

Exploring the Microbial Changes in Meibomian Gland Dysfunction through 16S rDNA Sequencing

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Abstract

Background: Meibomian gland dysfunction (MGD) is a common condition that occurs when the Meibomian glands, responsible for secreting tears in the eyes, do not produce enough oil or produce oil of poor quality. This can lead to various symptoms including dry eyes, discomfort, and poor ocular function. The study examines bacterial community diversity in patients with Meibomian Gland Dysfunction (MGD) using 16S rDNA sequencing, compared to healthy individuals. The goal is to understand microbial changes in MGD and provide insights into potential treatments.

Material and Methods: 27 16S rRNA gene sequences were obtained from the (EMBL-EBI) website, consisting of 3 sequences from healthy individuals, 7 sequences from individuals with mild Meibomian gland dysfunction, 6 sequences from individuals with moderate Meibomian gland dysfunction, and 11 sequences from individuals with severe Meibomian gland dysfunction. An algorithm utilizing machine learning was applied to identify the association with each sequence. A trained classifier was then used to create an OTU table.

Results: Our results found that there were significant differences in alpha diversity among individuals with severe (MGD) and healthy individuals. Furthermore, the microbial composition was found to be similar across all groups, regardless of their MGD status.

Conclusions: This study highlights the correlation between meibomian gland dysfunction (MGD) and imbalances in the bacterial microbiota on the ocular surface. The results suggest a role for *Staphylococcus*, *Corynebacterium*, and *Sphingomonas* in the development of MGD, with a positive correlation between MGD severity and bacterial abundance. The findings provide a basis for considering antibiotics in MGD treatment, with insights into the microbiome's role in the pathogenesis of the condition.

Keywords: Eye Microbiome; Meibomian Gland Dysfunction; MG; Microbiome; OSDI.

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Introduction

Meibomian gland dysfunction (MGD) is a chronic eye disease that results from inadequate production of oil (meibum) by the meibomian glands. This can lead to inflammation and negative changes in the ocular morphology and function, such as obstruction of terminal ducts, lipid deposition, blurred vision, and dry eye disease (DED) ¹⁻³ a total of 1015 participants with primary MGD, followed for at least 6 months, were enrolled. The participants were classified into the eyelid hygiene group and the control group. The participants who had stopped eyelid hygiene at any point in the observation period after the initial 2 months were classified into the withdrawal group. Analysis was conducted with a generalized linear mixed model. Treatment group, age, sex, ocular surface inflammation, anti-inflammatory treatments, and baseline MGD subtype were considered as fixed effects, and the individual factor was considered as a random effect. The MGD stage decreased significantly for the observational period in the eyelid hygiene group ($P < 0.001$). To maintain stability and prevent eye dryness, the meibomian glands produce and secrete lipids and proteins into the tear film of the eyes ⁴. These glands are located between the upper and lower eyelids in the tarsal conjunctiva. In severe cases of MGD, meibomian gland secretion is impaired, resulting in gland atrophy ⁵. MGD significantly reduces the quality of patients' lives because there is no understanding of its pathophysiology and no efficient treatment for it. ⁵

Studies have linked various eye diseases with ocular bacterial flora. Earlier research showed that ocular microbes contribute to endophthalmitis after cataract surgery. More recent studies have focused on the relationship between bacterial flora and dry eye diseases

such as anterior blepharitis, MGD, and keratitis. In healthy individuals, the ocular surface typically contains only commensal bacteria, primarily coagulase-negative Staphylococcus. However, patients with infectious eye diseases have been found to have a wider range of bacteria, including Staphylococcus aureus and Klebsiella spp. Based on the results of previous studies, the percentage of this disease has been reported differently in different countries, so the rate of MGD has not been accurately identified. ⁶. For individuals > 40 years of age, prevalence of MGD has been reported to range between 3.54 - 68 % ⁷⁻⁹. However, for participants in clinical studies in the same age range, prevalence has been reported to range between 18 - 47.5 %. In some studies, Asian populations are also more likely to suffer from MGD ^{10,11}. A reliable method to determine the severity of MGD is the Ocular Surface Disease Index (OSDI), which is a questionnaire survey that assesses vision function, eye symptoms, and environmental triggers ⁶. The OSDI is an effective diagnostic tool that provides a fast and accurate evaluation of symptoms related to MGD ⁷.

