



Effect of Liposomal-Lactoferrin-Based Eye Drops on the Conjunctival Microflora of Patients Undergoing Cataract Surgery

Giuseppe Giannaccare · Sofia Comis · Virginia Jannuzzi ·
Davide Camposampiero · Diego Ponzin · Sergio Cambria ·
Marcello Santocono · Nicola Pallozzi Lavorante · Chiara Del Noce ·
Vincenzo Scorgia · Carlo E. Traverso · Aldo Vagge

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ABSTRACT

Introduction: Postoperative endophthalmitis is typically caused by the patient's conjunctival bacterial flora. Povidone iodine solution (5%) is used perioperatively to obtain periocular and ocular antisepsis. However, an adjunctive prophylaxis procedure could further help control the conjunctival microbial load. Considering

the increase in antibiotic resistance, a progressive shift toward alternative methods would be desirable. Somilux[®] eye drops (Alfa Intes, lactoferrin-based eye drops) are medical devices containing liposomal lactoferrin (LF). This study evaluates the effects on conjunctival microflora of LF-based eye drops used in the preoperative phase in patients scheduled for cataract surgery.

Methods: LF-based eye drops or a vehicle solution (water solution) were instilled 4 times a day starting 3 days before cataract surgery. Before the therapy (T0) and at the time of surgery (T1), a conjunctival swab was performed in both eyes and processed to detect microbial growth, microbiological isolation, and species identification. The outcome was the quantification and characterization of the local microbial flora before and after using LF-based or vehicle-based eye drops. Safety of the treatments was also evaluated.

Results: 88 eyes of 44 patients (mean [\pm SD] age 75 [\pm 12.6] years) were enrolled. At baseline, 54 conjunctival swabs showed only saprophytic flora, 27 showed only potential pathogenic flora, and seven showed both of them. LF-based eye drops reduced the proportion of potentially pathogenic bacteria (36% at T0 vs. 9% at T1, $p = 0.008$) compared with the vehicle (41% at T0 vs. 55% at T1, $p = 0.302$) without altering the physiological ocular microbial composition. No adverse events have been reported.

G. Giannaccare (✉) · V. Scorgia
Department of Ophthalmology, University Magna
Graecia of Catanzaro, Catanzaro, Italy
e-mail: giuseppe.giannaccare@unicz.it

S. Comis · V. Jannuzzi · D. Camposampiero ·
D. Ponzin
Fondazione Banca degli Occhi del Veneto, Venice,
Italy

S. Cambria
Polilab Srl, Catania, Italy

M. Santocono
Centro Oculistico Dott. Marcello Santocono,
Catania, Italy

N. Pallozzi Lavorante · C. Del Noce ·
C. E. Traverso · A. Vagge
IRCCS Ospedale Policlinico San Martino, Genoa,
Italy

N. Pallozzi Lavorante · C. Del Noce ·
C. E. Traverso · A. Vagge
Università degli Studi di Genova, DiNOGMI, Clinica
Oculistica, Genoa, Italy

Conclusion: Our findings provide a novel contribution to the scientific knowledge on the role of LF in the ophthalmic field, supporting the use of LF-based eye drops as a safe and selective treatment to improve the ocular surface physiological defenses and control the bacterial ocular surface contamination prior to cataract surgery.

Keywords: Lactoferrin; Ocular microbiota; Endophthalmitis; Bacterial load; Cataract surgery

Key Summary Points

Why carry out this study?

In some circumstances (e.g., the preoperative setting), it is fundamental to improve ocular hygiene and control the microbial load on the ocular surface to reduce the risk of infection.

Prophylaxis with antibiotics has been widely used, but the increased antibiotic resistance pattern of conjunctival bacteria over the years has led to restrictions on their use.

A shift to alternative methods, such as the use of natural molecules that are able to stimulate the physiological defense mechanisms, would be desirable.

What was learned from the study?

LF-based eye drops provide a reduction in the proportion of potentially pathogenic bacteria compared with the vehicle (water solution) while not altering the physiological ocular microbial composition.

LF-based eye drops are a safe and selective treatment to improve the ocular surface physiological defenses and control bacterial ocular surface contamination prior to cataract surgery.

