

Efficacy of daily hygiene routine using cleansing wipes in the reduction of eyelid bacterial load.

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Abstract

Background- This study aims to evaluate the efficacy of using a cleansing eyelid wipe to reduce microbial load in healthy subjects.

Methods- A single-center, prospective study was conducted. Twenty healthy subjects were assigned to wipe their periocular area (eyelid, eyelashes and lid margin) twice a day with a commercial sterile wipe for 5 consecutive days. Bacterial load (total aerobic bacteria and *Staphylococcus* genus) was recovered from the same wipes and was measured on day 1, 3 and 5, with day 1 serving as an internal control. Both eyes were assessed independently, resulting in a total of 40 samples. Microbial evaluation was performed by culture on Tryptone Soy Agar (TSA) and Mannitol Salt Agar (MSA) plates.

Results- Positive and negative controls rendered expected results thus validating this innovative extraction procedure. Microbial growth on both media revealed that the great majority of microorganisms detected belong to the genus *Staphylococcus*.

Bacterial load reduction was significant (Mann-Whitney test $p < 0.05$) for both microorganisms between days 1 to 3, days 3 to 5 and days 1 to 5. Measurements at day 3 revealed a reduction on bacterial load of total aerobes and *Staphylococcus* spp. by 58.7% and 56.5% respectively. Moreover, on day 5, bacterial load showed a mean reduction of 80.8% and 82.7% on total aerobes and *Staphylococcus* spp. respectively. Furthermore, on day 5, 42.5% of the eyes showed $\geq 90\%$ reduction in total aerobes and 42.5% for *Staphylococcus* spp. On the third day 25% of the volunteers showed $\geq 90\%$ reduction of aerobes microbial load at least in one eye and the percentage was 15% for *Staphylococcus* spp. On the fifth day those values arise to 50% for total aerobes and 65% for *Staphylococcus* spp.

Conclusions- The study demonstrates the efficacy of the eyelid hygiene with commercial cleansing wipes in significantly reducing ($p < 0.05$) bacterial load in the periocular area after 3 days of use, with further reduction on the fifth day. Therefore, these wipes could be recommended in situations in which adequate of palpebral hygiene is needed.

Introduction

The normal microbiota of the eyelid and conjunctiva is present in non-pathological conditions and has been described as mainly bacterial. The most common bacteria isolated from the eyelids are Gram-positive bacteria, mostly coagulase-negative *Staphylococcus* spp [1–3].

Postoperative endophthalmitis is a rare but complicated risk derived from invasive ophthalmological interventions such as cataract surgery and intravitreal injections treatments as anti-Vascular Endothelial Growth Factor (VEGF) treatments for choroidal neovascularization related to age-related macular degeneration among other pathologies [4, 5].

Although postoperative endophthalmitis is considered a low rate complication, it is an important concern for surgeons as it can entail risks of great magnitude [5–7]. Postoperative endophthalmitis is generally caused by microorganisms from the eyelid margin, conjunctiva and tear film [6, 8]. Therefore, the reduction of the bacterial load prior to the intervention is an important goal for endophthalmitis prevention. Different prophylactic strategies have been applied such as the use of either topical or intracameral antibiotics, applied at different time points before, during or after the surgical procedure [9].

Also, antiseptics such as povidone-iodine (PVI) are applied to the periocular area and conjunctiva, showing to reduce microbiota in the conjunctiva [10]. Other approaches comprise the use of eyelid hygiene solutions to reduce the microbial flora before the intervention [11, 12]. This study examines the effect of specific cleansing eyelid wipes on reducing bacterial load after several days of use.

This analysis focused on the total load of microorganisms through the evaluation of total aerobic bacteria, which are those capable of decomposing organic matter at temperatures ranging between 30°C and 40°C [13, 14]. Total aerobic bacteria group includes different Gram-positive and Gram-negative bacterial genera and it may even include fungi and yeasts. To capture all its heterogeneity, the growth of total aerobes was carried out in the rich, non-selective, non-differential medium Tryptone and Soybean (TSA) [15].

This study analyzed specifically bacteria of the genus *Staphylococcus*, since these are the most frequently present microorganisms in the periocular area [1–3]. Considering previous studies, it was expected to detect the presence of *Staphylococcus epidermidis*, since this is the main colonizer of the human skin and wet mucosa [16], and is very abundant in ocular microbiota [17]. Although it is the least common cause of opportunistic infections, it is capable of generating ocular infections [16]. The genus *Staphylococcus* is formed by Gram-positive cocci, which express catalase. They are capable of growing in a variety of aerobic and anaerobic conditions in the presence of high concentration of NaCl and between 18 °C and 40 °C[18]. Therefore, to selectively grow *Staphylococcus* spp., Mannitol Salt Agar (MSA) medium was used.

Thus, this study aims to emphasize the relevance of eyelid hygiene as a complementary method of reducing the microbial load of the periocular area (eyelids, eyelashes and eyelid margin) and to motivate and potentially involve the patient in the care of the eyelid at home. The objective of this study is to determine the efficacy to decrease bacterial load after eyelid cleaning with sterile single-dose wipes.

Methods

Study design and participants-

This single-center, prospective study was conducted at Universidad de Barcelona. The present study adhered to the Declaration of Helsinki and was approved by the Bioethics Committee of the University of Barcelona. All individuals were previously informed of the design of the study and all volunteers provided

informed consent. Additionally, they were supplied with the annex to European Regulation 2016/679 that guarantees the processing of personal data.

Inclusion criteria included healthy volunteers over 18 years of age, with the capacity and commitment to correctly perform the usual daily eyelid hygiene and not to use makeup during the week of the study.

