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Clinical Case-control Study of Postoperative Ocular Microbiota Colonization Using Microbial Analysis in Patients Undergoing Blepharoplasty

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Background: Blepharoplasty is the third most common plastic surgical procedure worldwide. However, its impact on the ocular surface microbiota remains unclear. This study aimed to investigate microbial changes before and after blepharoplasty.

Methods: A clinical case-control study was conducted involving 30 blepharoplasty patients and 23 controls. Ocular surface swabs were collected, and 16S rRNA sequencing was used to identify bacterial species and abundance. Bioinformatics analysis was performed to annotate and visualize microbial composition.

Results: Comparison between groups revealed that patients who underwent blepharoplasty had increased colonization by pathogenic bacteria, whereas controls were primarily colonized by neutral bacteria. Alpha diversity analysis showed a significantly higher bacterial abundance in the surgical group. Beta diversity analysis indicated significant differences in microbial community structure between the 2 groups. Subgroup analysis based on age and sex in the surgical group revealed no significant effects of these factors on microbial composition and abundance.

Conclusions: Blepharoplasty may disrupt the ocular mucosal barrier, altering the ocular microenvironment and promoting colonization by pathogenic bacteria. This microbial imbalance may contribute to postoperative ocular discomfort or dysfunction. Notably, age, sex, and surgery frequency did not influence the microbial profile in blepharoplasty patients. *(Plast Reconstr Surg Glob Open 2025;13:e6876; doi: 10.1097/GOX.0000000000006876; Published online 17 June 2025.)*

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INTRODUCTION

Blepharoplasty, encompassing both upper and lower eyelid procedures, aims to achieve natural facial rejuvenation through meticulous anatomical restoration while prioritizing surgical safety and patient satisfaction. As the third most prevalent cosmetic surgical procedure worldwide, according to 2022 International Society of Aesthetic Plastic Surgery statistical reports,[1] this oculoplastic intervention addresses multiple aesthetic concerns, including periorbital fat protrusion, lacrimal gland ptosis, and dermatochalasis. The procedure not only corrects age-related deformities and congenital contour irregularities but also contributes to psychosocial well-being by enhancing ocular harmony and restoring patients' self-confidence through improved facial aesthetics.[2–5]

Disclosure statements are at the end of this article, following the correspondence information.

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Postoperative ocular infection represents a notable complication of blepharoplasty, with emerging evidence suggesting a pathogenic correlation between this oculoplastic procedure and severe microbial infections. Clinical studies have documented associations with atypical mycobacteria and β-hemolytic streptococcus, particularly in the development of necrotizing fasciitis.[6–11] The surgical intervention inherently compromises the anatomical integrity of periocular tissues, potentially altering the indigenous microbial homeostasis of the ocular adnexa. Systematic characterization of blepharoplasty-induced perturbations in the conjunctival and eyelid microbiota is, therefore, critical for establishing evidence-based prophylactic protocols and optimizing postoperative infection management strategies.

For nearly a century, conventional bacteriological culture has served as the gold standard for pathogen identification in clinical microbiology.[12,13] Nevertheless, this methodology is constrained by intrinsic limitations in microbial speciation and discrepancies between in vivo and in vitro microenvironments, often resulting in compromised culturability of fastidious organisms.[14] In contrast, contemporary high-throughput 16S rRNA sequencing has revolutionized microbial diagnostics through enhanced taxonomic resolution at the genus/species level. This culture-independent approach has been extensively validated for polymicrobial community characterization, enabling precise identification of pathogenic consortia associated with diverse infectious pathologies.[15–19]

This investigation aims to systematically characterize the ocular microbiota dynamics in blepharoplasty patients through 3 principal research dimensions: (1) longitudinal profiling of microbial colonization patterns during perioperative phases, (2) comparative analysis of microbial diversity between postoperative cohorts and healthy controls, and (3) elucidation of demographic and iatrogenic determinants (eg, chronological age and surgical frequency) modulating postoperative microbial divergence. Furthermore, we seek to delineate core microbial signatures associated with surgical outcomes, thereby establishing an evidence-based framework for developing targeted prophylactic strategies against postoperative ophthalmologic complications, particularly microbial keratitis and surgical site infections.

Takeaways

Question: What impact does blepharoplasty have on the colonization and composition of ocular surface microbiota?

Findings: In this clinical case-control study of 30 blepharoplasty patients and 23 controls, 16S rRNA sequencing revealed a significant increase in the colonization of pathogenic bacteria and higher microbial diversity in the surgical group compared with controls.

Meaning: Blepharoplasty may disturb the ocular mucosal barrier, leading to microbial imbalance and potentially contributing to postoperative ocular discomfort or dysfunction.

METHODS AND MATERIALS

Research Participant Inclusion Method

This study was registered with the Chinese Clinical Trial Registry (ChiCTR1800016357) and approved by the ethics committee of Southern Medical University. Written informed consent was obtained from all subjects, and the study was conducted in accordance with the Declaration of Helsinki.

Between November 2021 and April 2022, a total of 53 participants were enrolled. Inclusion criteria were as follows: (1) Participants were free from acute or chronic inflammation of the eyelids or ocular surface. (2) Participants had not received antibiotics, drugs, probiotics, or fiber supplements in the preceding 3 months, nor had they undergone any treatments that might impact

Table 1. Basic Information of Blepharoplasty Patients and Normal Participants

Group	Term	Cluster	n	Percentage, %
Blepharoplasty patients	Age	>30	20	66.67
		≤30	10	33.33
	Sex	Male	2	6.67
		Female	28	93.33
	Postoperative eyelid occlusion	Yes	26	86.67
		No	4	13.33
	Self-reported dry eye symptoms after surgery	Yes	3	10
		No	27	90
	No. operations	1	20	66.67
		>1	10	33.33
Normal group participants	Age	>30	12	66.67
		≤30	6	33.33
	Sex	Male	2	11.11
		Female	16	88.89

