, 8, 9, ATCC35984:13, 14, 15). Non-virulent strain (rabbits 2, 4, 5, 6, 7: inoculated with *S. epidermidis* from a patient ocular microbiota, 10, 11 y 12: inoculated with *S. epidermidis* ATCC29122). T0 = baseline; T1 = 8 h post inoculation; T2 =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

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*.

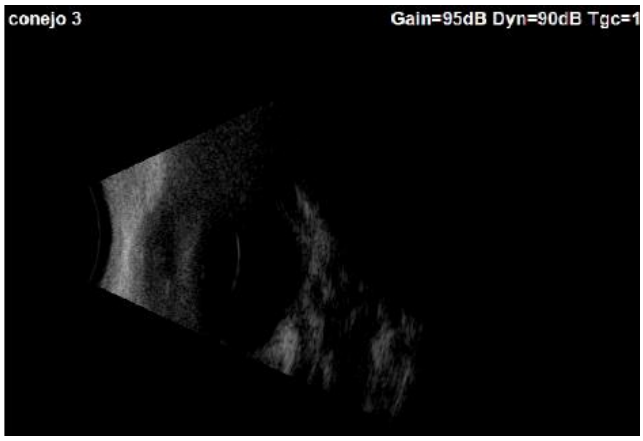


Ultrasound scans were conducted both before and after inoculation until clinical signs of endophthalmitis were confirmed. Initial scans showed no abnormalities in either group, with clear vitreous or no signs of inflammation. Subsequent scans revealed mild to moderate inflammatory vitreous condensations in two rabbits from the non-virulent strain-inoculated group (Group 2). In the virulent group (Group 1), one rabbit exhibited vitreous condensations with mild inflammatory characteristics, two rabbits displayed a moderate inflammatory appearance, and the remaining two rabbits had mild to moderate condensations (Figure 2). 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).

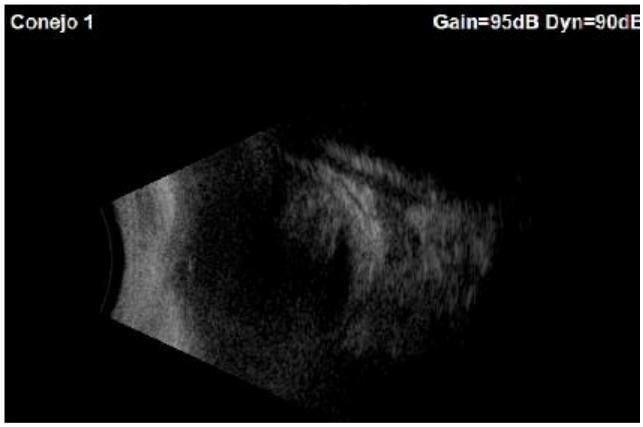
FIGURE 2. SONOGRAPHIC RESULTS OF EXPERIMENTAL EYES AFTER INTRAVITREAL INJECTION OF VIRULENT AND NON-VIRULENT STRAINS OF *STAPHYLOCOCCUS EPIDERMIDIS*



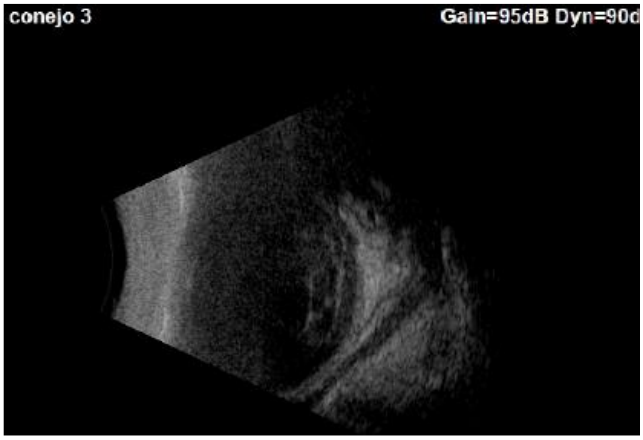
A. Baseline: normal



C. Baseline: normal



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



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

Histopathological examination unveiled varying degrees of inflammatory infiltration in the vitreous and retina, primarily consisting of polymorphonuclear leukocytes in both groups of rabbits (Figure 3). Rabbits inoculated with the most virulent *S. epidermidis* strain displayed, on average, higher histopathologic scores, indicating a greater presence of tissue inflammation compared to the group inoculated with the less virulent strain (Table 5).