INTRODUCTION

In physiological conditions, the ocular surface of the eye presents a significant microbial load, called “ocular microbiota” [1]. This is composed of Gram-positive resident microorganisms (staphylococci, streptococci, corynebacteria, and propionibacteria) and Gram-negative bacteria [2]. The role of the microbiota in the ocular surface is similar to that it plays in other body districts, namely interacting with epithelial and immune cells and the coordination of several functions, such as the preservation of the epithelial barrier, the inhibition of apoptosis and inflammation, the competitive exclusion of potential pathogens, and the maintenance of immune response homeostasis [2, 3].

In some circumstances, such as the preoperative setting, it is fundamental to improve ocular hygiene and control the microbial load on the ocular surface in order to reduce the risk of infection (e.g., endophthalmitis). Indeed, postoperative endophthalmitis is typically caused by the patient’s conjunctival bacterial flora [4–6]. Povidone iodine solution (5%) is commonly used perioperatively to obtain periocular and ocular antisepsis [5, 7]. However, an adjunctive prophylaxis procedure used in the preoperative days could further help control the conjunctival microbial load [8, 9]. For this task, prophylaxis with antibiotics has been widely used in the past. However, the increased antibiotic resistance pattern of conjunctival bacteria over the years has led to restrictions on their use [4]. The effectiveness of different disinfectant agents as prophylactic treatments was subsequently tested, but very rapid microbicidal activity was reported (which can be explained by their chemical composition), attesting to their aggressiveness towards the ocular microflora equilibrium [10, 11]. Consequently, a progressive shift toward alternative and microbiota-friendly methods for ocular prophylaxis, such as the use of natural molecules that are able to stimulate the physiological defense mechanisms, would be desirable.

Lactoferrin (LF) is an iron-binding multi-functional glycoprotein found in the blood circulation, mucosal surfaces, and various secretory fluids, such as colostrum, milk, tears, nasal and vaginal secretions, pancreatic juice, and saliva [12, 13]. LF is also produced by neutrophils, which are stored in secondary granules to be secreted at the sites of infection and inflammation [13]. LF has numerous well-known biological roles, including regulating iron absorption, the modulation of immune responses, and antimicrobial, antiviral, antioxidant, anticancer, and anti-inflammatory activities [14–16]. Moreover, LF is a critical component in mediating the immune response, particularly for the coordinated interactions between innate immunity and adaptive immunity [17, 18].

After colostrum and milk, the highest concentration of LF is found in the eye (about 2 mg/ml in tears), where it plays a key role in defense mechanisms [15, 19]. Of note, there is some evidence to support the therapeutic activity of LF at the ocular level [20]. In particular, besides its well-known antibacterial effect, novel interest has been growing in its potential application in the field of dry eye and infections [20–22]. However, a thorough understanding of the mechanisms of its beneficial effects on the ocular surface requires more in-depth research, and further studies are needed to characterize the role of LF in treating ocular pathological conditions or enhancing the ocular physiological defenses [15, 20].

Somilux[®] eye drops (Alfa Intes, hereafter termed “LF-based eye drops”) are a recently developed medical device containing liposomal LF, whose use is indicated in different eye conditions, including ocular infection, subconjunctival hemorrhage, ocular hyperemia, and dry eye disease. Incorporating LF into liposomes improves the molecule’s stability, as its poor aqueous stability and high nasolacrimal duct drainage may hinder its potential efficacy [23].

The present study aimed to evaluate the effects on conjunctival microflora of LF-based eye drops used in the preoperative phase in patients scheduled for cataract surgery.

METHODS

Study Design and Setting

This clinical study was conducted with the collaboration of the Mater Domini University Hospital (Catanzaro, Italy), the University Eye Clinic of Genoa, DINOGMI (Genoa, Italy), and the Veneto Eye Bank Foundation (Venice, Italy). Consecutive patients scheduled for senile cataract surgery were screened for enrollment at the Mater Domini University Hospital—Catanzaro. Exclusion criteria were a history of ocular infection in the affected eye in the last 3 months with or without the use of topical antimicrobial agents, the usage of glaucoma medications or other types of eye drops for the control of inflammatory conditions of the eye (including dry eye), diabetes, and autoimmune diseases.