The study of the bacterial makeup on the ocular surface and its impact on eye health can provide a deeper understanding of eye diseases. Knowledge of the interactions between bacteria, ocular surfaces, and disease mechanisms will lead to improved understanding and treatment of ocular surface conditions. There has been growing interest in the ocular surface microbiome (OSM) and its impact on ocular immune function ⁸. The OSM consists of various commensal bacteria that reside in the aqueous layer on the eye surface. The OSM serves as a barrier against the intrusion of pathogenic microorganisms and plays a crucial role in maintaining eye surface stability ^{9,10} who were

divided into groups of mild, moderate, and severe MGD, and 42 sex- and age-matched participants without MGD (control group). However, certain conditions can weaken the protective function of the OSM and lead to an overgrowth of opportunistic bacteria. Furthermore, alterations in meibomian gland secretion in MGD can result in an increase in lipid viscosity and the subsequent overgrowth of harmful bacteria ¹¹ microbial growth, associated skin disorders as well as potentially severe corneal complications culminate to make MGD a complex multifactorial disorder. It is probable that MGD is a heterogeneous condition arising from any combination of the following five separate pathophysiological mechanisms: eyelid inflammation, conjunctival inflammation, corneal damage, microbiological changes and DED resulting from tear film instability. The pathogenesis of both MGD and DED can be described in terms of a 'vicious circle': the underlying pathophysiological mechanisms of DED and MGD interact, resulting in a double vicious circle. The MGD vicious circle is self-stimulated by microbiological changes, which results in increased melting temperature of meibum and subsequent meibomian gland blockage, reinforcing the vicious circle of MGD. Meibomian gland blockage, dropout and inflammation directly link the two vicious circles. MGD-associated tear film instability provides an entry point into the vicious circle of DED and leads to hyperosmolarity and inflammation, which are both a cause and consequence of DED. Here we propose a new pathophysiological scheme for MGD in order to better identify the pathological mechanisms involved and to allow more efficient targeting of therapeutics. Through better understanding of this scheme, MGD may gain true disease status rather than being viewed as a mere dy

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itation”:¹¹.

The relationship between MGD severity and bacterial overgrowth has been the subject of recent studies. These studies have found a positive correlation between the two, as well as greater complexity in the conjunctival sac bacteria of MGD subjects compared to healthy subjects^{12,13} respectively. A higher rate of aerobic (44.0 %). Given this relationship, understanding the connection between MGD and the ocular surface microbiome (OSM) could lead to improved treatments for MGD. Considering that MGD can cause various eye disorders, increasing the secretion function of the meibomian gland and the integrity of the tear film in patients with MGD, can be considered a therapeutic goal¹⁴.

Meibum abnormalities are a hallmark of MGD. Meibum, which is produced by the meibomian glands and forms a layer on top of the tear film, helps to prevent evaporation and bacterial infections. It is widely accepted that bacteria-derived lipase and hydrolysis products play a role in altering meibum composition. This alteration can cause increased lipid viscosity and decreased fluidity, obstructing the ductal orifice and promoting the growth of bacteria. Changes in meibum composition can thus be considered a potential factor contributing to bacterial overgrowth on the ocular surface¹⁵. Despite evidence of meibum alterations in MGD patients, there is still a lack of definite proof of changes in the ocular surface microbiome in MGD¹⁵. Culturing results have shown a higher rate of positive bacterial cultures from the conjunctival sac in MGD patients compared to those without. While topical antibiotics have been effective in treating moderate to severe MGD, the underlying mechanisms are still unclear. Further research utilizing 16S rDNA sequencing technology to define the bacterial community on the ocular surface of

MGD patients could deepen understanding of the role of the ocular surface microbiome and contribute to the development of improved therapies for MGD¹⁶.

The aim of this study was to examine the potential of microbiome changes as a predictor of the onset of MGD, with the ultimate goal of identifying new and effective therapeutic options in the future for patients affected by this condition.

Material and methods

Study population and trial groups

27 samples of ocular surface microbiome from MGD patients (PRJNA642342) obtained from EMBL-EBI. All data were 16S rRNA gene sequence samples. The states of MGD were matched between four groups. Table 1 shows samples in each group.