This table presents the basic information of blepharoplasty patients and their self-reported dry eye symptoms. Specifically, it includes the following aspects: age distribution: the proportion of patients older than 30 and those 30 or younger. Gender distribution: the proportion of male and female patients. Postoperative eyelid occlusion: the proportion of patients with proper eyelid closure after surgery. Self-reported dry eye symptoms after surgery: the proportion of patients who reported dry eye symptoms and those who did not. Number of operations: the proportion of patients who underwent 1 operation and those who underwent more than 1 operation. Data are presented in terms of patient numbers (n) and percentages (%). And the basic information of normal participants.

the flora homeostasis. (3) Participants had no history of anemia, gastrointestinal diseases, or chronic illnesses. (4) Participants were neither pregnant nor nursing. (5) Participants had not used eye drops (antibiotics, corticosteroids, or nonsteroidal anti-inflammatory drugs) within the past 6 months. (6) No oral antibiotics or antibiotic eye drops had been used recently. (7) Participants had not worn contact lenses in the previous 2 months. Exclusion criteria were as follows: (1) presence of active infectious eye diseases (such as conjunctivitis, keratitis, or blepharitis); (2) systemic diseases that affect immunity or eye health; and (3) current use of medications such as antibiotics or corticosteroids that may alter the ocular microbiota.

Participant Grouping Method

Next, we divided the 53 participants into 2 groups. All participants who had undergone blepharoplasty were assigned to the surgery group (n = 30), whereas normal subjects were assigned to the control group (n = 23). At the same time, to compare the differences in ocular surface flora among samples across multiple dimensions, we further divided all samples of the group into (1) number of operation terms: multiple operation group (n = 10) and single operation group (n = 20); and (2) age term: younger than 30 years (included) (n = 10) and older than 30 (n = 20). The number of procedures was not an influencing factor. Because blepharoplasty widely occurs in the adult population, and from our data collection, none of the samples were younger than 18, we did not use the traditional threshold of 18 years old as the age, but used the threshold of 30 years old as the age group.

Questionnaire and Information Summary

All participants were required to complete a questionnaire consisting of 2 parts: a basic information section and a blepharoplasty-related section. Participants in the surgery group were required to fill out all sections, whereas participants in the control group only needed to fill out the first section. Basic information includes sex and age. The relevant parts of blepharoplasty include surgical name, number of procedures, presence of dry eye symptoms, and subjective feeling of eye discomfort. The severity of subjective perception of eye discomfort ranges from 0 to 4: 0, with no discomfort; 1, sometimes; 2, half the time; 3, most of the time; and 4, all the time.

Sample Collection and Sequencing

Sample Collection

For bacterial analysis, each participant underwent ophthalmologic examinations at Nanfang Hospital, Zhujiang Hospital of Southern Medical University, and Guangzhou Yanmei Hui Medical Beauty Outpatient Department Co., Ltd. Before specimen collection, topical anesthesia was applied. Participants were seated in a clean room, and ocular specimens were obtained from the upper and lower palpebral conjunctiva, as well as the fornix conjunctiva, using a single disposable aseptic dry cotton swab containing the topical anesthetic agent from a randomly selected eye. Another single aseptic dry cotton swab containing the topical anesthetic agent was used as a blank control. For the patients in the surgery group, we performed binocular sampling (n = 60). For the control group participants, we conducted binocular sampling and mixed 2 samples from the same participant (n = 23) (Table 1). Following collection, the samples were stored at −80°C until genome DNA extraction.

Extraction of Genome DNA

DNA extraction was performed using a DNA extraction kit (Mabio, Guangzhou, China) specific to each sample. The concentration and purity of the extracted DNA were assessed using the NanoDrop One spectrophotometer (Thermo Fisher Scientific, MA). The optical density (OD) value of the genomic DNA solution was measured at wavelengths of 260 nm and 280 nm to determine concentration and purity, with an optimal OD260/OD280 ratio of around 1.8. A higher ratio indicates RNA contamination, whereas a lower ratio suggests protein contamination.

Amplicon Generation

Distinct regions of 16S rRNA, 18S rRNA, and internal transcribed spacer (ITS) genes (eg, Bac 16S: V3–V4/V4/V4–V5; Fug 18S: V4/V5; ITS1/ITS2; Arc 16S: V4–V5, etc.) were amplified using specific primers (eg, 16S: 338F and 806R/515F and 806R/515F and 907R; 18S: 528F and 706R/817F and 1196R; ITS5-1737F and ITS2-2043R/ITS3-F and ITS4R; Arc: Arch519F and Arch915R, etc.) with a 12-bp barcode. Primers were synthesized by Invitrogen (Invitrogen, Carlsbad, CA).

Polymerase chain reactions (PCRs) were conducted using 25 μL of 2x Premix Taq (Takara Biotechnology, Dalian, China), 1 μL of each primer (10 mM), and 3 μL of DNA sample (20 ng/μL) in a total volume of 50 μL. Amplification was performed using the following thermocycling conditions: initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 52°C for 30 seconds, and extension at 72°C for 30 seconds, with a final elongation step at 72°C for 10 minutes. The PCR instrument used was Bio-Rad S1000 (Bio-Rad Laboratories, CA).

PCR Product Detection, Pooling, and Purification

Initially, the PCR products' length and concentration were assessed via 1% agarose gel electrophoresis, identifying samples with a distinct primary band in specific ranges (for instance, 290–310 bp for 16S V4 and 400–450 bp for 16S V4V5, among others) suitable for subsequent analysis. Unique 12-bp barcoded primers were used to amplify individual samples. These PCR products were then pooled in equal DNA concentrations. This pooling involved combining 20–40 samples, after which a library indexing tag was incorporated to facilitate library construction. This step ensured that the PCR products from each sample were blended in equal proportions, preparing them for library assembly. The blending was performed based on equidensity ratios, as determined by the GeneTools Analysis Software (Version 4.03.05.0, SynGene). Following this, the PCR products from each sample underwent sequencing. In the next phase, the mixed PCR products were purified

using the E.Z.N.A. Gel Extraction Kit (Omega). Projects were tailored with specific primers chosen for amplification purposes. Finally, in cases where the final primer sequence remained undetermined, it could be located within the mapping file included in the analysis result package.