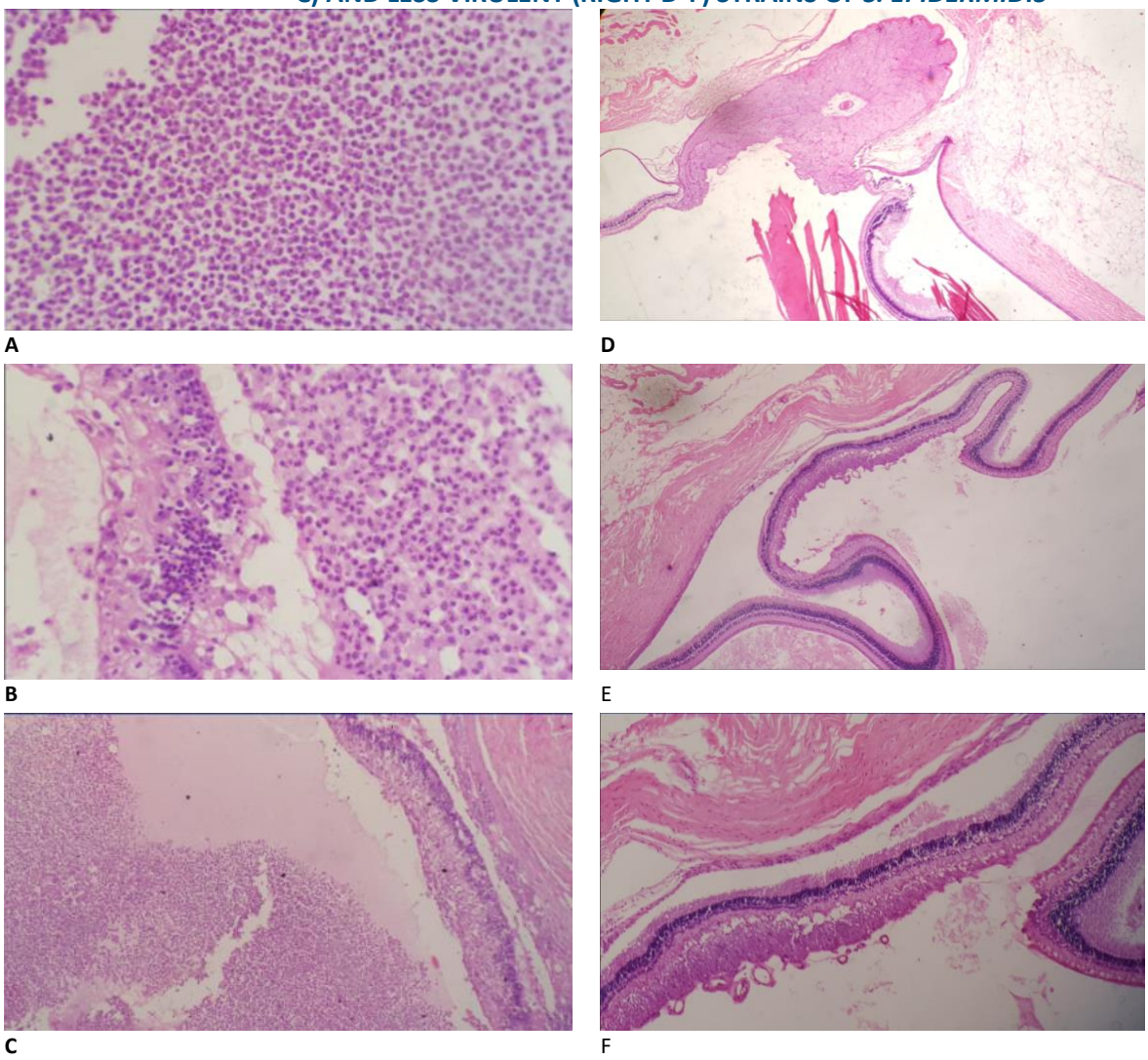
TABLE 5. HISTOPATHOLOGIC GRADING OF EXPERIMENTAL EYES AFTER INTRAVITREAL INJECTION OF THE DIFFERENT GROUPS OF *STAPHYLOCOCCUS EPIDERMIDIS*