Table 1: The MGD groups states in this study

Healthy	Mild	Moderate	Severe
3 (11.11 %)	7 (25.92 %)	6 (22.22 %)	11 (40.74 %)

To analyze the 16S rRNA sequencing data, we utilized the Quantitative Insights into Microbial Ecology software (QIIME2 v.1.9.1). From the sequencing of 27 samples, a total of 2436574 sequencing reads were generated. In order to process the 16S rRNA sequencing data, Dada2 and Deblur methods were used to enhance quality control by denoising 16S marker sequences (in both forward and reverse side) and distinguishing between true diversity and errors. Sequences with more than 97 % similarity were placed in a category and a representative of that category was selected as a reference sequence and mapped with reference databases to identify OTUs. Only high-quality reads for each sample were

selected for further analysis, while low-quality or non-bacterial 16S rRNA sequencing reads were omitted ⁸.

Microbial Diversity Analysis

This study uses trimming the taxonomy to V4 hypervariable region, and Greengene as the reference database. Clustering the sequences with 99 % sequence identity, and performing a Kruskal-Wallis test to analyze alpha diversity (Chao index, Shannon's index) and beta diversity were utilized. Also, the analysis aimed to detect microbial diversity in each sample, and analysis of Bray-Curtis differences between four genus groups was conducted. Besides that, a phylogenetic verification of communities was performed to infer the ocular microbiome metagenomes. Results were determined by considering a P value less than 0.05 ¹⁶.

Results

Sequences Quality Control and Feature Table Creation.

The impact of filtering on microbiome data analysis is shown in two diagrams: forward quality control and reverse quality control using fastq files from an MGD patient's microbiome dataset study. The process resulted in the generation of 2436574 sequencing reads from

27 samples. Low-quality or non-bacterial 16S rRNA sequencing reads were removed, and the high-quality reads were analyzed for each sample. (Figure 1).

Taxonomic classification

Taxonomic classification was performed on the 27 16SrRNA sequence samples to identify the taxonomic makeup of the microbes. No variations in the microbial community were found among the different states of MGD meibum at different taxonomic levels. Comparison between the three states of MGD and healthy groups showed the presence of some bacteria with varying abundance at the Phylum level in the different states of MGD meibum and healthy groups included Firmicutes, Actinobacteria, and Proteobacteria. Also, we can detect Bacilli, Actinobacteria, Gammaproteobacteria, Alphaproteobacteria, and Betaproteobacteria at the Class level. We can find some bacteria at the Order level like Actinomycetales, Bifidobacteriales, Clostridiales, and Pseudomonadales. (Figure 2).

Sample Diversity

Alpha-Diversity

The results of the Shannon and Faith_pd tests for the healthy, mild, moderate, and severe

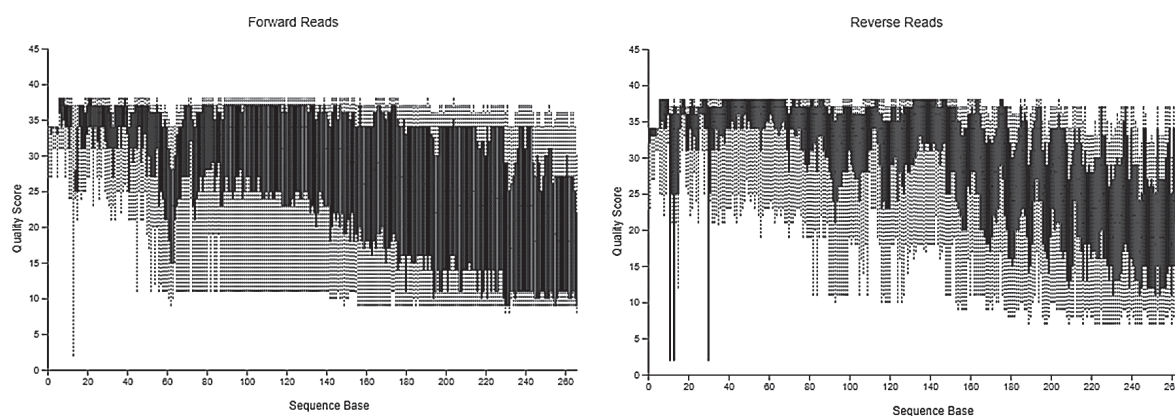


Figure 1: Sequence counts followed by a per-sample breakdown identified by the summarize action