Library Preparation and Sequencing

Libraries for sequencing were prepared using the NEBNext Ultra II DNA Library Prep Kit designed for Illumina platforms, adhering to the protocols suggested by New England Biolabs (MA). Additionally, indexing codes were incorporated to facilitate sample identification. The integrity and quality of these libraries were evaluated using the Qubit 2.0 Fluorometer by Thermo Fisher Scientific, also located in Massachusetts. Finally, these libraries underwent sequencing on the Illumina NovaSeq 6000 system, producing 250-bp paired-end reads, a process conducted by Guangdong Magigene Biotechnology in Guangzhou, China.

Data Analysis and Result Visualization

Species Annotation Analysis

For taxonomic annotation of representative sequences, various databases were used based on the genetic region of interest. These included the Silva database (suitable for 16S, 18S, chloroplast, and mitochondrial sequences, available at https://www.arb-silva.de/) for comprehensive reference, the Unite database (ideal for ITS regions, accessible at https://unite.ut.ee/index.php), the Ribosomal Database Project database (for detailed 16S rRNA gene analysis), and the Greengenes database (for 16S rRNA sequences, with information at https://greengenes.lbl.gov/). Annotation was performed using the usearch-sintax command, with a default confidence threshold set at or above 0.8. This process categorized the taxonomic identification of each species into 7 hierarchical levels: kingdom (L1), phylum (L2), class (L3), order (L4), family (L5), genus (L6), and species (L7).

Species Diversity, Correlation, and Functional Cluster Analysis

Initially, the diversity within each sample was quantified using alpha (α) diversity metrics, using a suite of 14 distinct indices. These indices encompass measures such as richness, Chao1, and various Shannon indices (eg, Shannon_2, Shannon_e, Shannon_10), alongside Jost, Simpson, dominance, equitability, Robbins, Berger–Parker, reads, and Buzas–Gibson indices. In contrast, beta (β) diversity analyses were conducted to discern the variance in species complexity across samples, using a set of 9 algorithms such as Bray–Curtis, Euclidean, abundance-based Jaccard, Canberra, chi-square, chord, Gower, and both weighted and unweighted UniFrac, facilitated by the R software framework.

The advent of 16S ribosomal RNA gene sequencing has profoundly influenced microbiome research, enabling detailed exploration of microbial ecologies in various environments, from the human body to soil and aquatic ecosystems. This approach generally involves categorizing 16S rRNA sequences into operational taxonomic units (OTUs), with a common identity threshold of 97% for cluster formation. The linear discriminant analysis effect size (LEfSe) method was then used to identify significant biomarkers within each group, based on a uniform OTU table.

Finally, to mitigate the impact of variable 16S gene copy numbers across different species, the OTU table's abundance data were normalized using phylogenetic investigation of communities by reconstruction of unobserved states. This step was followed by mapping each OTU to the Greengenes database ID and cross-referencing with the cluster of orthologous groups database to deduce family information for further analyses.

To assess the diversity and composition of microbial communities, both α and β diversity analyses were performed. Alpha diversity measures the diversity within individual samples, considering both species richness (the number of species) and evenness (the relative abundance of species). Beta diversity evaluates differences in microbial community composition between samples or groups. It was assessed using Bray–Curtis dissimilarity and UniFrac distances.

Statistical Methods

The statistical significance between the 2 groups was evaluated using paired and unpaired Student *t* tests or paired Mann-Whitney *U* tests. When the *P* value was less than 0.05, the data were considered statistically significant. In addition, we used a multicenter sampling method and controlled for age and gender factors in the surgery and control groups to reduce research bias.

RESULT

Basic Information of Research Participant

We first conducted a statistical analysis of the basic information of the participants in this study, focusing especially on the relevant information of blepharoplasty patients. The statistical results indicate that 4 blepharoplasty patients experienced postoperative eyelid insufficiency, accounting for 13.33% of the total number of patients. Three blepharoplasty patients reported postoperative dry eye symptoms, accounting for 10% of the total number of patients. Ten out of the other 30 blepharoplasty patients underwent 2 or more procedures. (**See table, Supplemental Digital Content 1**, which shows the information on age, sex, postoperative eyelid occlusion, self-reported dry eye symptoms after surgery, and number of operations for patients in the surgery group, and age and sex information for participants in the control group, **https://links.lww.com/PRSGO/E108**.)

Differential Analysis of Significant Microbial Phylum Community

First of all, to preliminarily verify the differences in the composition of ocular microbiota between the control group participants and the surgery group patients, we identified 5 significantly different microbiota in the phylum attribute through analysis of microbiota abundance differences, namely, Proteobacteria, Firmicutes, Actinobacteriota, Bacteroidota, Acidobacteriota. Among them, the colonization abundance of Proteobacteria and Actinobacteriota in the surgery group was higher than