Exclusion criteria included allergy to any of the components of the hygienic wipes, use of contact lenses, ophthalmic or eyelid problems (such as conjunctivitis, blepharitis, etc.), dermal problems (such as rosacea, acne, seborrheic dermatitis, etc.), recent ophthalmic surgery (during the last 3 months) or having been treated with antibiotics (nor topic nor oral) during the three months previous to the sample collection.

Materials- Participants were provided with the following materials:

Cleansing wipes: Each patient received 20 commercial sterile cleansing eye wipes (ESTILA® Angelini Farmacéutica, S.A., Barcelona, Spain), containing *Aloe barbadensis* and *Hamamelis virginiana* extracts, among other components.

Collection tubes: Each patient received six sterile 50 ml tubes marked with the volunteer number, right eye (RE) or left eye (LE) and the day of collection of the wipe (1, 3 or 5). Collection tubes contained 10 ml Ringer's buffer $\frac{1}{4}$.

Design- Twenty individuals were selected under the criteria defined above (n = 20). For each individual, both eyes were analyzed independently resulting in a n = 40.

Volunteers were asked to clean their eyelids with a cleansing wipe twice a day (morning and evening) for five consecutive days. Eyelid microbiota was collected on days 1, 3 and 5 from the cleansing wipes. Bacteria obtained from the wipes corresponding to day 1 were used as internal control. The wipes used to clean the two eyes on the morning of day 1 (Monday), 3 (Wednesday) and 5 (Friday) were placed in sterile 50 ml tubes and sent to the laboratory within less than 1 hour for immediate analysis. The wipes used to clean the eyes at other time points were discarded.

Instructions indicated that no cream or ointment should be applied in the periocular area throughout the duration of the study. Hands were washed vigorously with soap and water, dried with a clean towel and washed with the disinfectant solution containing ethanol. The wipe's envelope was opened, and the wipe was minimally manipulated. Each wipe served only for one eye that was cleaned by rubbing starting from the inside (lacrimal) moving horizontally above the lashes towards the outside. The procedure was repeated three to four times and the wipe was inserted in the sterile 50 ml tube. The same procedure was repeated with the other eye with a different wipe.

Recovery and enumeration of microorganisms- The microorganisms contained in the wipes used in the mornings on days 1, 3 and 5 were recovered. In total, 40 samples per day were evaluated (20 volunteers and 2 eyes per person). Thus, throughout the three days of the study, 120 samples were analyzed (Fig. 1).

Wipes were homogenized in 10 ml of sterile Ringer's buffer ¼ by vortex mixing for 1 min. The homogenate was further diluted 1:10, 1:100 and 1:1000 before plating.

TSA [15] and MSA [19] (its high salt and mannitol content allows the selective growth of *Staphylococcus* spp. while inhibiting the growth of other Gram-negative bacteria.) plates were inoculated with 0.1 ml of each dilution. Plating was performed in duplicate. After incubation of plates at 37 °C for 18 h, colonies were counted. Results were expressed in colony forming units (CFU/ml) of Ringer's buffer ¼.

Controls- In addition, on each day of analysis, the following positive and negative controls were performed:

- - Negative controls- To evaluate the sterility of Ringer's buffer ¼ and the TSA and MSA plates, a tube without inoculum and empty plates containing no inoculum were incubated each day of the assay.
- - Positive controls- Two strains from the laboratory collection (*Staphylococcus aureus* strain RN4220 [20] and *Escherichia coli* strain WG5 [21]) were inoculated on TSA and MSA plates, respectively to verify the growing media capacity to support the growth of the desired microorganisms.
- - Wipe and hands negative controls- To evaluate the sterility of the wipes, each day of study, one of them was picked from the envelope using sterile forceps and directly introduced in the 50 ml tube containing Ringer's buffer ¼. It was homogenized and inoculated on the TSA and MSA plates and incubated as described above.

To evaluate whether the protocol for washing the hands was effective reducing the commensal microbiota, after washing hands the envelope of a wipe was opened, and the wipe was only manipulated to place it directly in the 50 ml tube containing Ringer buffer ¼. Once inserted in the tube, it was homogenized, inoculated on the TSA and MSA plates and incubated as described above.

Statistical analysis-

Statistical analyses were performed using the R Foundation software [22]. To evaluate the normality of the data, the Shapiro-Wilk normality test and a Cullen and Frey chart were applied with a bootstrapping of 1000 samples as a screening method.

To evaluate if there were significant differences between the data obtained between the right and the left eye, the paired Mann-Whitney test and the Mood test were used to evaluate differences in the medians of the data obtained with both eyes.

Differences over time were evaluated with the Mann-Whitney test by pairing and unpairing the samples, by individual and by time. Differences among counts between days 1 and 3, between days 3 and 5 and between days 1 and 5 were evaluated.

Results

The study included 20 healthy volunteers (9 men, 11 women) with ages ranging from 19 to 62 years and an average age of 35.1 years who correctly followed the instructions for the periocular hygiene for five days. The hygienic protocol consisting on wiping their eyelids twice a day with an independent cleansing wipe for each eye (right and left eye were included). The cleansing wipe served both to wipe the eyelid and to collect the sample eyelid bacterial load of each eye of the volunteers was collected and further analyzed on day 1, 3 and 5. (Fig. 1). The first wipe employed on day 1 was used as internal control

Colony morphology

Eyelid microbiota recovered from the right and left eyes separately of 20 volunteers were grown both in TSA and MSA medium in order to both assess bacterial load and observe colony morphology, which denote the heterogeneity of the sample. TSA is a non-specific or differential medium that allows the growth of white colonies of varied morphology belonging to various taxonomic groups. However, the morphologies observed in TSA in this study were quite homogeneous (Fig. 2), suggesting that there was not much variability within the analyzed microbiota that should be composed by few predominant bacterial groups. On the contrary, the MSA is specific for *Staphylococcus* spp. and allowed the growth of white small opaque colonies showing in general a uniform morphology (Fig. 2). The fact that the phenol red contained in the MSA plates did not turn yellow suggests that most *Staphylococcus* spp. recovered were catalase negative *Staphylococcus* spp. / *Staphylococcus epidermidis*.