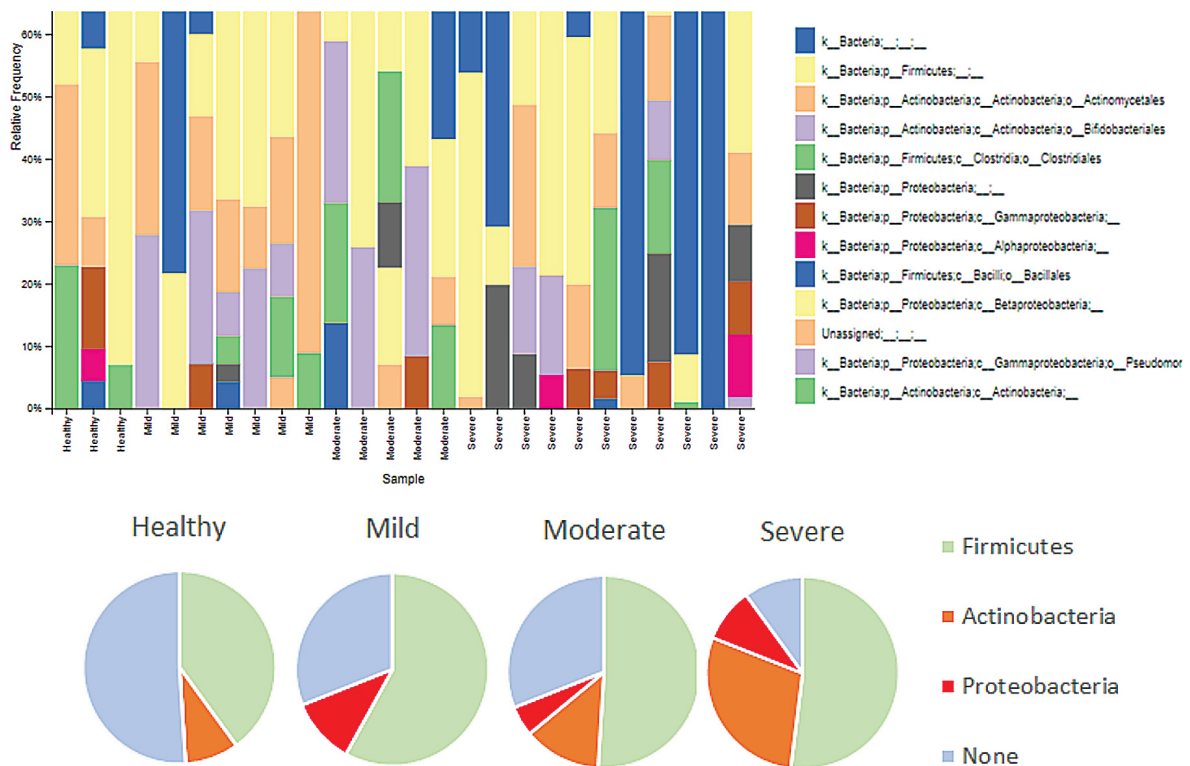


Figure 2: Plot of taxonomic classifications. Microbiome taxonomic profiles at Order level for surface samples from MGD patients. Proteobacteria are sorted into four categories: mild, moderate, and severe, then by their abundance

groups are displayed in figure 3. The mild and moderate groups had similar levels of bacterial population and diversity, while statistical analysis revealed significant differences between the healthy and severe MGD patients in terms of the diversity and abundance of bacteria on their ocular surfaces.

Bacterial Beta-Diversity

The β -diversity index was calculated to compare the structure of microbiome communities. There was no significant difference in the ocular surface flora diversity in the MGD and healthy groups, suggesting that MGD did not significantly affect it. (diversity indices: β diversity, p -value=0.81) (Figure 4).

To identify alterations to the ocular microbial population, total sequence samples were continuously examined in this study. Individual microbiomes of the four states were similar in

terms of how many bacteria were present in the meibum. The microbiome population did not change significantly in different states ($P > 0.05$). But regarding Bacilli, there was a significant change in the population among different states of the disease ($P < 0.01$).