The study complied with the protocol, good clinical practices, the Declaration of Helsinki, and local legal and regulatory requirements. Informed consent was obtained from all participants. The Ethics Committee of the Calabria Region—Center Area Section approved the study on 17 February 2022.

Study Treatment

LF-based eye drops (Somilux[®], Alfa Intes, Casoria, Italy) were instilled 4 times a day starting from 3 days before surgery in the eye to be operated on; a vehicle solution (water solution) was used according to the same posology in the contralateral eye. Before starting the prophylactic therapy (T0), and at the time of surgery before 5% povidone iodine solution application (T1), a conjunctival swab was performed in both the eye to be operated on (the treated eye) and the other eye (the control eye). The conjunctival swab was processed for microbial growth detection and subsequent microbiological isolation and identification of the microbial species. The examining centers (Veneto Eye Bank Foundation, Ophthalmology Center of Catania, and Polilab Srl) were unaware of the treatment used by each patient in both eyes. The normal

surgical practice was not altered by the implementation of this clinical study, as the extra activity was limited to two tear film samples with a special non-invasive and non-harmful conjunctival swab.

Study Measures and Procedures

The study's outcome was the quantitative evaluation and characterization of the local microbial flora before and after using LF-based eye drops or vehicle. The safety of treatment with LF-based eye drops was also evaluated.

After collection, the conjunctival swab was mixed with Amies transport medium for 15 s and incubated at 32 °C for 30 min. After incubation, the swab was re-mixed for 15 s, and 500 µL of Amies medium were seeded into an HB&L[®] culture bottle (Alifax Srl, Italy) under sterile conditions. The culture bottles were then placed in an HB&L[®] instrument (Alifax Srl, Italy) and incubated for 24 h. The bacterial flora was quantified by evaluating the growth curve based on the initial values of nephelometric readings (CFU/mL) and the corresponding growth curve provided by the HB&L[®] instrument.

In the case of a positive swab for microbial growth, the sample was inoculated into 1 mL brain heart infusion broth (Biomérieux SA), incubated at 30 °C for 15 min, and then spread on selective media. Tryptic soy agar or Sabouraud dextrose agar plates were used for non-exigent bacteria or fungi, respectively. In contrast, Baird-Parker agar and cetrinide agar plates were used to isolate *Staphylococcus* spp. and *Pseudomonas* spp., respectively. Chocolate agar and 5% sheep blood agar plates were used to encourage the growth of exigent bacteria. Plates were incubated at 37 °C for 24–48 h for microorganisms and at 22–25 °C for 5 days for fungi.

Isolated bacteria were identified by MicroScan Specialty ID Panels (Beckman Coulter[®]) and classified as saprophytic or potentially pathogenic according to *Koneman's Color Atlas and Textbook of Diagnostic Microbiology* (4th edition) and the *Dizionario di batteriologia umana normale*

Table 1 Bacteria classification

Saprophytic flora

Staphylococcus auricularis
Staphylococcus epidermidis
Staphylococcus haemolyticus
Staphylococcus hyicus
Staphylococcus intermedius
Staphylococcus lugdunensis
Staphylococcus sciuri
Staphylococcus simulans
Staphylococcus warneri
Staphylococcus xylosus

Potential pathogenic flora

Alcaligenes faecalis
Candida spp.
Moraxella lacunata
Morganella morgani
Pseudomonas fluorescens
Ralstonia pickettii
Serratia marcescens
Staphylococcus aureus
Methicillin-resistant *Staphylococcus aureus* (MRSA)
Methicillin-resistant *Staphylococcus epidermidis* (MRSE)
Stenotrophomonas maltophilia

e patologica (*Dictionary of Normal and Pathological Human Bacteriology*) (Table 1).

In vitro susceptibility testing for commercially available topical ophthalmic antibiotics (netilmicin, tobramycin, ofloxacin, levofloxacin, moxifloxacin, chloramphenicol, and azithromycin) plus oxacillin and cefuroxime was performed using the Kirby–Bauer disk diffusion method, following the National Committee for Clinical Laboratory Standard Institute protocols.