Comparison between both eyes

We evaluated the data obtained from both the right and left eye of each volunteer to statistically evaluate significant differences.

Results showed that there were almost no differences between the right and left eyes for either of the evaluated microorganisms at all time points (Fig. 3). Those data indicate that each eye can be evaluated independently, therefore rendering a n = 40.

Microbial enumeration

Total values for total aerobic bacteria and *Staphylococcus* spp. counts on day 1, 3 and 5 are shown in Table 1. For all the assessed days, the average of CFU/ml per day (n = 40) showed that load of total aerobes was higher than the one corresponding to *Staphylococcus* spp. (day 1: $3.5 \cdot 10^4$ vs $2.0 \cdot 10^4$ respectively, day 3: $1.1 \cdot 10^4$ vs $7.8 \cdot 10^3$, and day 5: $4.7 \cdot 10^3$ vs $2.7 \cdot 10^3$).

The results led to the inference that the great majority of flora detected belonged to the genus *Staphylococcus* and only a small fraction belonged to other bacterial genera. This is supported by the observation of the morphology of the colonies grown in the TSA medium, which allows the growth of different bacterial genera, however, the morphology of the colonies appeared quite homogeneous, indicating that mostly the same genera of bacteria were isolated.

Data showed that already on day 3, total aerobes and *Staphylococcus* spp. load were reduced by 58.71% and 56.50% respectively. This bacterial load was further reduced by 42.15% (aerobes) and 50.37% (*Staphylococcus* spp.) from day 3 to 5, achieving a total reduction from day 1 to 5 of 80.76% and 82.71% for total aerobes and *Staphylococcus* spp. respectively. These results which are depicted in Table 1 can also be visualized in Figure 4.

Moreover, it could be observed that on day 5, the percentage of eyes harboring > 90% reduced bacterial load was 42.5% for total aerobes and 42.5% for *Staphylococcus* spp. (Table 1 and Fig 5).

Positive and negative controls indicate that the employed methodology is valid to collect and determine eyelid bacterial load. The contamination which can be carried to the wipe through the previously disinfected hands is insignificant, since the values obtained for total aerobes from control wipes are several orders of magnitude below the values obtained from eyelid samples (data not shown).

Individual counts

Results visualized according to each individual (Fig. 6) showed differences between the initial levels of microorganisms at day 1, with bacterial loads ranging between 2 orders of magnitude (volunteers 7, 8 and 20 harbored a bacterial load almost 2 orders of magnitude above the bacterial load present in the eyes from volunteer 15).

Shapiro-Wilk's test of normality and Cullen and Frey's chart revealed that not all data could be considered normal. Therefore, non-parametric statistics were used to evaluate both parameters (total aerobic bacteria and *Staphylococcus* spp.). A significant decrease (Mann Whitney Test, $p < 0.05$) in bacterial load was observed, with values consistently close to 90% for both microorganisms between days 1 and 5, despite inter-individual differences. Some eyes presented a greater reduction between days 1 to 3 (volunteer n° 5 RE, volunteer n° 6 LE, and volunteers n° 14, 16 and 19 both eyes) while in others the greatest reduction occurred between days 3 to 5 (volunteer n° 8 RE and volunteer n° 17 RE and LE) (Fig. 6).

When assessed by individuals, bacterial load quantification on day 1 and 5 showed that 3 individuals (15%) (volunteers n° 5, 7 and 14) showed $\geq 90\%$ reduction for the two microorganisms in both eyes and 4 more individuals (volunteers n° 1, 6, 8 and 17) showed reduction values $\geq 89\%$ for the two microorganisms in both eyes. Moreover, the percentage of individuals showing either total aerobic bacteria or *Staphylococcus* spp. load reduction of $\geq 90\%$ in both eyes were 40% (volunteers n° 1, 5, 6, 7, 8,

12, 14 and 17). Additionally, the percentage of individuals who showed a reduction of $\geq 90\%$ for aerobes or for *Staphylococcus* spp. in at least one eye was 65% on day 5 (volunteers n° 1, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 17 and 20). Furthermore, 50% of individuals showed more than a 90% reduction for aerobes in one of the two eyes, and this percentage increased to 65% in the case of *Staphylococcus* spp. (Fig. 5 and Table 2). On day 3, the percentages of individuals showing bacterial load reduction $\geq 90\%$ were lower but still significant (Table 2).

All individuals showed some reduction in total aerobes and *Staphylococcus* spp. bacterial load after 5 days of eyelid wiping. The lowest reduction observed corresponded to 13.5% (volunteer n° 11, RE). Volunteers n° 3, 4, 11 and 18 had the lowest values of reduction (Fig. 5).

Moreover, significant reduction of bacterial load for both total aerobic bacteria and *Staphylococcus* spp. occurred not only between the initial (day 1) and the last day (day 5) of the study, but also between days 1 and 3, as indicated by Mann-Whitney Test ($p < 0.05$).

These results show a significant decrease in bacterial load on day 3, which further decrease on day 5, demonstrating that eyelid cleansing wipes are effective in reducing bacterial load in both periods of time.

Discussion

In this study, the efficacy of wiping the periocular area with cleansing wipes has been evaluated. For this purpose, bacterial load reduction was measured. In addition, the rapidity of this process was also assessed.