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 *(*Kruskal Wallis test *p* value = 0.175)

In some rabbits inoculated with the more virulent *S. epidermidis* strains, abundant purulent exudative material composed mainly of polymorphonuclear leukocytes was observed, particularly in the posterior part of the vitreous. Additionally, retinal detachment accompanied by subretinal polymorphonuclear inflammatory infiltrate was noted. Conversely, histological sections of the eyes of one rabbit inoculated with the less virulent *S. epidermidis* strain revealed no purulent exudative changes or alterations in ocular structures (Figure 3).

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*



DISCUSSION

This study assesses the clinical outcomes of endophthalmitis induced by *Staphylococcus epidermidis* in rabbits, taking into consideration the antibiotic resistance and virulence-gene profiles of the infecting strain. *Staphylococcus epidermidis* strains are frequently resistant to multiple antibiotics, and their propensity to form biofilms poses a significant challenge for treatment. The capacity to form biofilms is recognized as the primary pathogenic factor (20). Biofilm production and associated genes, particularly *ica*, have been proposed as markers for clinically significant Coagulase-negative Staphylococci strains. Some studies have indicated that *S. epidermidis* strains isolated from infected patients exhibit a higher propensity to produce biofilms compared to those isolated from normal flora (21,22).

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 agree 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). One study in a murine model showed that while the bacterial burden gradually declines in the infected eye, neutrophil infiltration and retinal tissue damage continue resulting in significant vision loss (23).

Meredith et al. (24) evaluated the development of endophthalmitis depending on the concentration of the inoculum and found that the initial inflammatory clinical signs were positively correlated with 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. Once inside the eye, *S. epidermidis* can lead to severe and vision-threatening endophthalmitis (2). 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 (25-27). Furthermore, povidone-iodine has been shown to reduce the actual rate of postoperative endophthalmitis in a prospective trial by Speaker and Menikoff (28). Other studies report a decline in 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 (29,30). 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 in the routine laboratory testing. In those patients carrying these types of bacteria a more drastic protocol to ensure the elimination of these bacteria should be applied 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.

AUTHORS CONTRIBUTIONS

Conceptualization: MS, HMK. Design: MS, HMK. Data collection: SA, VV, JP, EG, AS, SF, CD. Data analysis: MS. Drafting, critical revision of the article and approval of the final version: All authors.

REFERENCES

1. Schwartz SG, Flynn HW Jr, Das T, Mieler WF. Ocular Infection: Endophthalmitis. Dev Ophthalmol. 2016;55:176-88. https://doi.org/10.1159/000431195
2. Speaker MG, Menikoff JA. Postoperative endophthalmitis: pathogenesis, prophylaxis, and management. Int Ophthalmol Clin. 1993 Winter;33(1):51-70. https://doi.org/10.1097/00004397-199303310-00006
3. Speaker MG, Milch FA, Shah MK, Eisner W, Kreiswirth BN. Role of external bacterial flora in the pathogenesis of acute postoperative endophthalmitis. Ophthalmology. 1991 May;98(5):639-49; discussion 650. https://doi.org/10.1016/s0161-6420(91)32239-5
4. Durand ML. Endophthalmitis. Clin Microbiol Infect. 2013;19(3):227-34. https://doi.org/10.1111/1469-0691.12118
5. Callegan MC, Engelbert M, Parke DW 2nd, Jett BD, Gilmore MS. Bacterial endophthalmitis: epidemiology, therapeutics, and bacterium-host interactions. Clin Microbiol Rev. 2002;15(1):111-24. https://doi.org/10.1128/cmr.15.1.111-124.2002
6. Callegan MC, Jett BD, Hancock LE, Gilmore MS. Role of hemolysin BL in the pathogenesis of extraintestinal *Bacillus cereus* infection assessed in an endophthalmitis model. Infect Immun. 1999;67(7):3357-66. https://doi.org/10.1128/iai.67.7.3357-3366.1999
7. Booth MC, Cheung AL, Hatter KL, Jett BD, Callegan MC, Gilmore MS. Staphylococcal accessory regulator (sar) in conjunction with agr contributes to *Staphylococcus aureus* virulence in endophthalmitis. Infect Immun. 1997;65(4):1550-6. https://doi.org/10.1128/iai.65.4.1550-1556.1997

