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Culture- and non-culture-based approaches reveal unique features of the ocular microbiome in dry eye patients

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ABSTRACT

Purpose: The purpose of this study was to investigate the ocular microbiome in individuals with dry eye disease and to identify features of their ocular microbiome of possible health and diagnostic significance.
Methods: Conjunctival samples were collected from both eyes in duplicate from 91 individuals (61 dry eye, 30 healthy) and used for both culture-dependent and culture-independent analyses. Samples were either analysed using next generation sequencing (V3-V4 16S rDNA) or inoculated on a wide range of agar types and grown under a broad range of conditions to maximize recovery. Isolates were identified by partial sequencing of the 16S rDNA and rpoB genes and tested for antibiotic susceptibility. We applied a L2-regularized logistic regression model on the next generation sequencing data to investigate any potential association between severe dry eye disease and the ocular microbiome.
Results: Culture-dependent analysis showed the highest number of colony forming units in healthy individuals. The majority of isolates recovered from the samples were *Corynebacterium, Micrococcus* sp., *Staphylococcus epidermidis*, and *Cutibacterium acnes*. Culture independent analysis revealed 24 phyla, of which *Actinobacteria, Firmicutes* and *Proteobacteria* were the most abundant. Over 405 genera were detected of which *Corynebacterium* was the most dominant, followed by *Staphylococcus* and *Cutibacterium.* The L2-regularized logistic regression model indicated that *Blautia* and *Corynebacterium* sp. may be associated with severe DED.
Conclusions: Our study indicates that the ocular microbiome has characteristic features in severe DED patients. Certain *Corynebacterium* species and *Blautia* are of particular interest for future studies.

1. Introduction

The ocular microbiome has been investigated using both culture [1–3] and culture-independent approaches [2,4,5]. These have shown the presence of a wide range of bacteria and fungi [6]. Imbalances in microbial compositions (dysbioses) are considered to be an underlying mechanism in disease development [7–9]. Dry eye disease (DED) affects millions of people worldwide [10,11]. It is caused by loss of tear film homeostasis, resulting in inflammation, cell damage and pain [12,13]. Dysbioses have previously been reported to be associated with other ocular surface disorders such as staphylococcal blepharitis [5,14].

Although the presence of an ocular microbiome has been established, it is still debated whether a "core" group of commensals are continuously present [15,16]. It has been suggested that the ocular surface has a stable and low diversity "minimal" core microbiome, in which all individuals share a few number of taxa [6]. The majority of studies report the presence of *Corynebacterium*, *Cutibacterium*, and coagulase-negative *Staphylococcus* [17]. Several studies have shown that the innate immune system of the ocular surface epithelium can differentiate between pathogenic and non-pathogenic bacteria [18,19]. Commensals contribute to maintaining ocular health by inhibiting apoptosis, barrier preservation and by interacting with the host immune system [16,20]. For example, the commensal *Corynebacterium* has been shown to induce interleukin-17 production from T-cells present in the conjunctiva [9].

The purpose of this study was to characterize the ocular microbiome in individuals with either no self-reported symptoms of DED or with varying degrees of DED symptoms and thereby identify the compositional changes that characterize DED. To determine if there is an

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association between the ocular microbiome and DED, a subgroup of participants characterized as having severe DED based on tear breakup time (TBUT) and Schirmer's scores, were used in a L2-regularized logistic regression model. We have also sought to characterize cultured bacteria with respect to antibiotic sensitivity, as antibiotic treatment is sometimes indicated in certain forms of meibomian gland dysfunction and blepharitis [21].

2. Materials and methods

2.1. Participants and assessment of DED symptoms/ethics statement

The study was approved by the Regional Committee of Medical Research Ethics (REK number: 350387) and adhered to the principles of the Declaration of Helsinki. Written informed consents were obtained for all participants. Participants were recruited from Oslo Metropolitan University (OsloMet) and the Norwegian Dry Eye clinic (NDEC). General exclusion criteria were as follows: below 18 years of age, Sjogren's syndrome, antibiotics use in the past 4 weeks, ongoing, active ocular infection, including conjunctivitis. OsloMet participants were also required to not have any prior history with DED. Participants were asked to fill out the Ocular Surface Disease Index (OSDI) and the Dry Eye Disease Questionnaire 5 (DEQ5) which were used to assess the DED severity, as recommended by the Nordic guidelines [22]. Participants scores between 0 and 22 were considered normal-mild, 23–32 were classified as moderate, and severe was $\geq$33. Schirmer's test was performed on both eyes and the average was calculated for all participants for use in the study. Further details are available in supplementary methods 1.1.