The protocol consisting of two washing steps per day for five days showed to be effective since it reduced the skin microorganisms approximately to 90% in 50 to 65% of the individuals.

Although diverse microbiota might be present in eyelids and conjunctiva, the total aerobic bacteria colonies observed in TSA plates showed homogeneous morphology with few different colonies observed in each plate. The counting values of *Staphylococcus* spp., grown on MSA plates were close to the ones obtained on TSA plates. Furthermore, the morphology of the colonies grown on TSA and MSA plates were similar. This indicated that *Staphylococcus* spp. was one of the most relevant contributors to the microflora. These observations are comparable with the ones detected in studies performed in the setting of the ocular microbiota, which show that the most commonly isolated bacteria are coagulase-negative *Staphylococcus* spp [1–3].

On average, there was a statistically significant reduction in the number of microorganisms (both total aerobes and *Staphylococcus* spp.) from day 1 to day 3. This reduction continued until day 5, when higher reduction levels were achieved. Even though some individuals did not show a reduction until day 5, in some individuals a reduction of 90% was detected at day 3. This means that although five days of eyelid hygiene is highly recommended, if for any reason a cleaning protocol of 5 days could not be performed, 3

days could accomplish effective and significant bacteria load reductions in a substantial part of the population, therefore rendering benefits in a shorter time frame.

The fact that eyelid wiping reduces bacterial load, and more specifically *Staphylococcus* spp. is highly relevant given the role that catalase-negative *Staphylococcus* spp. have in the pathology of endophthalmitis. Several studies have shown that catalase-negative *Staphylococcus* spp. are the microorganisms responsible for about 70% of post-cataract surgery endophthalmitis, followed by *S. aureus* and others [6, 16, 23]. Therefore, it is very important to maintain preoperative and postoperative asepsis of both eyelid and conjunctiva. However, not all the antibiotic prophylaxis has been reported to be clearly effective and, additionally, there is an increase of resistant bacteria that difficult the success of these strategies [24–26]. Therefore, apart from other prophylactic choices, periocular skin hygiene might contribute to reduce the risk of ocular infections associated to the surgery. As this study shows hygiene with commercial cleansing wipes has proven to be an accessible and efficient option to reduce bacterial load, that might be considered in cases when high standard eyelid hygiene is needed and can be used alone or in a complementary manner to the already existing hygienic strategies.

This study has some limitations. A relatively small number of samples has been analyzed (n = 40) and only an internal control (day 1) has been used. In addition, an observer bias might have been introduced, since the samples arrived at the laboratory within 1 h after collection, meaning the investigators were aware of the time point they were evaluating.

This study also presents some additional strengths apart from having proved effective in reducing bacterial load. The method has proven robust since positive and negative controls have behaved as expected. Hand disinfection before wipe handling has led to insignificant contamination of the sample. Moreover, the simplicity of the procedure, which involves just one step for both cleaning and collecting the sample is of great importance for the use of this method in future studies.

This information could be the basis for further studies not only in healthy subjects, but also in particular situations where hygiene of the periocular area is recommended such as in anterior and posterior blepharitis and conjunctivitis.

Conclusion

The use of sterile wipes for periocular hygiene for several days provides significant bacterial load reductions in healthy volunteers. Also, these wipes have several advantages since they can be easily used by patients at home and its application is less restrictive than antimicrobial compounds as they can be used together with other prophylactic strategies.

Abbreviations

CFU

Colony Forming Unit

LE

Left Eye

MSA

Mannitol Salt Agar

RE

Right Eye

TSA

Tryptone Soy Agar

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Tables

Table 1 Logarithmic bacterial load reduction.

	Day 1		Day 3		Day 5	
	Aerobic	Staph	Aerobic	Staph	Aerobic	Staph
-	-	-	-	-	-	-
Average (Log ₁₀ CFU/ml)	4.29	4.16	3.77	3.68	3.35	3.19
SD	0.51	0.38	0.50	0.47	0.58	0.47
3 1-3 Log reduction			0.52	0.48		
% reduction			58.7%	56.5%		
% wipes >90% reduction			17.5%	10%		
3 3-5 Log reduction					0.42	0.49
% reduction					42.1%	50.4%
% wipes >90% reduction					12.5%	12.5%
3 1-5 Log reduction					0.93	0.97
% Reduction					80.8%	82.7%
% wipes >90% reduction					42.5%	50%

Logarithmic reduction (\log_{10} No CFU/ml day 1 - Nt CFU/ml day 5) of the number of total bacteria (Aerobic) or Staphylococcus (Staph), in which No is the number of microorganisms on the initial day (day 1 or 3) and Nt is the number of microorganisms on the final day (day 3 or day 5).

Table 2 Individuals showing $\geq 90\%$ bacterial load reduction.

	Both eyes		At least one eye	
	Days 1 to 3	Days 1 to 5	Days 1 to 3	Days 1 to 5
Aerobes	5%	35%	25%	50%
<i>Staphylococcus</i>	5%	20%	15%	65%
Both	5%	15%	15%	50%

Percentages of volunteers who showed $\geq 90\%$ reduction in bacterial load (total aerobes, Staphylococcus or both), on days 3 and 5, in one or both eyes.

Declarations

Ethical approval and consent to participate:

This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was granted by the Bioethics Committee of the University of Barcelona (approval number IRB00003099). Written

informed consents were obtained from all participants.

Consent for publication:

Not applicable.

Availability of data and materials:

The datasets analysed during the current study are available from the corresponding author on reasonable request.

Competing interests:

The authors declare that they have no competing interests.

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Authors' contributions:

CG: Data Analysis.

JM: Data Analysis and Manuscript preparation.

MM: Research Design, data analysis and manuscript preparation.

All authors read and approved the final version of this manuscript.