Statistical Analysis

Means, medians, and standard deviations (SDs) were calculated for continuous variables, while absolute values and frequencies (percentages) were calculated for categorical variables. The comparison of continuous values between groups was performed using the Mann–Whitney *U* test, while the comparison of continuous values within the group was performed using the Wilcoxon signed-ranks test. The comparison of proportions within the group was performed using McNemar's test, and the relationship between treatments and the presence (or absence) of potentially pathogenic bacteria was assessed using the odds ratio (OR). $p < 0.05$ was considered statistically significant. A post-hoc power analysis based on McNemar's test was calculated considering OR = 0.1 (eyes with potentially pathogenic bacteria, treated vs. vehicle), $\alpha = 0.05$, and the proportion of discordant pairs = 0.3.

RESULTS

Overall, 88 eyes of 44 patients (25 males, 19 females, mean [\pm SD] age 75 [\pm 12.6] years) were enrolled in the study. At baseline, 54 conjunctival swabs (61.4% of the total) showed only saprophytic flora, 27 (30.7%) only potential pathogenic flora, and 7 (8.0%) both saprophytic and potentially pathogenic bacteria (mixed flora). The distribution of the detected bacteria in the whole sample is reported in Table 2. The in vitro susceptibility test highlighted the presence of resistance profiles among isolated bacteria toward some commonly used antibiotics. In particular, the reported methicillin resistance was useful for identifying MRSA (detected in 8.0% of conjunctival swabs at the baseline) and MRSE (detected in 6.8% of conjunctival swabs at the baseline) species (Table 2).

Microbial Load

At the baseline visit, the microbial load was equal to 40×10^6 UFC/mL (IQR range: 50×10^6) in both treated and control eyes. At

Table 2 Distribution of detected bacteria in the whole sample before and after the treatments ($n = 88$)

Bacteria*	T0, <i>n</i> (%)	T1, <i>n</i> (%)	
		LF-based eye drops	Vehicle
<i>Alcaligenes faecalis</i>	0	0	1 (1.1)
<i>Candida</i> spp.	0	1 (1.1)	0
<i>Moraxella lacunata</i>	1 (1.1)	0	1 (1.1)
<i>Morganella morganii</i>	1 (1.1)	0	0
<i>Pseudomonas fluorescens</i>	2 (2.3)	0	1 (1.1)
<i>Ralstonia pickettii</i>	1 (1.1)	0	0
<i>Serratia marcescens</i>	1 (1.1)	0	0
<i>Staphylococcus auricularis</i>	0	1 (1.1)	1 (1.1)
<i>Staphylococcus aureus</i>	13 (14.8)	3 (3.4)	11 (12.5)
<i>Staphylococcus aureus</i> (MRSA)	7 (8.0)	0	4 (4.5)
<i>Staphylococcus epidermidis</i>	18 (20.5)	13 (14.8)	7 (8.0)
<i>Staphylococcus epidermidis</i> (MRSE)	6 (6.8)	0	5 (5.7)
<i>Staphylococcus haemolyticus</i>	5 (5.7)	2 (2.3)	0
<i>Staphylococcus hyicus</i>	1 (1.1)	4 (4.6)	2 (2.3)
<i>Staphylococcus intermedius</i>	16 (18.2)	11 (12.5)	6 (6.8)
<i>Staphylococcus lugdunensis</i>	6 (6.8)	1 (1.1)	1 (1.1)
<i>Staphylococcus sciuri</i>	0	0	1 (1.1)
<i>Staphylococcus simulans</i>	3 (3.4)	0	1 (1.1)
<i>Staphylococcus warneri</i>	2 (2.3)	0	0
<i>Staphylococcus xylosum</i>	9 (10.2)	5 (5.7)	3 (3.4)
<i>Stenotrophomonas maltophilia</i>	2 (2.3)	0	0