2.2. Conjunctival sampling

The lower conjunctival sac was sampled using sterile nylon swabs (FloqSwabs, Copan, Italy), without any topical anaesthetic. Tear samples were taken from both eyes as follows: first a Floq swab was applied to both eyes and this was swab used for DNA analysis. Subsequently, a new Floq swab was applied to both eyes and this was used for culture analysis. Samples were taken in a standardized fashion by the same dry eye expert physician. Swabs were sealed in containers without any medium and placed on ice immediately after sample collection and during transportation to the laboratory. Thereafter, swabs were either used to inoculate agar growth medium immediately after arrival at the laboratory (maximally 6 h) or stored at −80 °C for DNA extraction and sequencing. Three unused swabs were also processed in downstream analysis as negative controls both in culture and non-culture-based approaches.

2.3. Bacterial culture

Swabs with tear samples and unused swabs were cut above the nylon head using sterile flamed scissors and added to 1 ml MSwab medium (Copan, Italy) and vortexed for 30 s. One hundred µl of Mswab suspension was plated on MacConkey agar (Thermo Fisher Scientific), Brain heart infusion (BHI) agar (Thermo Fisher Scientific), R_2A agar (Thermo Fisher Scientific) supplemented with 5 µg/ml of vancomycin, Sabouraud glucose agar with 50 µg/ml chloramphenicol (Sigma Aldrich) and Columbia agar with chocolate horse blood (CHB) (Thermo Fisher Scientific). All the above agar plates were incubated at 37 °C for 1 week under normal atmospheric conditions. Additionally, 100 µl of Mswab suspension was plated on Schaedler anaerobe agar (Thermo Fisher Scientific) and CHB and these were incubated anaerobically at 37 °C for 1 week. Lastly, 100 µl of Mswab was plated on CHB and plates were incubated in 5% CO_2 for 1 week at 37 °C. The number and morphologically different types of colonies on each agar were recorded. Macroscopically different colonies as judged by two researchers were picked and stored at −80 °C in 20% glycerol for further analysis and identification; usually two colonies of each morphological type were stored.

2.4. Identification of isolates based on partial sequencing of the 16S rDNA and rpoB genes

Colonies isolated from the primary plates (MacConkey agar, and CHB incubated aerobically, in an enriched CO_2 environment and anaerobically) were regrown for identification based on Sanger sequencing of the 16S rDNA. Colonies identified as *Corynebacterium* by 16S rRNA were also identified by partial sequencing of the rpoB gene as previous studies have shown that rpoB provides better species discrimination (supplementary materials l.2).

Accession numbers for the 16S rDNA sequences: PP345631 - PP345718.

Accession numbers for the rpoB gene sequences: PP372578 - PP372605.

2.5. Confirmatory tests for Enterococcus sp., Staphylococcus aureus and the MRSA phenotype

The appearance of short chains of cocci and deep-red coloured colonies after incubation on *Enterococcus* selective agar (Merck, cat. No. 45183) were taken as indicating the presence of *Enterococcus* sp. *Enterococcus faecalis* ATCC 29212 was used as positive control.

Isolates identified as belonging to the genus *Staphylococcus* by sequencing were tested for the production of DNase on agar (ThermoFisher scientific, cat. no CM0321) and for bound coagulase (clumping factor) and/or protein A using the PROLEX™ STAPH LATEX KIT (Prolab diagnostics). Isolates testing positive in both analyses were considered to be confirmed *S. aureus.*

Confirmed *S. aureus*, an MRSA control (DSM 11729) and the negative control DSM 799 were tested for the MRSA phenotype using Brilliance MRSA agar 2 (ThermoFisher). After incubation the development of blue colonies was taken to indicate presumptive MRSA.

2.6. Antimicrobial susceptibility testing

Susceptibility testing of *Staphylococcus* sp. and *Enterococcus* sp. was performed using GPALL1F plates (Sensititre; Thermo Scientific) as described in the product protocol (supplementary materials. 1.3).

2.7. DNA extraction and sequencing

Conjunctival swabs were cut above the nylon head using sterile scissors and added to 1 ml DNA/RNA shield (Nordic BioSite, Sweden) before sending with cooling to a commercial laboratory (Zymobiomics Ltd.) for analysis (supplementary materials 1.4).