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Not applicable.

Figures

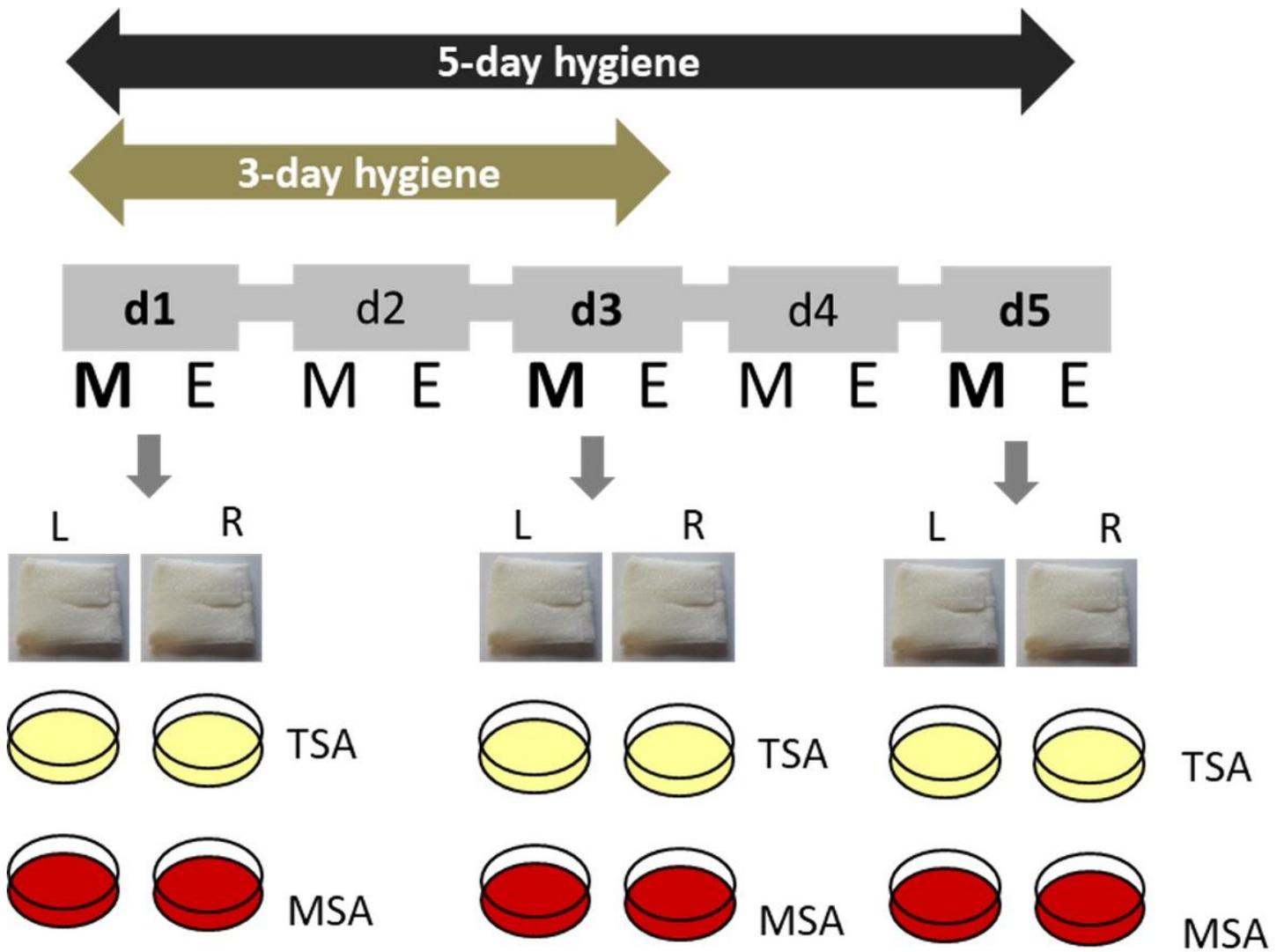


Figure 1

Schematic representation of the hygienic protocol using commercial cleansing wipes. Periocular area (eyelid, eyelashes and lid margin) is cleaned twice a day (in the morning (M) and in the evening (E)), of both eyes separately (Right R and Left L) for 5 consecutive days. Samples (cleansing wipes) are obtained on the morning of day 1, day 3 and day 5 to assess the impact of a 3-day and 5-day hygiene and were incubated in two different mediums: TSA (Trypticase soy agar) for Total aerobes and MSA (Mannitol salt agar) for *Staphylococcus* spp.

TSA: Total aerobic bacteria

MSA: *Staphylococcus* spp.

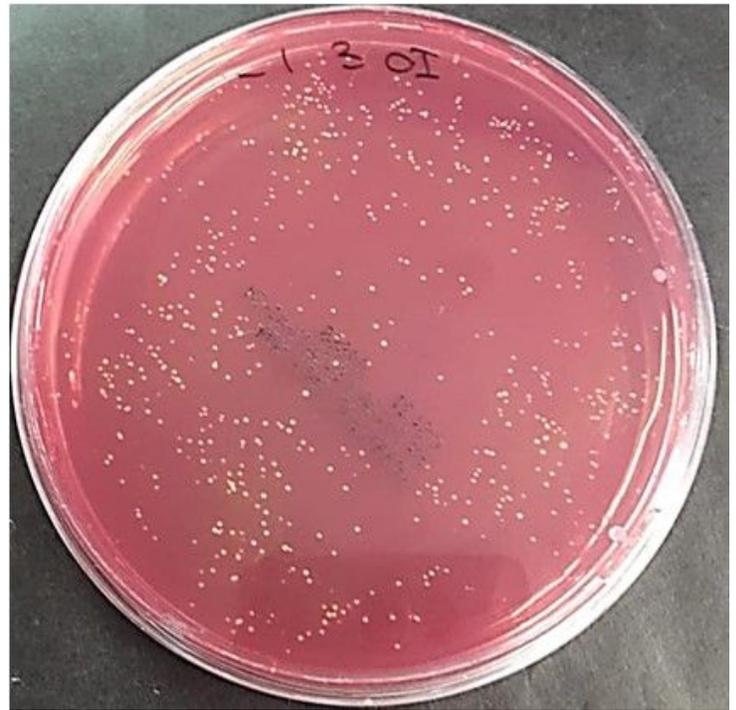


Figure 2

Colony morphology. Two representative plates with colonies grown on TSA and MSA media.