Discussion

The discussion presents the findings from previous studies regarding the presence of Bacilli in patients with dry eye disease and its association with Meibomian Gland Dysfunction (MGD), Zilliox et al.¹⁷ observational study to characterize the ocular surface microbiome (OSM), 2020, and Andersson et al.¹⁸ $n = 21$, respectively, 2021 have shown that Bacilli are a dominant presence in patients with dry eye disease. However, our results indicate a positive correlation between the severity of MGD and a higher abundance

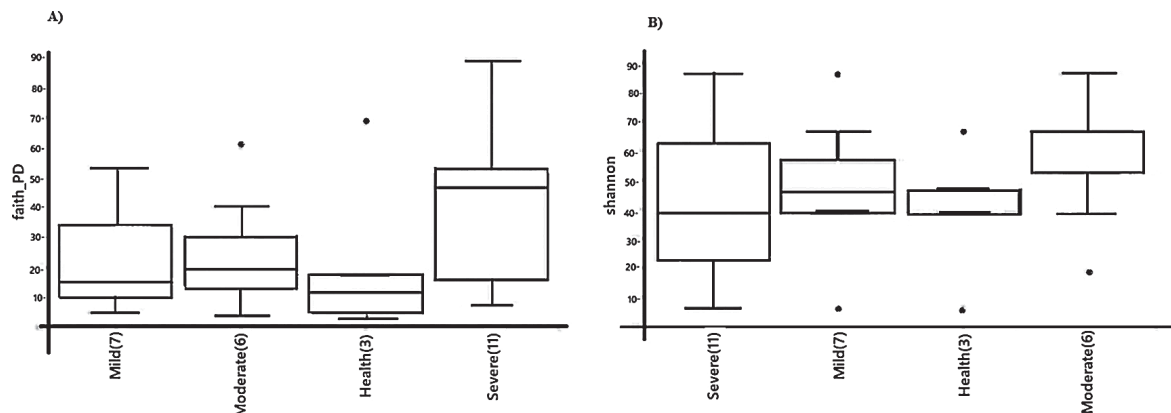


Figure 3: Alpha_diversity in all category. A) faith_PD; B) Shannon

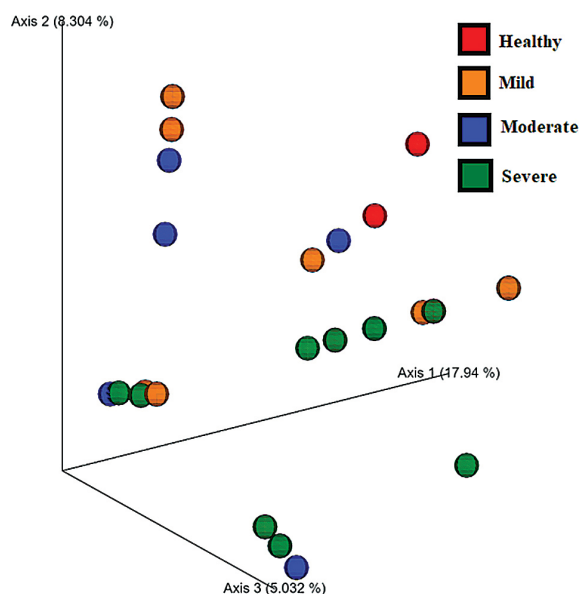


Figure 4: Indicators of diversity: β diversity. Multiple groups were analyzed using beta diversity analysis. MGD patient samples were tested for significant differences in the microbiome diversity of their ocular surfaces. In the metadata file, there are groups of samples that have been analyzed statistically. For the Bray-Curtis and weighted UniFrac distance measures, beta-diversity distance matrices were generated, as well as principal coordinates analyses based on these distance measures. A three-dimensional ordination plot was used to display these visualization files

of Bacilli. We also detected the presence of Bacilli in healthy individuals. Therefore, the mere existence of Bacilli does not necessarily result in illness. However, it may contribute to the severity of the disease. Further clinical studies are needed to confirm this. Firmicute was also identified as a potential contributor to the pathophysiology of MGD. The study aimed to examine the relationship between the severity of Meibomian Gland Dysfunction (MGD) and the presence of bacteria⁸. Results showed that a correlation exists between the severity of MGD and the diversity and population of bacteria found in the secretions of the CS and segmental MG.