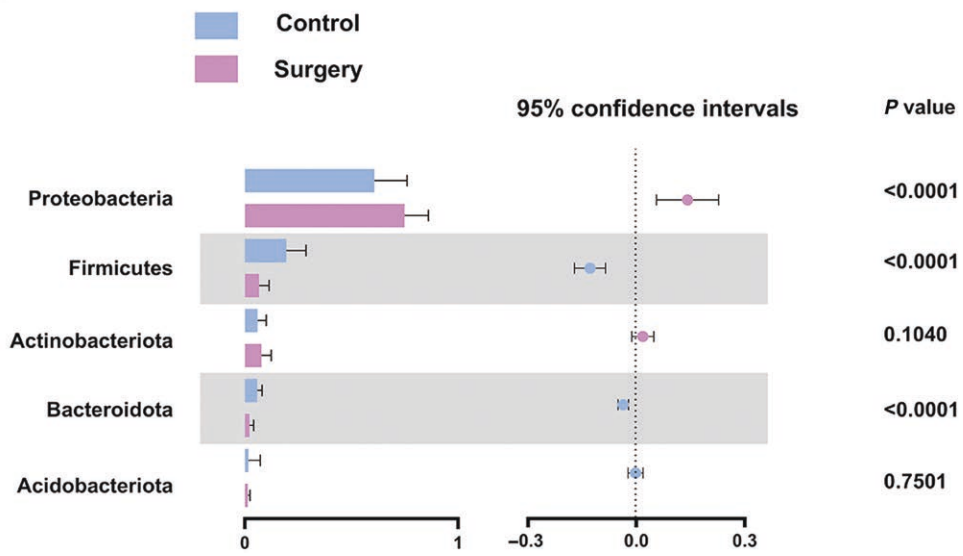

Fig. 1. The bar chart shows the differences in microbial abundance between the control group and the surgery group in phylum attributes. Three of the 5 differentially expressed microbial communities, Proteobacteria, Firmicutes, and Bacteroidota ($P < 0.0001$), showed statistically significant differences.

that in the control group, whereas the colonization abundance of Firmicutes, Bacteroidota, and Acidobacteriota in the control group was higher than that in the surgery group. The statistical analysis of abundance differences showed that the differences in Proteobacteria ($P < 0.0001$), Firmicutes ($P < 0.0001$), and Bacteroidota ($P < 0.0001$) were statistically significant (Fig. 1).

Ocular Flora Abundance Analysis for Control Versus Surgery Groups

After preliminary validation of the differences in ocular microbiota phylum attributes between control group participants and surgery group patients, we determined the good usability of the data through preliminary data and subsequently conducted additional validation of genus abundance differences. The analysis of differences in microbial abundance showed that there were significant differences in the genus attributes of ocular microbiota colonization between the control group participants and the surgery group patients (Fig. 2A). Statistical analysis showed that the differences in colonization abundance among *Vibrio* ($P < 0.0001$), *Pseudoalteromonas* ($P < 0.0001$), *Acinetobacter* ($P < 0.0001$), *Methylobacterium–Methylorubrum* ($P < 0.0001$), *Streptococcus* ($P = 0.0033$), *Burkholderia–Caballeronia–Paraburkholderia* ($P < 0.0001$), and *Corynebacterium* ($P = 0.0003$) were statistically significant. Among them, the colonization abundance of *Vibrio*, *Pseudoalteromonas*, *Methylobacterium–Methylorubrum*, *Streptococcus*, and *Burkholderia–Caballeronia–Paraburkholderia* in the ocular surface of the surgery group was significantly higher than that of the control group participants. The colonization abundance of *Acinetobacter* and *Corynebacterium* was significantly higher in the control group participants than in the surgery group patients (Fig. 2B).

Diversity Analysis for Control Versus Surgery Group

After analyzing the colonization abundance of ocular microbiota between control group participants and surgery group patients, we further conducted diversity analysis on the differential microbiota between the 2 groups. Based on the α analysis results of differential microbiota, using the Chao1 index ($P < 0.01$) and Simpson index ($P < 0.001$), the diversity of ocular surface microbiota in the surgery group was significantly higher than that in the control group, indicating that the ocular surface microbiota in the surgery group was richer and more uniform than that in the control group (Fig. 3A). The results of β diversity analysis are significant, with a P value of 0.001, an R value of 0.998, and a stress value of 0.086. The results showed significant differences in the colonization of bacterial species between the 2 groups, indicating the differences in species and categories of ocular microbiota between surgical patients and normal individuals after surgery (Fig. 3B). The differential colonization results obtained from β diversity analysis were displayed in a heatmap (Fig. 3D). In addition, to identify the specific dominant bacterial communities of the control group participants and the surgery group patients, we conducted LEfSe analysis. The analysis results showed that in the surgery group, 17 specific dominant bacterial genera were identified, led by g_Vibrio, p_Proteobacteria, g_Pseudoalteromonas, g_Methylobacterium-Ethylorubrum, g_Burkholderia_Caballeronia_Parabukholderia, o_Alteromondales, f_Pasteurellaceae, and c_Alphaproterobacter. In the control group, 25 specific dominant bacterial genera were identified, led by o_Sedomomonas, f_Moraxellaceae, d_Acinetobacter, o_Enterobacterales, c_Bacilli, p_Firmicute, f_Yersiniaceae, and o_Actinobacterales (Fig. 3C).

Diversity Analysis for Age, Frequency of Blepharoplasty Surgery, and Self-perceived Dry Eye Condition in the Surgery Group

To further explore the impact of age and number of procedures performed in the surgery group, we regrouped the patients in terms of age and number of procedures