2.8. Bioinformatics

The samples were processed and analysed using the ZymoBIOMICS® Targeted Sequencing Service (Zymo Research) as detailed in supplementary materials 1.5. Prior to analysis, the dataset was processed with the Decontam R package to identify potential contaminating sequences based on the DNA extracted from unused swabs [23,24]. To determine which taxa may be associated with severe DED we used the mikropml package from R [25] to perform L2-regularized logistic regression. The model was used on a subset of NDEC subjects with severe DED and OsloMet subjects defined as a healthy group (supplementary materials 1.5). The participants with severe DED were subsetted using the average TBUT (below 5 s) and Schirmer's score (below 10 mm) based on the Nordic guidelines [22].

3. Results

3.1. Participants

In total 91 participants were included in this study, of which 61 were recruited from the NDEC and 30 were recruited from staff and students at the university (Table 1). Participants were characterized as normal or mild and severe DED (see supplementary results 2.1).

To investigate which microbial taxa may be associated with DED, a subgroup (n = 20) of NDEC subjects was picked based on TBUT below 5 s and Schirmer's score below 10 mm. These participants were considered as severe DED patients based on the Nordic guidelines [22].

3.2. Culture dependent analysis of the ocular microbiome

Conjunctival samples collected from the NDEC (n = 55) and OsloMet (n = 30) were cultured on both selective and nonselective agars in different atmospheric conditions in order to maximize recovery. In total 92% of the swabs showed growth of at least one colony type (n = 78). Seven NDEC subjects (11%), and 1 OsloMet subject (3%) scored negative for microbial growth on all agar plates. No fungi were isolated from any samples on Sabouraud agar. Only 1 colony was obtained from R_2A-vancomycin agar from each group. The OsloMet group had significantly higher numbers ($p < 0.05$) of colony forming units (CFUs) compared to the NDEC group on all other agar types (Fig. 1). No colonies were recovered from the 3 unused swabs.

3.3. Identification of cultured colonies

In total 190 colonies were identified from MacConkey agar and CHB based on partial sequencing of the 16S rDNA and rpoB genes. Overall, 6 major genera were identified: *Corynebacterium, Staphylococcus, Streptococcus, Enterococcus, Cutibacterium* and *Micrococcus*. In addition, a small number of isolates were identified as *Schaalia* (n = 2), *Ruminococcaceae* (n = 1), *Enhyrobacter* (n = 1), *Dermacoccus* (n = 1) and *Kocuria* (n = 1). Supplementary Table 1 provides identifications of all colonies which were successfully recovered from freezer cultures with the exceptions of samples identified as putative *Micrococcus* sp., *S. epidermidis* and *C. acnes* which being common skin commensals were considered as of lesser interest.

3.4. Antibiotic susceptibility testing

Results of susceptibility testing for *Staphylococcus* and *Enterococcus* are given in Supplementary Tables 2A–C. The cefoxitin screen (Supplementary Table 2A) and the results of growth on MRSA chromogenic agar, indicated that none of the isolates were MRSA. The susceptibility profiles of the isolates and control strains were generally similar; no *S. aureus* strain showed complete resistance to any antibiotic other than penicillin. *Enterococcus* strains were susceptible to penicillin and vancomycin.

Table 1
Participant characteristics based on recruitment site. Male to female ratio is provided based on recruitment site and age. OSDI and Schirmer's scores were recorded to assess the prevalence of dry eye in subjects.

Characteristic	NDEC	OsloMet
Sex (M: F)	1:2.5	1:1.7
Age (M: F)		
50 years or younger	1:2	1:1.1
Mean ± S.D	66 ± 7	57 ± 4
51 years or older	1:3.4	1:4.5
Mean ± S.D	37 ± 7	32 ± 9
Dry eye classification based on questionnaires (n = 91 participants)		
Normal to mild symptoms	13	30
Moderate to severe	48	0
Schirmer's test (n = 91 participants)		
Over 10 mm	35	29
Under 10 mm	26	1

3.5. Culture independent bacterial community analysis

Sequencing of the V3-V4 region of the 16S rDNA gene was used to characterize the ocular microbiome.