In this study, the positive bacterial isolation rate for meibomian gland (MG) secretions was 54.3 %, consistent with other published studies, where the isolation rates ranged from 36.9 % to 75.6 %. This highlights that the sterilization procedure performed prior to sampling may not effectively eliminate bacteria in the MG, particularly in patients with meibomian gland dysfunction (MGD)¹⁹ McMonnies survey, goblet cell density, and meibomian gland assessment. Conventional bacterial culture and broad-range 16S rDNA PCR, cloning, and DNA sequencing were used for bacterial identification. Repeated sampling

was performed in a subset of subjects over a 3-month period. The association between goblet cell loss and bacterial counts in a subgroup of subjects was assessed. RESULTS: Most of the bacteria identified by culture were coagulase negative staphylococci, whereas molecular methods demonstrated a considerable number of additional bacteria. Atypical ocular surface bacteria including *Rhodococcus erythropolis*, *Klebsiella oxytoca*, and *Erwinia* sp., were identified in cases of overt inflammation and, surprisingly, on the normal ocular surface. The same bacteria remained on the ocular surface after repeated sampling. Increased bacterial flora was associated with reduced goblet cell density. CONCLUSIONS: Molecular analysis revealed a diverse ocular surface bacterial population. In addition to the normal flora, various potentially pathogenic bacteria were identified. The detection of known pathogens in both normal and dry eyes, with minimal signs of infection, presents a diagnostic dilemma. It remains unknown whether their presence is associated with inflammation and reduced goblet cell density or whether they adversely affect the ocular surface predisposing it to abnormal microbial colonization. In the absence of overt clinical infection, it is unknown whether such results should prompt intervention with therapy.”, “author”: [{“dropping-particle”: “”, “family”: “Graham”, “given”: “Joanna E”, “non-dropping-particle”: “”, “parse-names”: false, “suffix”: “”}], {“dropping-particle”: “”, “family”: “Moore”, “given”: “Jonathan E”, “non-dropping-particle”: “”, “parse-names”: false, “suffix”: “”}], {“dropping-particle”: “”, “family”: “Jiru”, “given”: “Xu”, “non-dropping-particle”: “”, “parse-names”: false, “suffix”: “”}], {“dropping-particle”: “”, “family”: “Moore”, “given”: “John E”, “non-dropping-particle”: “”, “parse-names”: false, “suffix”: “”}], {“dropping-particle”: “”, “family”: “Goodall”, “given”: “Edward A”, “non-dropping-particle”: “”, “parse-names”: false, “suffix”: “”}], {“dropping-particle”: “”, “family”: “Dooley”, “given”: “James S G”, “non-dropping-particle”: “”, “parse-names”: false, “suffix”: “”}], {“dropping-particle”: “”, “family”: “Hayes”, “given”: “Velma E A”, “non-dropping-particle”: “”, “parse-names”: false, “suffix”: “”}], {“dropping-particle”: “”, “family”: “Dartt”, “given”: “Darlene A”, “non-dropping-particle”: “”, “parse-names”: false, “suffix”: “”}], {“dropping-particle”: “”, “family”: “Downes”, “given”: “C Stephen”, “non-dropping-particle”: “”, “parse-names”: false, “suffix”: “”}], {“dropping-particle”: “”, “family”: “Moore”, “given”: “Tara C B”, “non-dropping-particle”: “”, “parse-names”: false, “suffix”: “”}], “container-title”: “Investigative ophthalmology & visual science”, “id”: “ITEM-1”, “issue”: “12”, “issued”: {“date-parts”: [[“2007”, “12”]]}, “language”: “eng”, “page”: “5616-5623”, “publisher-place”: “United States”, “title”: “Ocular pathogen or commensal: a PCR-based study of surface bacterial flora in normal and dry eyes.”, “type”: “article-journal”, “volume”: “48”}], “uris”: [“http://www.mendeley.com/documents/?uuid=09523ae3-fe6d-4f5f-8b82-09690f70f2a6”]]], “mendeley”: {“formattedCitation”: “19. Despite implementing a standardized pre-operation sterilization procedure, bacteria in the MG were not eradicated. During surgery, contaminated MG secretions may lead to severe consequences such as endophthalmitis. Our findings (Figures 3 and 4) revealed a strong correlation between the bacterial isolation rate and the severity of MGD. Severe MGD increases the risk of infection, making it imperative to perform a stricter pre-operation evaluation of the MG. To reduce the risk of post-operation disease, especially during lengthy surgical procedures, such as vitreous

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and retina surgery, it is recommended to use a surgical adhesive membrane to cover the orifices of the MG.