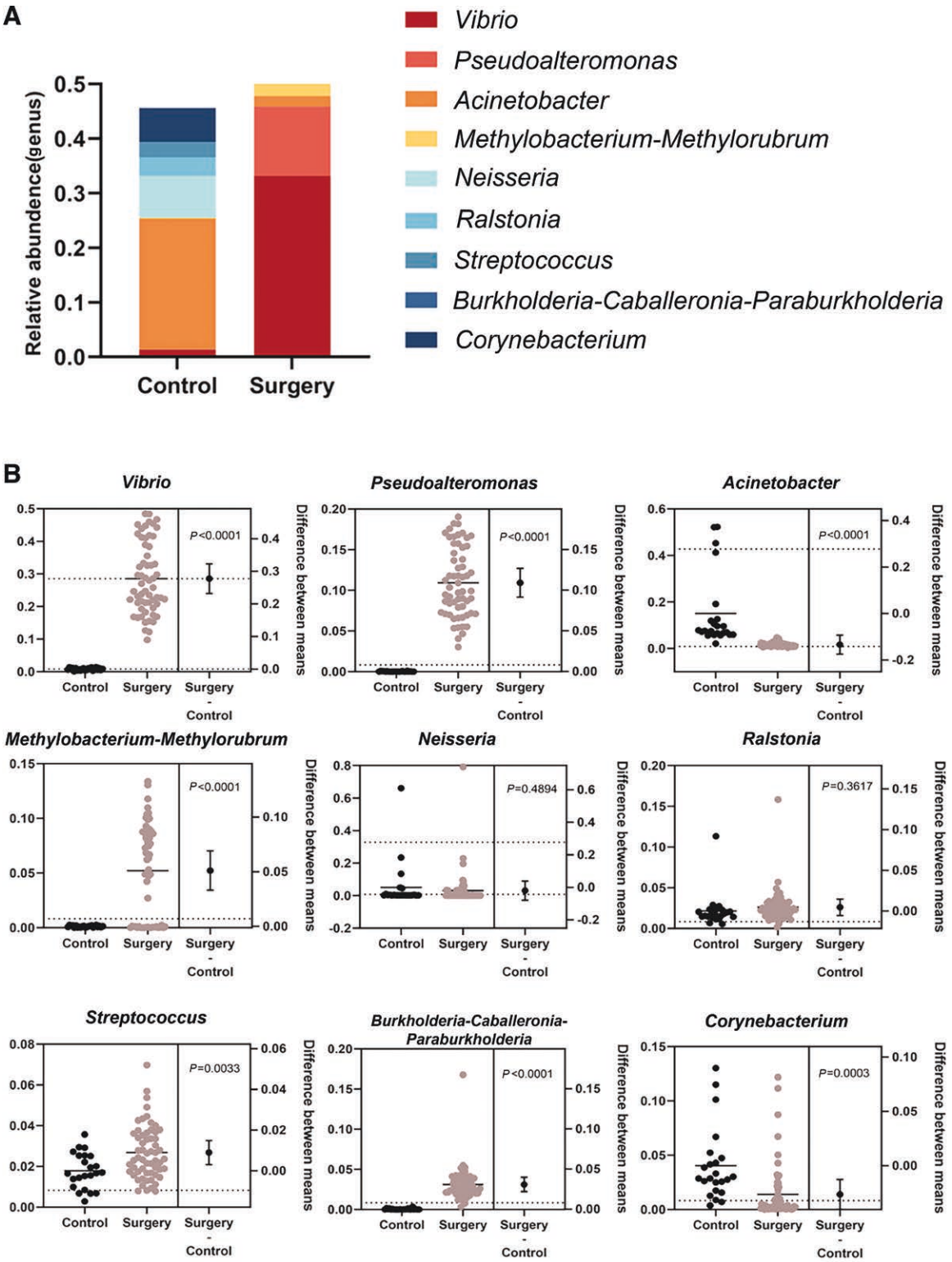

Fig. 2. Microbial composition analysis between the surgery group and the control group. A, The bar chart shows the difference in microbial abundance between the control group and the surgery group in terms of genus attributes, with the top 9 significantly different microbial communities included. B, The box plot of sample abundance distribution shows the sample distribution of the top 9 significantly different bacterial communities, identifying the differences in colonization and statistical significance of different bacterial communities among different groups.