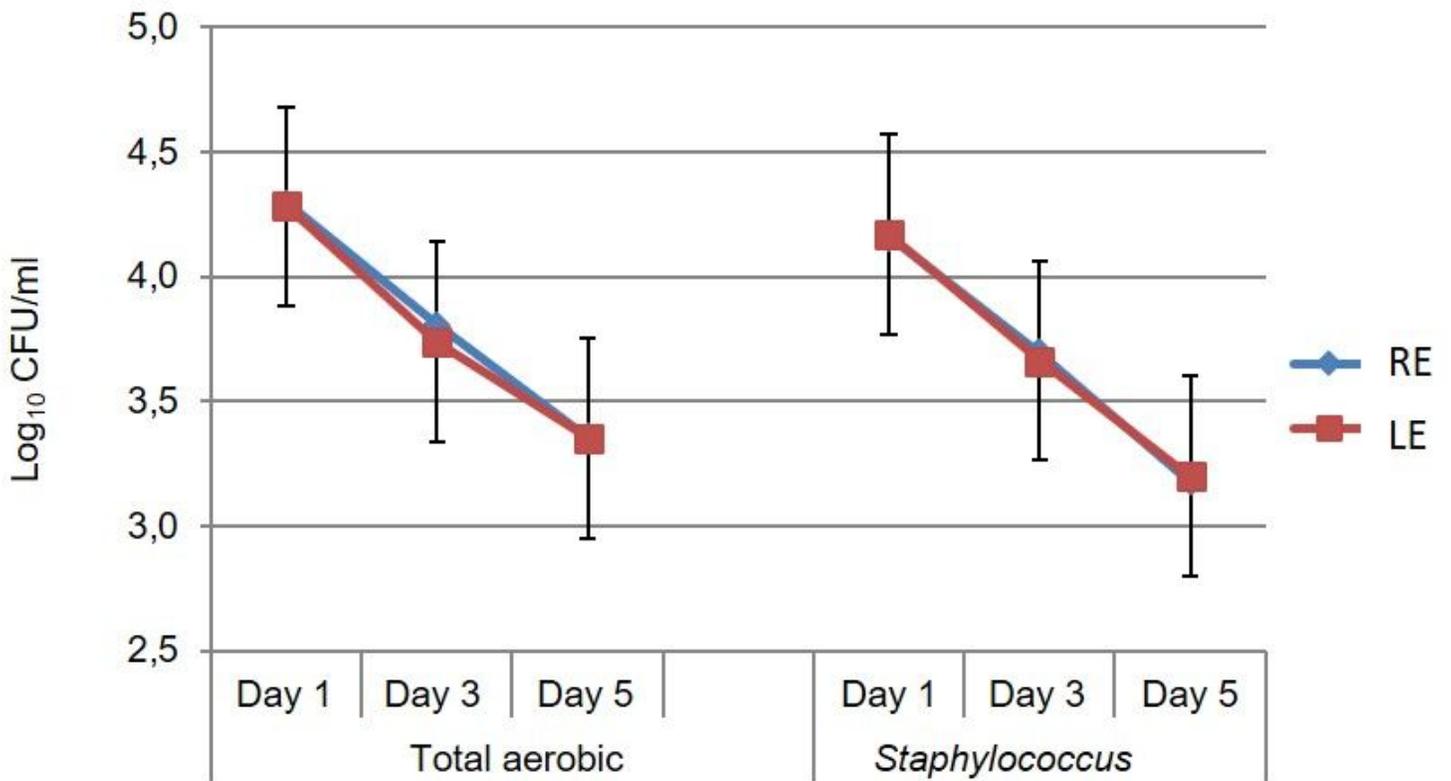


Figure 3

Comparison of bacterial load reduction between right and left eyes. The graph shows average values of the bacterial load for total aerobes and *Staphylococcus* spp. at each day of assay for both right eyes (REs) and left eyes (LEs). Error bars indicate standard deviations.