Table 2 continued

Bacteria*	T0, n (%)	T1, n (%)	
		LF-based eye drops	Vehicle
No growth	1 (1.1)	2 (3.4)	1 (1.1)
Saprophytic flora	54 (61.4)	40 (45.5)	21 (23.9)
Potential pathogenic flora	27 (30.7)	4 (4.6)	22 (25)
Mixed flora	7 (8.0)	2 (3.4)	1 (1.1)

Species shown in bold are potentially pathogenic flora

T1, the microbial load was not statistically different from that at T0 in both groups. In detail, the microbial load was equal to 15×10^6 UFC/mL (IQR range: 50×10^6) in the treated eyes ($p = 0.279$) and to 17.5×10^6 UFC/mL (IQR range: 40×10^6) in the control eyes ($p = 0.253$). The list of bacteria detected at T1 according to the treatment is reported in Table 2.

Characterization of the Ocular Microbiota

A statistically significant reduction in the proportion of potentially pathogenic bacteria was reported after the treatment with LF-based eye drops compared with the baseline evaluation (36% at T0 vs. 9% at T1, $p = 0.008$). Conversely, this difference was not statistically significant after the treatment with the vehicle (41% at T0 vs. 55% at T1, $p = 0.302$) (Fig. 1). The post-hoc power analysis based on McNemar's test indicated a power of 87%.

Combining the presence or absence of potentially pathogenic strains at T0 and T1 (Table 3), it was observed that eyes treated with LF-based eye drops had a significantly higher chance of reducing the presence of potentially pathogenic strains (OR: 4.0 [1.3–12.4]; $p = 0.015$), a significantly lower risk of increasing the presence of potentially pathogenic strains (OR: 0.2 [0.06–0.98], $p = 0.046$), a significantly higher chance of maintaining saprophytic flora (OR: 5.2 [1.2–21.9]; $p = 0.024$), and a significantly higher chance of turning back to

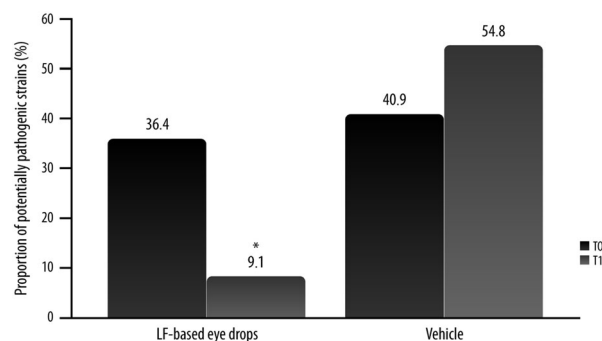


Fig. 1 Presence of potentially pathogenic strains according to time and treatment. A significant reduction in the proportion of potentially pathogenic strains was reported in eye swabs after the treatment with LF-based eye drops ($*p = 0.008$)

Table 3 Presence of potentially pathogenic bacterial strains

	T0 yes, T1 no	T0 no, T1 yes	T0 no, T1 no	T0 yes, T1 yes
LF-based eye drops, n (%)	15 (34.1)	3 (6.8)	25 (56.8)	1 (2.3)
Vehicle, n (%)	5 (11.4)	10 (22.7)	16 (36.4)	13 (29.5)

saprophytic flora (OR: 39.0 [4.0–378.2]; $p = 0.002$) than eyes treated with the vehicle (Fig. 2).

LF-based eye drops had the ability to modulate the potentially pathogenic strains and favor the saprophytic flora affected by both Gram-positive (25% at T0 vs. 7% at T1, $p = 0.039$) and Gram-negative (14% at T0 vs. 0% at T1; $p = 0.039$) bacteria (Table 4).

Safety

All patients used the study treatment and the vehicle as per our instructions. No adverse events were reported during the study period for either group.