The composition of eye microbiota has been widely studied in individuals with MGD, with a focus on the changes in the Bacteroidetes and Firmicutes phylum. Findings from previous studies indicate that individuals with moderate to severe dry eye disease show a decrease in the Firmicutes/Bacteroidetes ratio compared to healthy individuals. Additionally, there is a decrease in the genus *Faecalibacterium*, while some studies have reported an increase in the genus *Prevotella* and a decrease in the genus *Bifidobacterium* and *Bacteroides*.²⁰ 003 quality DNA reads, corresponding to 221 species-level phylotypes per subject. The combined bacterial community classified into 5 phyla and 59 distinct genera. However, 31 % of all DNA reads belonged to unclassified or novel bacteria. The intersubject variability of individual OS microbiomes was very significant. Regardless, 12 genera—*Pseudomonas*, *Propionibacterium*, *Bradyrhizobium*, *Corynebacterium*, *Acinetobacter*, *Brevundimonas*, *Staphylococci*, *Aquabacterium*, *Sphingomonas*, *Streptococcus*, *Streptophyta*, and *Methylobacterium*—were ubiquitous among the analyzed cohort and represented the putative “core” of conjunctival microbiota. The other 47 genera accounted for < 4 % of the classified portion of this microbiome. Unexpectedly, healthy conjunctiva contained many genera that are commonly identified as ocular surface pathogens. **CONCLUSIONS:** The first DNA sequencing-based survey of bacterial population at the conjunctiva have revealed an unexpectedly diverse microbial community. All analyzed samples contained ubiquitous (core). However, the results of these studies are inconsistent, with disparity seen among clinical studies, particularly when it comes to

identifying a specific bacterium responsible for dry eye disease. This suggests that external factors may play a role in shaping the gut microbiome and that future studies must be well-controlled to accurately assess the impact of gut microbiota on dry eye disease. Despite a common investigation of the tear film, there is a disparity in the patterns of microbiome dysbiosis among the studies. This highlights the need for future studies in severe subjects to be well aware of and strictly control external factors.¹⁹ McMonnies survey, goblet cell density, and meibomian gland assessment. Conventional bacterial culture and broad-range 16S rDNA PCR, cloning, and DNA sequencing were used for bacterial identification. Repeated sampling was performed in a subset of subjects over a 3-month period. The association between goblet cell loss and bacterial counts in a subgroup of subjects was assessed. **RESULTS:** Most of the bacteria identified by culture were coagulase negative staphylococci, whereas molecular methods demonstrated a considerable number of additional bacteria. Atypical ocular surface bacteria including *Rhodococcus erythropolis*, *Klebsiella oxytoca*, and *Erwinia* sp., were identified in cases of overt inflammation and, surprisingly, on the normal ocular surface. The same bacteria remained on the ocular surface after repeated sampling. Increased bacterial flora was associated with reduced goblet cell density. **CONCLUSIONS:** Molecular analysis revealed a diverse ocular surface bacterial population. In addition to the normal flora, various potentially pathogenic bacteria were identified. The detection of known pathogens in both normal and dry eyes, with minimal signs of infection, presents a diagnostic dilemma. It remains unknown whether their presence is associated with inflammation and reduced goblet cell