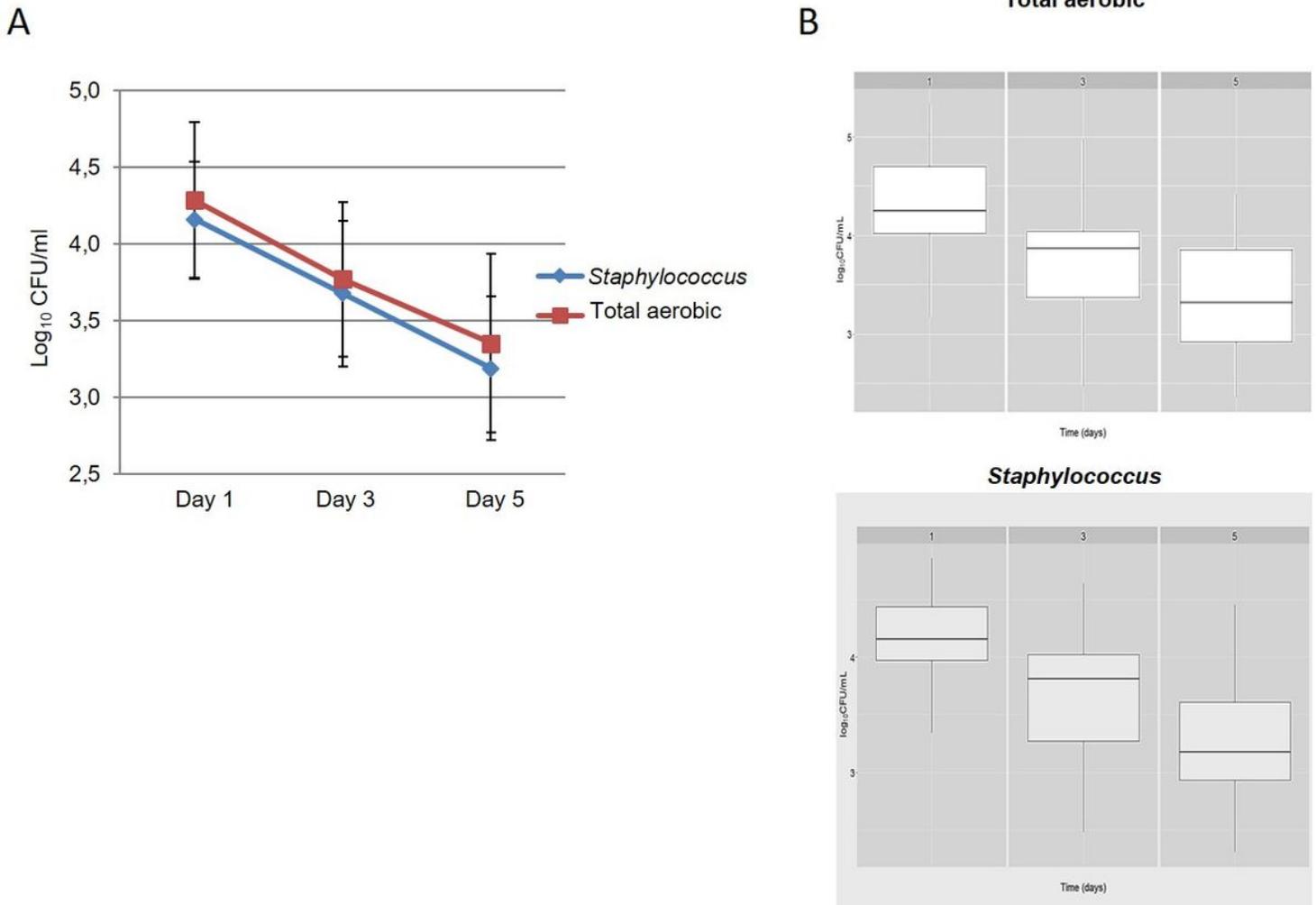


Figure 4

Comparison of bacterial load reduction between aerobes and *Staphylococcus* at different timepoints. (A) Linear graph of average values (log₁₀ CFU/ml) of the wipes for each microorganism at each day of assay. (B) The data (log₁₀ CFU/ml) is represented in box diagrams. In this graph, the upper squares include samples that present values within the 75th percentile, and in the lower squares the values are shown within the 25th percentile. The line that crosses the box indicates the average values and the upper and lower error bars indicate the maximum and minimum values.