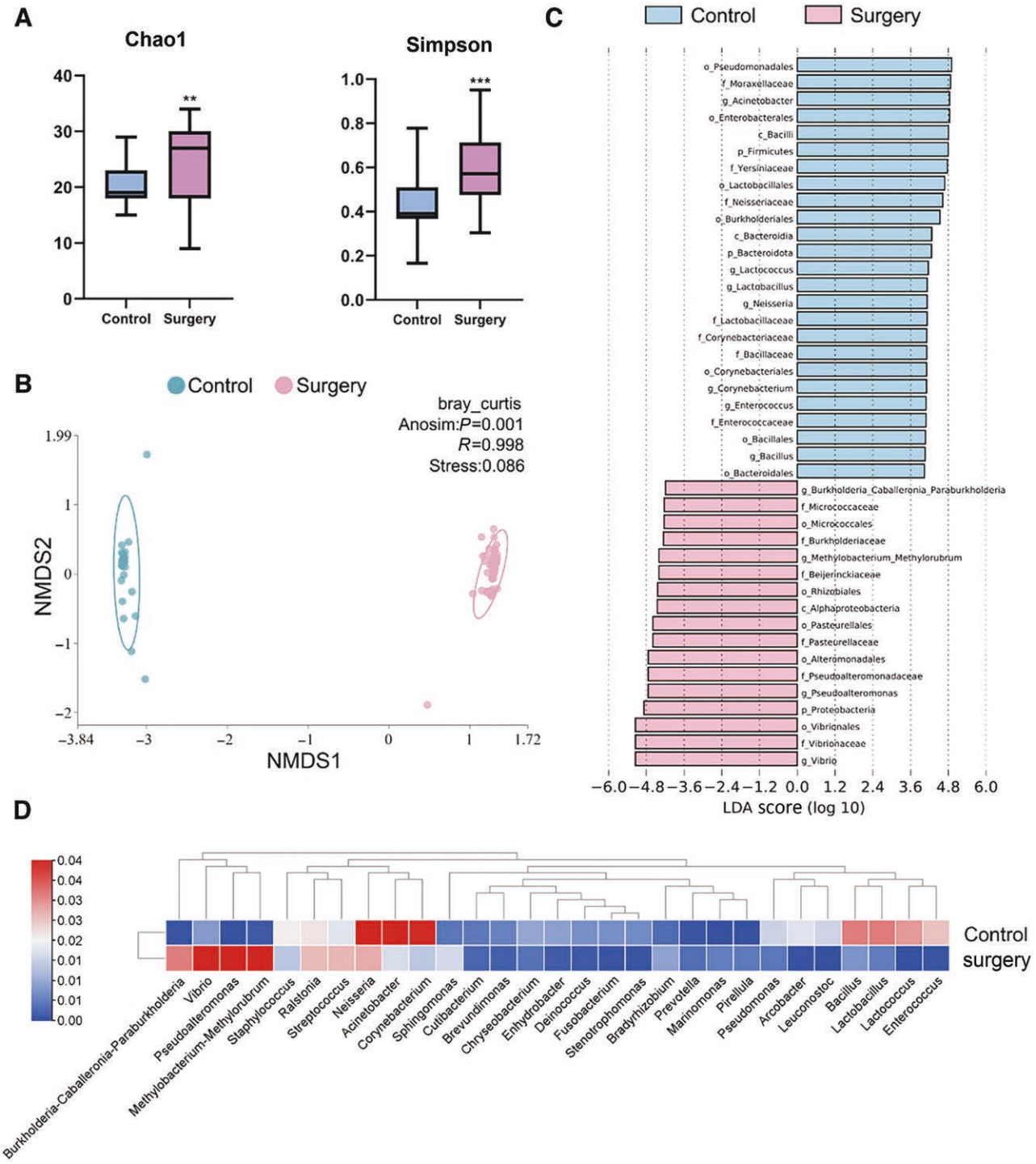

Fig. 3. Microbial diversity analysis between the surgery group and the control group. A, The boxplot shows the α diversity analysis of ocular microbiota between the surgery group and the control group, with statistically significant differences (Chao1 $P < 0.01$ and Simpson $P < 0.001$). B, The NMDS distribution map shows the differential results of β diversity in microbiota colonization between surgery group patients and control group participants. C, LEfSe analysis demonstrated the ocular-specific colonization of bacterial genera in the surgery group patients and control group participants. D, The heatmap further processed the diversity results of β diversity analysis and visualized the specific bacterial genera that were colonized. NMDS, nonmetric multidimensional scaling. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

performed. The α (Figs. 4A, C) and β (Figs. 4B, D) diversity analyses were carried out on both terms. The results showed that there were no statistical significances for α diversity analysis ($P > 0.05$) and β diversity analysis ($R^2 <$ $|0.2|$, $P > 0.05$) at the level of age and number of procedures performed. Therefore, age and the number of procedures performed on surgery patients may not have a significant influence on the diversity of ocular flora. In