Richness and Shannon diversity were calculated using the vegan package from R (Fig. 2A). α-diversity indices were similar for NDEC (including the subsetted group) and OsloMet subjects except for Richness. OsloMet subjects had a median value of 60.5 while NDEC with moderate symptoms and severe symptoms had median values of 41 and 40, respectively which was significantly lower ($p < 0.05$) than the OsloMet group. Bray-Curtis dissimilarity was calculated to quantify the β-diversity and nonmetric dimensional scaling (NMDS) was used to visualize the data (Fig. 2B). No clear grouping or clustering was observed for samples in either group. To assess dissimilarity between groups, we used PERMNOVA which was significant ($p < 0.05$). We also tested for variance in dispersion which was significantly different ($p < 0.05$).

3.6. Taxonomy and bacterial composition of the ocular surface microbiome

The decontaminated dataset was classified at phylum, genus, and amplicon sequence variants (ASV) levels and the relative abundances were calculated. Taxa present at a relative abundance below 2% were classified together as "Other". Overall, 24 phyla were detected in the samples. The most abundant phylum was *Actinobacteria* (NDEC; 48%, OsloMet; 50%), followed by *Firmicutes* (NDEC; 31%, OsloMet; 30%), and *Proteobacteria* (NDEC; 16%, OsloMet; 15%). These together accounted for more than 90% of the composition in both groups.

At the genus level, a total of 405 different genera were detected for both groups taken together. Only 4 genera had a mean relative abundance above 2% in both the NDEC and OsloMet groups: *Corynebacterium* (39% and 35%), *Staphylococcus* (15% and 22%), *Cutibacterium* (5% and 10%), and an unclassified genus belonging to Neisseriaceae (8% and 10%) (Supplementary Fig. 1).

DADA2 provides ASV which increases the resolution of the data. We examined the data at ASV level (Fig. 3) which revealed interesting differences between the groups. The 3 most abundant ASVs in the NDEC group (ASV 1, ASV 4 and ASV 5) belonged to the genus *Corynebacterium*. In contrast, the most abundant ASVs in the OsloMet subjects (ASV 2 and 6) were identified as *S. capitis-caprae-epidermidis* (13%) and *C. acnes* (9 %). A significant amount of the ASVs were present at much lower levels, but collectively contributed to over 40% in both groups.

We further looked for the presence of a dominant organism (defined as an ASV with relative abundance >50%) in individual samples (Supplementary Fig. 2). In total, 33% of NDEC subjects showed the presence of a dominant organism. In 90% of these samples the dominating ASV was a *Corynebacterium,* and the remaining were dominated by either *Moraxella* or *Staphylococcus*. Twenty percent of OsloMet subjects showed one dominant organism, which in 66% of cases was a *Corynebacterium*. The remaining were dominated by either *Staphylococcus* or an unclassified genus belonging to the *Neisseriaceae* family. These findings illustrate that the ocular microbiome consists primarily of *Corynebacterium*, *Staphylococcus* and *Cutibacterium*, regardless of DED status.

3.7. Association between ocular microbiome and severe DED

To investigate the potential link between severe DED and the ocular microbiome, we subsetted the NDEC group based on Schirmers score and TBUT (n = 20) and used a L2-regularized logistic regression model with the decontaminated data on ASV level, age, and sex. This model was chosen as we prioritized interpretability over performance. The mean area under the curve (AUC) for the testing data after 100 splits was