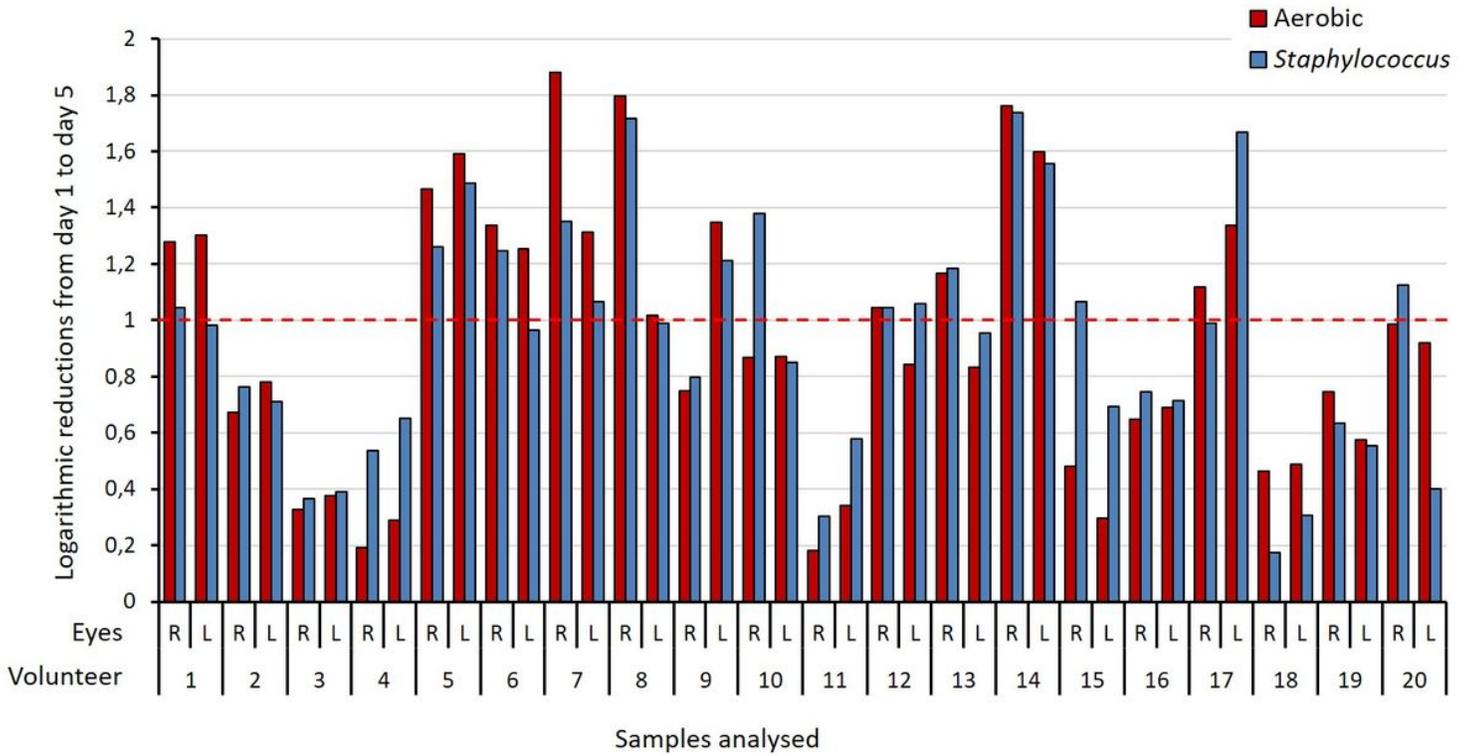


Figure 5

Logarithmic reductions of bacterial load from each eye. Columns represent the total aerobes and *Staphylococcus* spp. logarithmic reductions from day 1 to day 5 (\log_{10} No CFU/ml day 1 - \log_{10} Nt CFU/ml day 5). The dotted line indicates a reduction of 1 logarithmic unit (\log_{10}), what means a reduction of 90%.

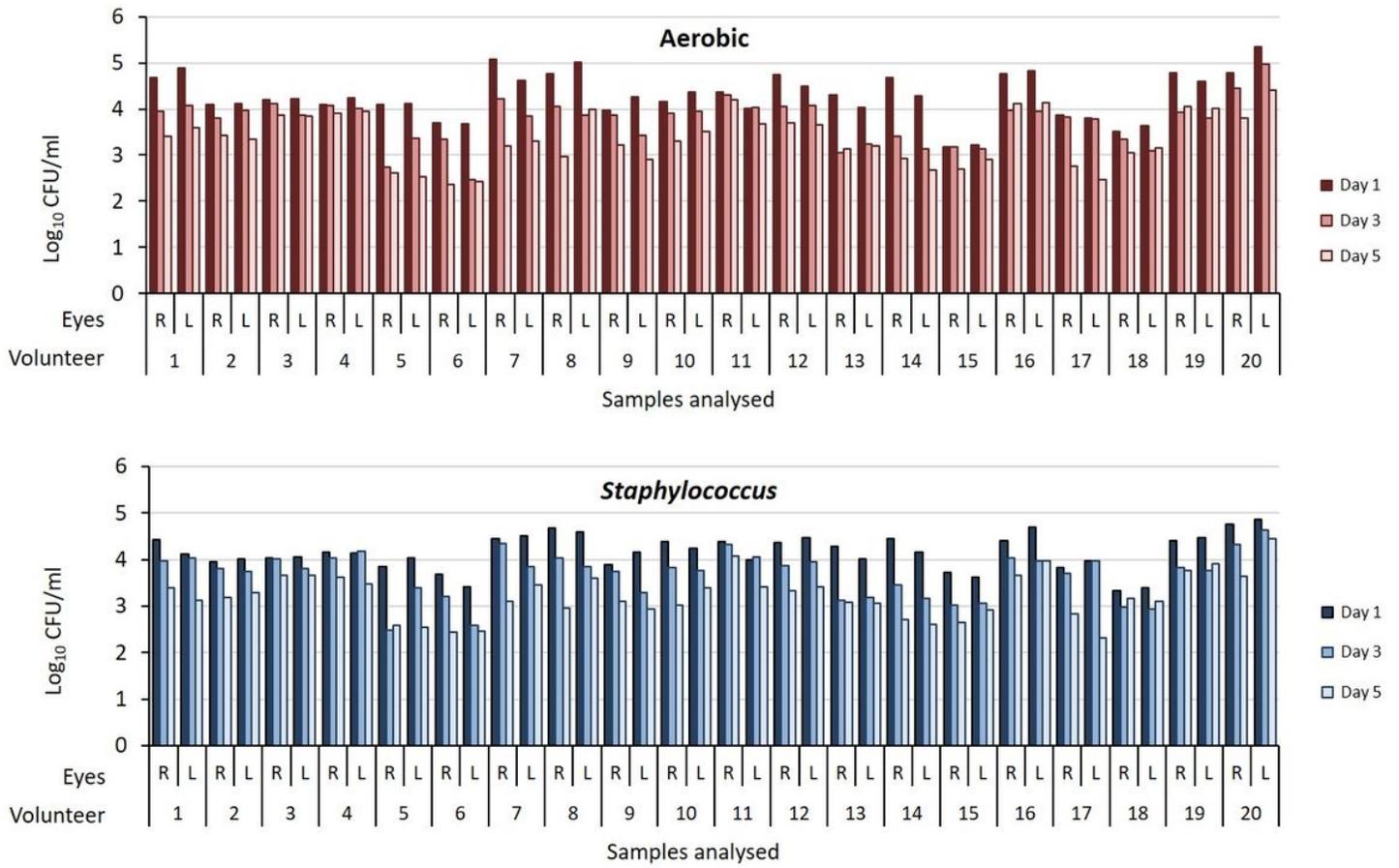


Figure 6

Microbial load on the different timepoints. Columns represent the total aerobes and *Staphylococcus* spp. load present in the left and right eyes of each of the 20 volunteers on day 1, 3 and 5 of the study.