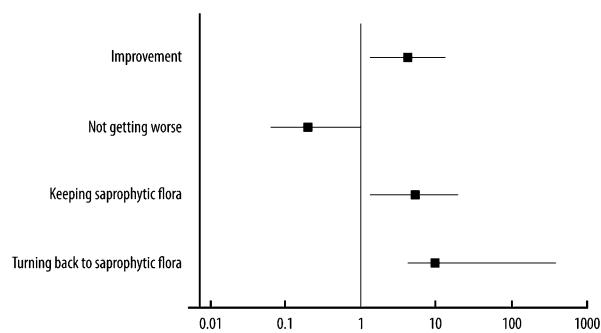


Fig. 2 Odds ratio for change in bacterial flora over time. In the treatment group, eyes had a better chance of reducing the presence of potentially pathogenic strains (improve; odds ratio, OR: 4.0 [1.3–12.4]), a lower risk of increasing the presence of potentially pathogenic strains (not getting worse; OR: 0.2 [0.06–0.98]), and a better chance of keeping saprophytic flora (OR: 5.2 [1.2–21.9]) and turning back to saprophytic flora (OR: 39.0 [4.0–378.2])

Table 4 Presence of potentially pathogenic bacterial strains

	T0 (<i>n</i> = 44), <i>n</i> (%)	T1 (<i>n</i> = 44), <i>n</i> (%)	<i>p</i> value
Gram-positive potential pathogenic strains			
LF-based eye drops	11 (25.0)	3 (6.8)	0.039
Vehicle	16 (36.3)	20 (45.5)	0.424
Gram-negative potential pathogenic strains			
LF-based eye drops	6 (14.0)	0	0.039
Vehicle	2 (4.5)	3 (6.8)	0.424

DISCUSSION

Even though ocular surgery is performed with sterile instruments and under aseptic conditions, postoperative endophthalmitis represents an unavoidable risk [24]. It is widely known that the patient's ocular flora is the main source of the microbes responsible for most cases of intraocular infection [4–6]. Therefore, reducing the microbes on the ocular surface and eliminating potential pathogenic flora in the

preoperative phase are useful strategies to decrease the risk of endophthalmitis [4]. For this purpose, prophylaxis with various classes of topical antibiotics has been widely used in recent decades [25]. However, several studies have evaluated the antibiotic resistance pattern of conjunctival bacterial flora in patients undergoing cataract surgery. These studies suggest that an increase in bacterial resistance has been registered due to the inappropriate and widespread use of antibiotics and argue for the need for alternative prophylaxis methods [4, 26–28].

In recent years, a progressive shift toward different types of disinfectants that are able to reduce, without selectivity, the microbial load of the conjunctiva has been taking place [29–31].

In the present study, using LF-based eye drops for 4 days significantly reduced the proportion of potentially pathogenic bacteria compared with the vehicle without altering the physiological ocular microbial composition. In addition, our findings suggest a higher chance of reducing the presence of potentially pathogenic bacterial strains, a lower risk of increasing the presence of potentially pathogenic bacterial strains, and a higher chance of maintaining or turning back to saprophytic flora during the treatment with LF-based eye drops than with the vehicle. According to this activity, unlike conventional disinfectants, the LF-based eye drops did not produce profound changes in the saprophytic flora (e.g., some coagulase-negative *Staphylococci* spp.) that is known to have a physiological role in the regulation of the immune homeostasis of the ocular surface system. At the same time, in our cohort, the susceptibility test reported the presence of MRSA and MRSE species in 8.0% and 6.8% of conjunctival swabs at the baseline, respectively, which were not retrieved after the LF-based treatment. These pathogens have been frequently associated with ocular infections; thus, controlling them represents an important therapeutic objective, as these species are often associated with high resistance to other antibiotic classes [32, 33].

No adverse events were reported during the study, in agreement with the safety results reported in preclinical studies [23].

The observed protective effect of LF can be explained by the peculiar activity of this molecule in preventing inflammation and acting against microbial infections [17]. LF directly affects pathogens through iron sequestration and its ability to bind lipopolysaccharide and block interactions with receptors used for entry into host cells [34]. Furthermore, it has been reported that LF plays a critical mediating role in the interactions between innate and adaptive immunity [17, 18]. Indeed, LF is a pleiotropic molecule that can directly contribute to the influence of presenting cells for the development of T-helper cell polarization [17, 35, 36]. The discovery of LF receptors on a wide variety of immune cells and their demonstrated capability to bind LF further support the potential of this molecule to modulate and influence both innate and adaptive immune system responses [37, 38].