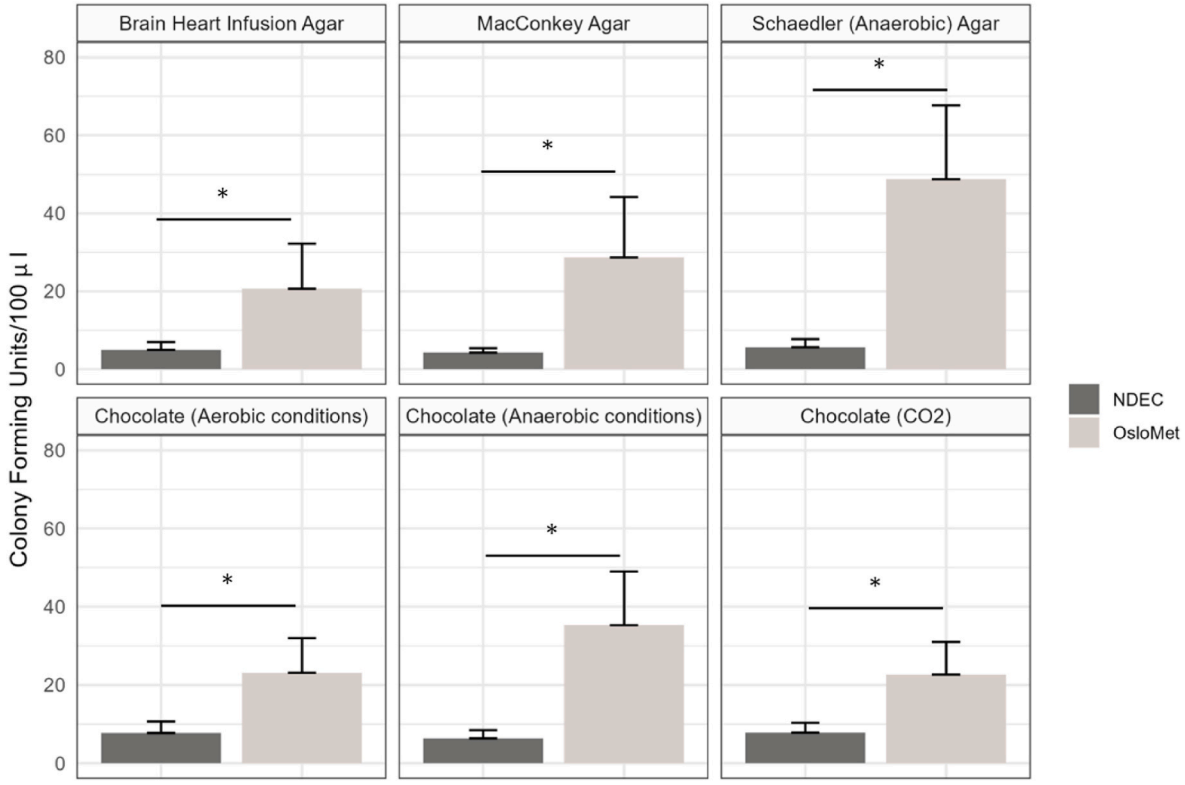

Fig. 1. Cultivatable microbes present on the ocular surface. Average number of CFUs/100 µl (±SEM) from subjects recruited from the NDEC and OsloMet. All plates were incubated at 37 °C for 1 week. * Indicates significance.

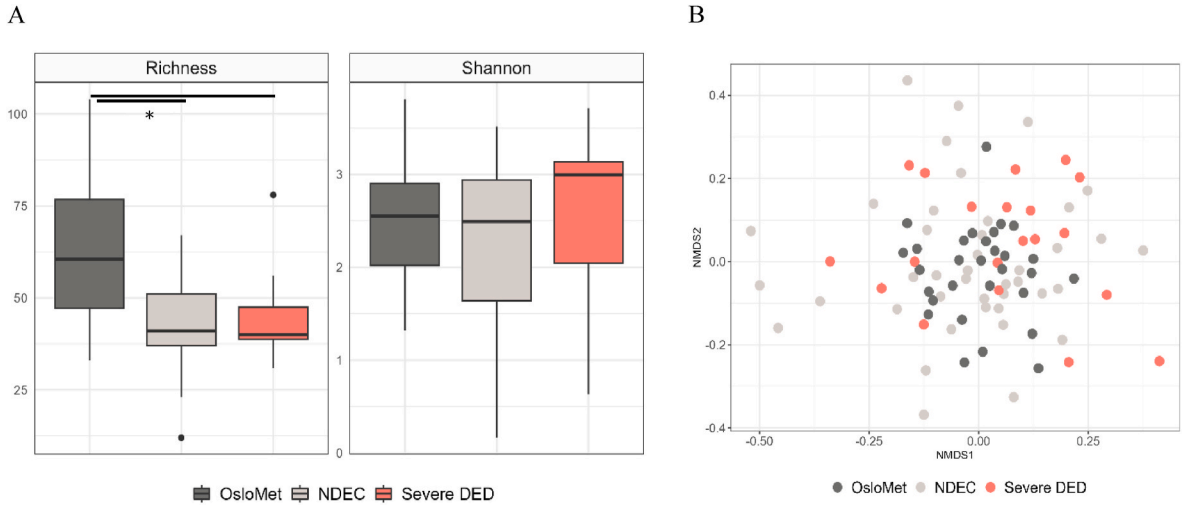

Fig. 2. A) Alpha diversity of the ocular samples obtained from NDEC and OsloMet subjects. Richness was significantly different between the OsloMet participants and the NDEC as well as between OsloMet and Severe DED ($p < 0.05$). No statistically significant differences in the Shannon diversity were observed between the groups. **B) Nonmetric dimensional scaling (NMDS) of Bray-Curtis dissimilarity.** NMDS was used to visualize the Bray-Curtis dissimilarity between the samples. Black, grey and red points indicate samples obtained from OsloMet, NDEC and Severe DED subjects, respectively.