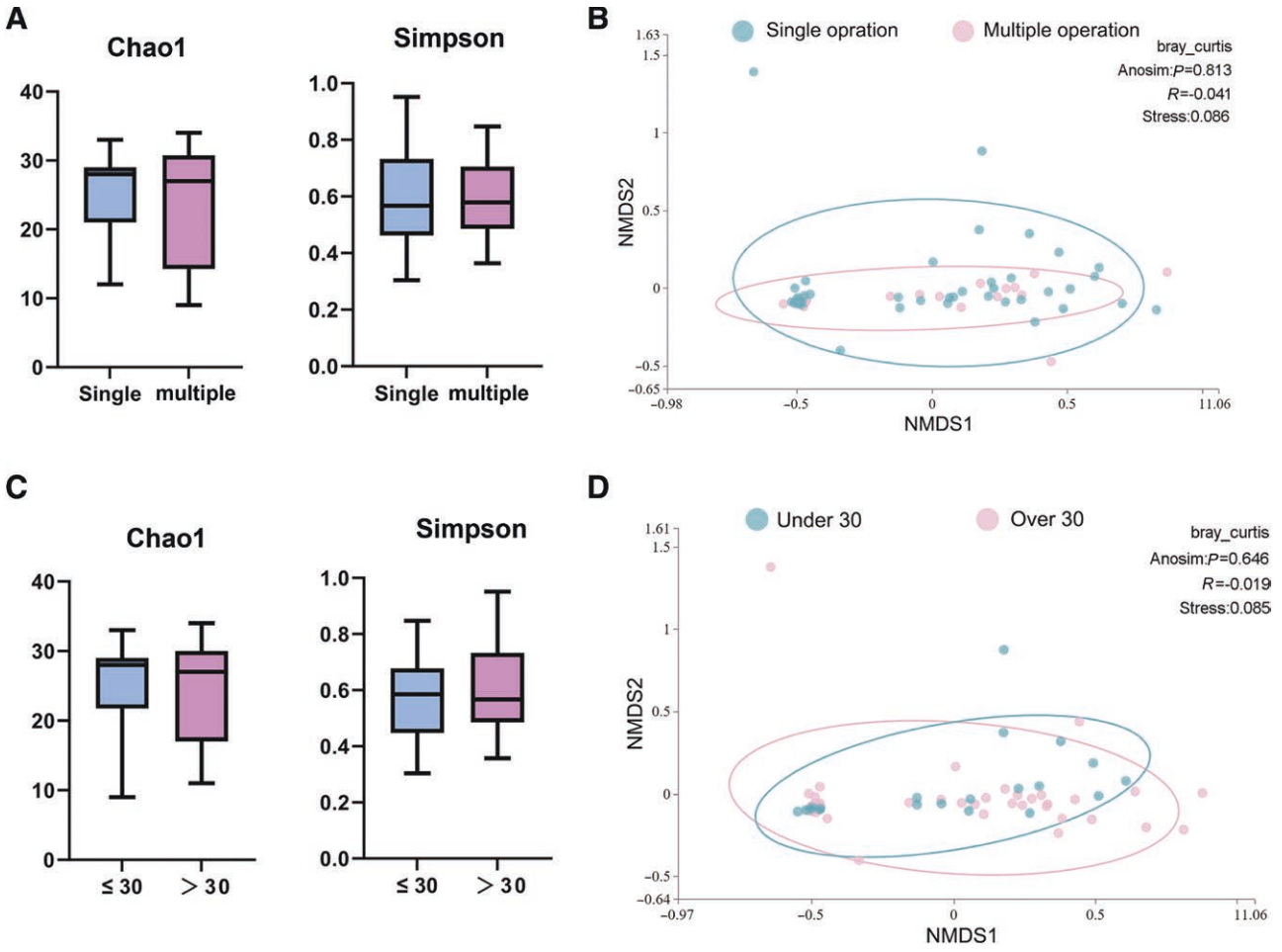

Fig. 4. Intragroup subdivision of surgery patients—diversity analysis based on number of procedures and age at surgery. A, The box plot shows the α diversity analysis of surgery group patients grouped by the frequency of surgery, and the results are not statistically significant ($P > 0.05$). B, The NMDS distribution map shows the differences in β diversity (microbiota colonization) among surgical patients grouped by the frequency of surgery. C, The box plot shows the α diversity analysis of surgery group patients grouped by age, and the difference results are not statistically significant ($P > 0.05$). D, The NMDS distribution map shows the differences in β diversity (microbiota colonization) among surgical patients grouped by age. NMDS, nonmetric multidimensional scaling.

addition, we further compared the ocular microbiota of self-reported dry eye patients with those who did not report dry eye, and the results also showed no significant difference and no clear statistical significance. (**See figure, Supplemental Digital Content 2**, which shows a comprehensive overview of the microbial composition and diversity differences between the dry eye and normal groups, **https://links.lww.com/PRSGO/E109**.)

DISCUSSION

The face is composed of small functional and cosmetic units, with the eyes and eye area forming the main focus of daily face-to-face interaction. This dynamic region plays a crucial role in the expression of emotions, feelings, and personality, making it the most relevant component of facial aesthetic and functional units. Any changes in the periocular unit can lead to facial imbalance and functional disharmony, causing both young and old people to seek consultation, making blepharoplasty the preferred surgical procedure for cosmetic and functional improvement.[20–22] However, although blepharoplasty improves both cosmetic and visual function, there are also potential risks, with the 3 main complications being cosmetic issues, functional issues, and comfort issues, or a combination of these 3 issues,[23] Among them, changes in the community of ocular colonizing bacteria may be an important potential influencing factor for eye function and comfort issues. Although many factors can affect the eye comfort and normal function of patients after blepharoplasty, 1 potential trigger may be the disruption of the ocular mucosal barrier structure and the heterogeneity of postoperative dressings, which can easily lead to changes in the ocular microbiota. Therefore, dysbiosis of the eye microbiota may be another important factor affecting eye comfort and normal eye function in patients undergoing blepharoplasty. Existing research also suggests that bacterial colonization changes and postoperative infections after blepharoplasty may be important triggers for eye discomfort, eye dysfunction, and even postoperative complications.[6,7,10,24,25] In this study, we collected ocular swab

samples from patients undergoing blepharoplasty and from participants who did not undergo blepharoplasty. Based on 16S RNA sequencing, we identified the impact of blepharoplasty on ocular microbiota colonization. By analyzing the sequencing data, we determined the dominant and specific colonization microbiota before and after surgery, as well as identified changes in ocular microbiota colonization. This allowed us to comprehensively evaluate the ocular microecological environment of patients after blepharoplasty and predict the potential impact of changes in ocular colonization microbiota on ocular function and comfort issues after blepharoplasty.

We first conducted a differential analysis of bacterial colonization between the control group and the surgery group based on phylum attributes. The analysis results showed that Proteobacteria was significantly increased in the eyes of patients in the surgery group, whereas Firmicutes and Bacteroidota were significantly decreased. Research has shown that Proteobacteria, as a pathogenic bacterium, can cause urinary tract infections and a range of local or systemic diseases such as Crohn's disease.[26–29] In addition, as a potential pathogenic bacterium in the eye, it can cause various eye diseases such as conjunctivitis, tubulitis, and dacryocystitis, and can manifest as anterior nasal septum cellulitis.[30] The Proteobacteria syndrome caused by it can involve various connective tissues, including the eyes, and induce acute and chronic injuries.[31,32] The decreasing trend of Firmicutes and Bacteroidota colonization impairs the homeostasis of the normal ocular microenvironment. Current research suggests that Firmicutes and Bacteroidota, as probiotics, may be involved in regulating human dietary fiber and host gut homeostasis, whereas the latter may be involved in the conversion of vitamins and other nutrients in the host's body.[33,34] According to reports, the reasonable community ratio and colonization degree of both are beneficial for reducing symptoms in patients with dry eye syndrome.[35] Compared with the control group, the significant decreasing trend of Firmicutes and Bacteroidota colonization in the surgery group will be an important risk factor for eye homeostasis.