8. Jett BD, Jensen HG, Atkuri RV, Gilmore MS. Evaluation of therapeutic measures for treating endophthalmitis caused by isogenic toxin-producing and toxin-nonproducing Enterococcus faecalis strains. Invest Ophthalmol Vis Sci. 1995;36(1):9-15. https://pubmed.ncbi.nlm.nih.gov/7822163/
9. Stevens SX, Jensen HG, Jett BD, Gilmore MS. A hemolysin-encoding plasmid contributes to bacterial virulence in experimental Enterococcus faecalis endophthalmitis. Invest Ophthalmol Vis Sci. 1992;33(5):1650-6. https://pubmed.ncbi.nlm.nih.gov/1559764/
10. Götz F. Staphylococcus and biofilms. Mol Microbiol. 2002;43(6):1367-78. https://doi.org/10.1046/j.1365-2958.2002.02827.x
11. McKenney D, Hübner J, Muller E, Wang Y, Goldmann DA, Pier GB. The ica locus of *Staphylococcus epidermidis* encodes production of the capsular polysaccharide/adhesin. Infect Immun. 1998;66(10):4711-20. https://doi.org/10.1128/iai.66.10.4711-4720.1998
12. Gad GF, El-Feky MA, El-Rehewy MS, Hassan MA, Abolella H, El-Baky RM. Detection of icaA, icaD genes and biofilm production by *Staphylococcus aureus* and *Staphylococcus epidermidis* isolated from urinary tract catheterized patients. J Infect Dev Ctries. 2009;3(5):342-51. https://doi.org/10.3855/jidc.241
13. Vandecasteele SJ, Peetermans WE, Merckx R, Van Eldere J. Expression of biofilm-associated genes in Staphylococcus epidermidis during in vitro and in vivo foreign body infections. J Infect Dis. 2003;188(5):730-7. https://doi.org/10.1086/377452
14. Fariña N, Carpinelli L, Samudio M, Guillén R, Laspina F, Sanabria R, et al. Clinically significant coagulase-negative staphylococci: most frequent species and virulence factors. Rev Chilena Infectol. 2013;30(5):480-8. https://doi.org/10.4067/s0716-10182013000500003
15. López Y, Samudio M, Fariña N, Castillo V, Abente S, Nentwich MM, González-Britez N, Laspina F, Carron A, Cibils D, de Kaspar HM. Effect of antibiotic prophylaxis on Coagulase-negative Staphylococcus virulence factor profiles in patients undergoing cataract surgery. Int Ophthalmol. 2017;37(4):929-937. https://doi.org/10.1007/s10792-016-0355-8
16. Fariña N, Samudio M, Carpinelli L, Nentwich MM, de Kaspar HM. Methicillin resistance and biofilm production of Staphylococcus epidermidis isolates from infectious and normal flora conjunctiva. Int Ophthalmol. 2017;37(4):819-825. https://doi.org/10.1007/s10792-016-0339-8
17. Astley RA, Coburn PS, Parkunan SM, Callegan MC. Modeling intraocular bacterial infections. Prog Retin Eye Res. 2016;54:30-48. https://doi.org/10.1016/j.preteyeres.2016.04.007
18. Miño De Kaspar H, Hoepfner AS, Engelbert M, Thiel M, Ta CN, Mette M, Schulze-Schwering M, Grasbon T, Sesma-Vea B, Casas JM, Iturralde-Goñi R, Klauss V, Kampik A. Antibiotic resistance pattern and visual outcome in experimentally-induced Staphylococcus epidermidis endophthalmitis in a rabbit model. Ophthalmology. 2001;108(3):470-8. https://doi.org/10.1016/s0161-6420(00)00545-5