0.845 (IQR 0.781–0.927). To interpret the importance of each feature we plotted the mean weights of the coefficients and their corresponding interquartile range (Fig. 4). Age was most associated with DED, and male sex least associated. We found that ASV 29 belonging to *Blautia* had the highest weight in the model after age, although its relative abundance was found to be below 2%. *Corynebacterium,* depending on the species, was found to be either positively or negatively associated with DED (Fig. 4).

4. Discussion

In this study we examined the ocular microbiome using culture-and non-culture-based methods for 91 participants. These were patients with varying degrees of DED recruited from the NDEC, and university staff and students with no previous history of ocular disease. We did not find fungal growth from any participants. Previous studies have reported low or sporadic growth of fungi using culture dependent methods [26,27]. Although sequencing studies have shown the presence of fungal

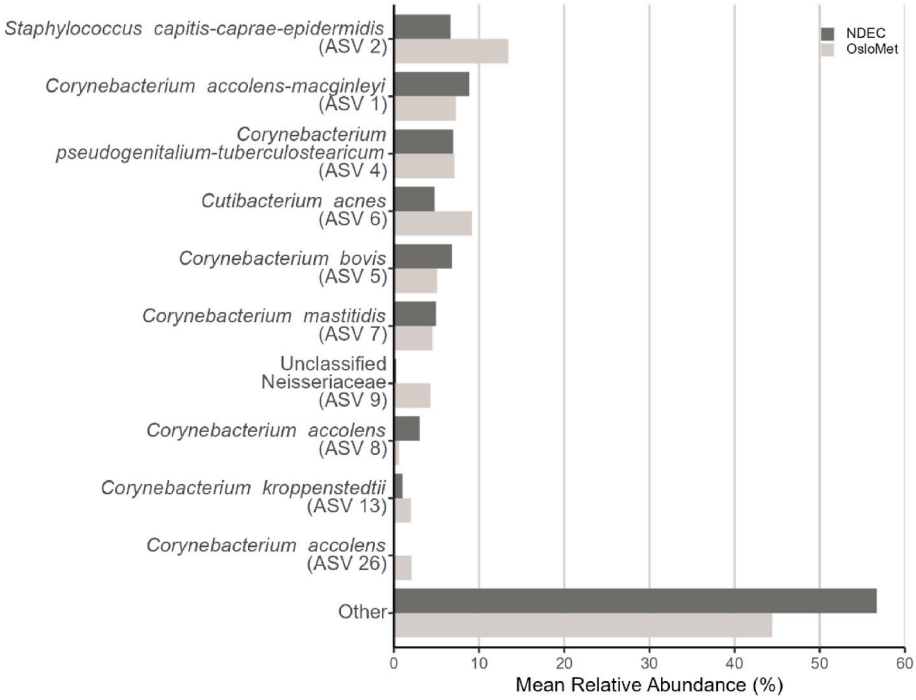

Fig. 3. Mean relative abundance of ASVs. The majority of ASVs belonged to the *Corynebacterium* genus, whereas both *Staphylococcus* and *Cutibacterium* consisted of one ASV with a mean relative abundance over 2%. Any ASV with a mean relative abundance below 2% is grouped in the "Other" category.