The protective effects of LF have been described in different human districts thanks to its presence in various fluids. Human colostrum, a key player in the bacterial colonization of the neonatal gut, contains the highest LF concentrations [39]. Several studies have shown the usefulness of LF supplementation in children, particularly preterm infants, in reducing the incidence of late-onset sepsis and necrotizing enterocolitis by strengthening the intestinal epithelial barrier, promoting antagonism toward enteropathogens, and balancing anti- and pro-inflammatory responses [40–42]. LF also showed anti-inflammatory, immunomodulatory, and antimicrobial activities relevant to maintaining gut microbiota–host mutualism [43, 44].

In addition, it has been reported that salivary LF can counteract oral pathogen growth, including that of *Streptococcus* spp., *Candida albicans*, and other anaerobic periodontopathic bacteria (*Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia*) residing in the biofilm of supragingival and subgingival plaque, respectively [45, 46]. Interestingly, LF also inhibits the biofilm formation of these bacteria at physiological concentrations [16].

Protective activities of LF have also been reported during vaginal dysbiosis, characterized by low amounts of vaginal lactobacilli and increased levels of endogenous anaerobic

bacteria [47]. In this condition, increased levels of LF have been reported and related to an immune-modulator action of LF, similar to the role normally played by healthy microbiota in vaginal mucosa [47, 48].

These mechanisms could explain the control activity of LF against potentially pathogenic microorganisms observed in our study, thus providing preliminary evidence of the natural protective and immunomodulatory activity of LF in the ocular district as well.

Obtained results support the use of LF-based eye drops as a safe and specific treatment to prepare the ocular surface for surgery through the improvement of the ocular physiological defenses and the control of bacterial contamination. Moreover, our findings suggest that the LF-based eye drops can be useful for restoring the homeostasis of the ocular surface during the postoperative course, or in the case of recurrent/chronic infections (e.g., blepharitis, blepharoconjunctivitis). In addition, the LF-based eye drops can be used after standard medical therapy for the management of postoperative ocular conditions characterized by inflammation and/or an altered microbial profile (e.g., cataract surgery). This hypothesis is supported by previous studies on cell culture or preclinical assays in which topical eye drops of LF-loaded biodegradable nanoparticles were effective in treating various conditions characterized by ocular inflammatory processes [23]. However, any difference in the reconstitution of the bacterial flora in the postoperative phase should be assessed in the future after treatment with LF-based eye drops to further characterize its activity.

The present study suffers from some limitations that deserve mentioning and are related to the relatively small sample size and the non-randomized design. In addition, the clinical relevance of this treatment to the endophthalmitis rate, as well as to other parameters related to an altered ocular microbial profile, was not addressed and will represent the subject of future large studies. However, our findings suggest that LF-based eye drops can be useful to stimulate the ocular physiological defenses and modulate the microbial equilibrium toward the saprophytic component. This action is carried

out without altering the biodiversity of the ocular microbiome, whose equilibrium is fundamental to immunomodulatory function and the maintenance of relationships with other microbes and organs, and LF-based eye drops thus represent a natural, safe, and selective prophylactic treatment [3].

CONCLUSIONS

LF is an ingredient in many supplements and medicines, but a thorough understanding of the mechanisms for its beneficial effects requires further in-depth research, especially in the ocular tissues. Our findings provide a novel contribution to the knowledge on the role of LF in the ophthalmic field, supporting the use of LF-based eye drops as a safe and specific treatment to improve the ocular surface physiological defenses and control bacterial ocular surface contamination before cataract surgery. More in-depth studies focusing on LF-induced changes in the ocular microbiome may further confirm these observations and extend the application of this protocol from the prevention of post-surgical eye infections to the management of postsurgical ocular conditions characterized by an altered microbial community profile.

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Author Contributions. Study design: GG, VS, AV, NPL, CDN, CET; data collection and interpretation: all; manuscript writing: GG, AV; manuscript editing: all; approval to submit: all.

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Compliance with Ethics Guidelines. The study was conducted within the protocol approved by the Ethics Committee of the Calabria Region (Sezione Area Centro) on February 17, 2022. It adhered to the principles of the Declaration of Helsinki. All the participants signed an informed consent form.

Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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