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In recent studies, researchers have been investigating the surface microbial diversity of human eyes, particularly in healthy subjects. The main genus of bacteria found on the surface of healthy eyes includes *Propionibacterium*, *Bacillus*, *Erwinia*, *Rhodococcus*, *Staphylococcus*, *Corynebacterium*, and *Klebsiella*. However, research has also shown that different bacterial species can be prevalent in different individuals. For example, *Novosphingobium*, *Lactobacillus*, and *Candidatus*. *Microthrix* have been found to be prevalent in healthy eyes by Xiaotian et al. ²¹ constituting a complex interaction. This study aims to explore how ocular surface *Demodex* infestation (DI) while Kugadas et al. ²² found that *Staphylococcus* was the most prevalent bacterium. Other studies have also investigated the prevalent genera in the conjunctival microbiome ²³. Dong et al. ²⁴ but no study has directly compared different sampling methods applied to the same eyes to one another or to a reference standard of corneal epithelial biopsy. We addressed this

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lack by comparing the microbiome from three conjunctival swabs with those of corneal epithelial biopsy. **METHODS:** Twelve eyes (11 patients found five predominant genera) (*Pseudomonas*, *Bradyrhizobium*, *Propionibacterium*, *Corynebacterium*, and *Acinetobacter*), while Zhou et al.²⁵ on the ocular surface (OS) found that *Corynebacterium*, *Streptococcus*, *Propionibacterium*, *Bacillus*, and *Staphylococcus* were the five most prevalent genera in healthy conjunctiva²⁰ 003 quality DNA reads, corresponding to 221 species-level phylotypes per subject. The combined bacterial community classified into 5 phyla and 59 distinct genera. However, 31 % of all DNA reads belonged to unclassified or novel bacteria. The intersubject variability of individual OS microbiomes was very significant. Regardless, 12 genera-*Pseudomonas*, *Propionibacterium*, *Bradyrhizobium*, *Corynebacterium*, *Acinetobacter*, *Brevundimonas*, *Staphylococci*, *Aquabacterium*, *Sphingomonas*, *Streptococcus*, *Streptophyta*, and *Methylobacterium*-were ubiquitous among the analyzed cohort and represented the putative "core" of conjunctival microbiota. The other 47 genera accounted for < 4 % of the classified portion of this microbiome. Unexpectedly, healthy conjunctiva contained many genera that are commonly identified as ocular surface pathogens. **CONCLUSIONS:** The first DNA sequencing-based survey of bacterial population at the conjunctiva have revealed an unexpectedly diverse microbial community. All analyzed samples contained ubiquitous (core. In our study, we found that while *Staphylococcus* and *Novosphingobium* were more abundant in MGD patients than in healthy control subjects, *Corynebacterium* abundance were lower²¹ constituting a complex interaction. This study aims to explore how

ocular surface *Demodex* infestation (DI. Overall, the distribution of microorganisms on ocular surfaces was found to be similar between MGD patients and healthy subjects. Several studies have also emphasized the importance of microbial populations on the ocular surface and in the meibomian gland for maintaining permanent ocular function. To understand the microbiome of the ocular surface, researchers have used 16S rRNA gene sequencing to compare the microbial population compositions of subjects with and without dysfunction of the meibomian gland (MGD). Although some differences were found, no significant differences were found between the OSM populations of MGD and healthy groups. The microbial populations on the ocular surface and in the meibomian gland play a significant role in maintaining permanent ocular function, as per several studies¹⁴. Our study found that the *Staphylococcus* genre from the Firmicutes phylum was more abundant in the MGD group compared to healthy control subjects, even though *Actinobacterium* in phylum levels were lower. However, the distribution of microorganisms on ocular surfaces was found to be similar between MGD patients and healthy subjects.

Conclusion

In conclusion, Bacilli were detected in both healthy and sick individuals, their mere presence cannot be considered as the sole cause of the disease. However, the severity of the disease is directly related to the abundance of Bacilli. Additional clinical research is required to validate this. The study confirms previous findings that Bacilli are a dominant presence in these patients and that Firmicute may play a role in the pathophysiology of MGD. These findings further emphasize the

importance of understanding the microbiome of the ocular surface and its relationship with MGD. Further research is necessary to fully understand the impact of bacteria on the ocular surface and to develop effective interventions for MGD.

Availability of Data and Material

The dataset generated and analyzed during the current study are available in the European Bioinformatics Institute (EMBL-EBI) website repository under the Primary accession number PRJNA642342

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Footnotes and Financial Disclosures

Conflict of interest:

The authors have no conflict of interest with the subject matter of the present manuscript.