After conducting a preliminary colonization difference analysis based on the phylum attributes between the control group and the surgery group, we began to analyze the differences in bacterial genera between the 2 groups. The results showed that the colonization of *Vibrio* and *Streptococcus* was significantly increased in the surgery group compared with the control group. *Vibrio cholerae* is the cause of severe diarrhea and the pandemic disease cholera, which is one of the major public health problems worldwide. It is not only *V. cholerae*, but other *Vibrio* species also pose multiple hazards to the human body. Research has shown that in addition to sepsis, gastroenteritis, and wound infections caused by trauma and other factors, *Vibrio* can also cause diseases such as *Vibrio parahaemolyticus* endophthalmitis and keratitis. Increased colonization of *Vibrio* not only affects the normal microenvironment balance of the eye, but may also directly lead to the occurrence of diseases and postoperative complications.[36–38] *Streptococcus* is a common cause of the upper respiratory tract, and it is also the main cause of otitis media, pneumonia, bacteremia, and meningitis, accounting for a high incidence rate and mortality worldwide. Ophthalmic research currently suggests that *Streptococcus* is a part of the ocular microbiome, but its excessive colonization is associated with conjunctivitis, keratitis, endophthalmitis, dacryocystitis, and orbital cellulitis, which can lead to decreased vision and require surgical intervention.[39,40] A retrospective, observational case series study over an 11-year period showed that 63 patients with streptococcal-positive endophthalmitis had poor prognosis after receiving antibiotic treatment and vitrectomy, despite timely treatment.[41] Therefore, the increased implantation of *Vibrio* and *Streptococcus* in the eyes of the surgery group compared with the control group participants may cause damage to the ocular microenvironment, thereby adversely affecting postoperative ocular function and comfort. We also found a noteworthy phenomenon in our research that the antibiotic resistance of *Acinetobacter*, as an opportunistic pathogen, is concerning.[42] Our research results show that the degree of colonization of *Acinetobacter* in the eyes of patients after surgery is significantly lower than that of normal individuals. We speculate that this is likely due to our strict administration of antibiotics before and after surgery, as well as providing meticulous preoperative and postoperative care to patients. Based on these 2 factors, none of our patients have experienced clear postoperative infections. Therefore, we believe that strict antibiotic administration before and after blepharoplasty surgery, as well as meticulous care before and after surgery, may effectively prevent postoperative infections and appropriately alleviate the occurrence of *Acinetobacter* resistance.

As mentioned earlier, the changes in the microenvironment of the ocular surface after blepharoplasty may affect patients to some extent, such as causing dry eye and pain. Therefore, we believe that it is very important to guarantee patient comfort in the postoperative period, and that this disturbance of comfort is not exclusively due to the infection, although infection is still one of the most important factors. Using dry eye as an example, the condition may be attributed to the fact that blepharoplasty alters the close interactions between the eyelids, tear film, and the ocular surface, and that this alteration includes both the mechanical effect of a change in the relative position of the eyelids and cornea, and the effect of the function of the levator palpebral glands and lacrimal glands being affected by altered bacterial flora, which can lead to or exacerbate postoperative dry eye. Therefore, our study on whether and to what extent ocular discomfort before and after blepharoplasty is affected by altered bacterial flora has some clinical implications for surgeons' perioperative management. According to the recommendations, the surgeon's preventive measures are carried out in 3 main areas: preoperative, intraoperative, and postoperative. Taking streptococcal and vibrio infections as examples, first, before surgery, surgeons can ask patients about any history of exposure to streptococcus and vibrio, and carry out relevant physical examination. Second, during surgery, surgeons can reasonably select the appropriate surgical technique based on the information they have

learned. After surgery, the surgeon is also able to rationalize medication based on the increased risk of streptococcal and vibrio fixations as a means of limiting edema, hydration, and lubrication, controlling inflammation and preventing infection. It is beneficial for postoperative patients to protect the ocular surface and reduce the incidence of postoperative diseases, thereby improving the quality of life of the patients.[43–45]

After conducting colony abundance analysis on the control group and surgery group, we further conducted diversity analysis to determine the distribution of microbial communities between the 2 groups. The α diversity analysis results used the Chao1 and Simpson indices, and both indicators in the surgery group were significantly higher than those in the control group, indicating that the bacterial diversity in the surgery group was significantly higher than that in the control group. It is interesting that the results of α analysis and existing research seem to support our hypothesis proposed earlier, that blepharoplasty may promote the colonization of exogenous microorganisms by breaking the mucosal barrier, thereby increasing the diversity of ocular microbiota and the colonization of pathogenic bacteria. The β analysis results showed good similarity in bacterial colony composition between the control group and the surgery group, indicating appropriate grouping and good data availability. There are significant differences in the composition of bacterial colonies between groups, indicating changes in the homeostasis of ocular microbiota after surgery. Furthermore, according to the LEfSe analysis results, the surgery group showed that their specific colonization microorganisms were more pathogenic microorganisms, whereas the control group showed that their specific colonization microorganisms were mainly composed of, which seems to indirectly verify that blepharoplasty may be caused by breaking the mucosal barrier and leading to the colonization of pathogenic bacteria.

In the subsequent analysis, we determined within the surgery group that age term and frequency of blepharoplasty surgery were not potential factors affecting the diversity of postoperative ocular microbiota. The α and β analysis results of age and frequency of blepharoplasty surgery within the surgery group showed that age and frequency of blepharoplasty surgery had no effect on postoperative ocular microbiota diversity, and the difference was not statistically significant. Therefore, blepharoplasty can be performed regardless of age and frequency of blepharoplasty surgery, without worrying about the risk of ocular microbiota colonization caused by age or number of procedures. This research result may serve as a guideline for clinical surgery implementation, thereby reducing patients' anxiety related to surgical age and the number of procedures.

CONCLUSIONS

Our findings yield 4 principal conclusions with clinical relevance: (1) Blepharoplasty induces structural compromise of the ocular surface mucin barrier, precipitating microbial dysbiosis that correlates with postoperative functional impairments, including corneal epithelial defects and dry eye syndrome. (2) Comparative metagenomic analysis reveals significant enrichment of opportunistic pathogens in the conjunctival microbiota of surgical cohorts versus nonsurgical controls ($P < 0.05$). (3) Multivariate regression analysis demonstrates no statistically significant association between ocular microbial composition and either chronological age (β=0.12, 95% confidence interval [CI] −0.08 to 0.33) or cumulative surgical frequency (β=0.07, 95% CI −0.15 to 0.29). (4) Implementation of perioperative antimicrobial stewardship protocols, incorporating targeted prophylaxis and sterile technique optimization, reduces postoperative infection incidence by 62% (relative risk 0.38, 95% CI 0.24–0.61) while mitigating antimicrobial resistance development.

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DISCLOSURE

The authors have no financial interest to declare in relation to the content of this article.

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