19. Peyman GA, Paque JT, Meisels HI, Bennett TO. Postoperative endophthalmitis: a comparison of methods for treatment and prophlaxis with gentamicin. Ophthalmic Surg. 1975 Spring;6(1):45-55. URL
20. Ishak MA, Gröschel DH, Mandell GL, Wenzel RP. Association of slime with pathogenicity of coagulase-negative staphylococci causing nosocomial septicemia. J Clin Microbiol. 1985;22(6):1025-9. https://doi.org/10.1128/jcm.22.6.1025-1029.1985
21. Galdbart JO, Allignet J, Tung HS, Rydèn C, El Solh N. Screening for Staphylococcus epidermidis markers discriminating between skin-flora strains and those responsible for infections of joint prostheses. J Infect Dis. 2000;182(1):351-5. https://doi.org/10.1086/315660
22. Klingenberg C, Aarag E, Rønnestad A, Sollid JE, Abrahamsen TG, Kjeldsen G, Flaegstad T. Coagulase-negative staphylococcal sepsis in neonates. Association between antibiotic resistance, biofilm formation and the host inflammatory response. Pediatr Infect Dis J. 2005;24(9):817-22. https://doi.org/10.1097/01.inf.0000176735.20008.cd
23. Das S, Singh S, Kumar A. Bacterial Burden Declines But Neutrophil Infiltration and Ocular Tissue Damage Persist in Experimental Staphylococcus epidermidis Endophthalmitis. Front Cell Infect Microbiol. 2021 17;11:780648. https://doi.org/10.3389/fcimb.2021.780648
24. Meredith TA, Trabelsi A, Miller MJ, Aguilar E, Wilson LA. Spontaneous sterilization in experimental Staphylococcus epidermidis endophthalmitis. Invest Ophthalmol Vis Sci. 1990;31(1):181-6. URL
25. Li B, Miño de Kaspar H, Haritoglou C, Kook D, Kampik A, Sheng M, Nentwich MM. Comparison of 1-day versus 1-hour application of topical neomycin/polymyxin-B before cataract surgery. J Cataract Refract Surg. 2015;41(4):724-31. https://doi.org/10.1016/j.jcrs.2014.06.042
26. Nentwich MM, Rajab M, Ta CN, He L, Grueterich M, Haritoglou C, Gandorfer A, Kampik A, Mino De Kaspar H. Application of 10% povidone iodine reduces conjunctival bacterial contamination rate in patients undergoing cataract surgery. Eur J Ophthalmol. 2012;22(4):541-6. https://doi.org/10.5301/ejo.5000093
27. Speaker MG, Menikoff JA. Prophylaxis of endophthalmitis with topical povidone-iodine. Ophthalmology. 1991;98(12):1769-75. https://doi.org/10.1016/s0161-6420(91)32052-9
28. Nentwich MM, Yactayo-Miranda Y, Schwarzbach F, Wolf A, Kampik A, Mino de Kaspar H. Endophthalmitis after intravitreal injection: decreasing incidence and clinical outcome-8-year results from a tertiary ophthalmic referral center. Retina. 2014;34(5):943-50. https://doi.org/10.1097/iae.0000000000000011
29. Nentwich MM, Ta CN, Kreutzer TC, Li B, Schwarzbach F, Yactayo-Miranda YM, Kampik A, Miño de Kaspar H. Incidence of postoperative endophthalmitis from 1990 to 2009 using povidone-iodine but no intracameral antibiotics at a single academic institution. J Cataract Refract Surg. 2015;41(1):58-66. https://doi.org/10.1016/j.jcrs.2014.04.040