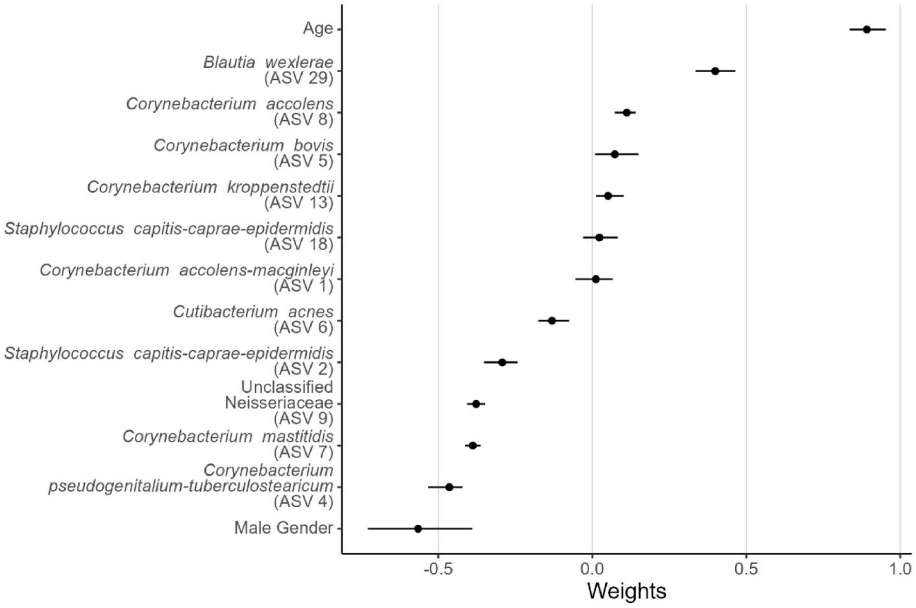

Fig. 4. The feature weights included in the L2-regularized logistic regression model. *Blautia wexlerae* was found to be the most associated with DED followed by different *Corynebacterium* species. *C. pseudogenitalium-tuberculostearicum* and *C. mastitidis* were found to be negatively associated with DED, and positively associated with a healthy ocular surface.

operational taxonomic units (OTUs), it is uncertain if fungi are present continuously on the ocular surface and are a part of the core ocular microbiome [27]. Notwithstanding, the present analysis indicates that culturable fungi are not significant colonizers of the healthy or DED ocular surface.

We found coagulase-negative staphylococci such as *S. epidermidis* to be the most abundant bacterial type followed by variously *C. acnes*, *Corynebacterium, Streptococcus,* and *Micrococcus.* This is generally in line with previous investigations [2,3,28,29]. There are few studies that have used culture-based methods to compare DED and healthy individuals. The majority of these report significantly higher number of CFUs in the DED group compared to the controls [1,2,30]. In contrast, we found a significantly higher number of CFUs in the non-dry eye group. This could be explained by the wide range of media used in the present study, revealing taxa not cultured in previous work.

The α and β diversities in NGS data were calculated prior to taxonomy classification. We examined the α-diversity for species richness and evenness. Only species richness was significantly different between the groups and indicated that NDEC subjects had a less diverse microbiome. Similar observations have been reported previously and are in

accordance with the dysbiosis hypothesis [31]. Evenness measured by the Shannon index was similar in the NDEC and the OsloMet group [32]. This is in contrast to a previous study examining the closed eye microbiome which found higher species richness in tears of DED patients compared to controls [11]. Our β-diversity analyses using Bray-Curtis was visualized using NMDS and although no apparent clustering was visible, there was a significant difference between the groups which could be explained by large variations within the groups [33]. Many of the taxa shown to be most abundant in the NGS data were also among the cultured bacteria such as *Corynebacterium, Cutibacterium* and *Staphylococcus,* suggesting the analyses were mutually supportive. Systematic and direct comparisons between NGS and culture-based analyses are made difficult by the small number of known taxa able to be grown in the laboratory on standard media. Interestingly, of the 190 randomly chosen colonies for identification, none were gram-negative species. Most NGS ocular studies show *Proteobacteria* to make up a significant proportion of the relative abundance. The reason for this is unknown, but one explanation could be that many of the *Proteobacteria* found in NGS libraries are contaminating sequences or not easily cultured.

In terms of ocular health and the possible effects of antibiotic treatment, it was relevant to look at the susceptibility profiles of the staphylococcal and other ocular isolates. The resistance profiles of *S. aureus* isolated from DED patients did not differ from those generally recorded for this species. All were completely sensitive to chloramphenicol which together with fusidic acid are the first-choice antibiotics for treating staphylococcal conjunctivitis in Norway [21].

All of the cultured *Enterococcus* strains originated from one NDEC participant (P40, Supplementary Table 2). The finding is supported by the NGS data where this species made up 11% of the sequences for P40, whilst occurring rarely in other participants. Thus, it would appear that the genus is not intimately associated with DED. The data suggests that P40 with severe DED, may have had an ocular infection with *E. faecalis*. All strains were susceptible to penicillin and vancomycin.

We further used a logistic regression model to determine if the ocular microbiome is associated with the most severe cases of DED. We stratified NDEC subjects to delineate the most severe DED cases, independently of subjective self-reported symptoms. The mikropml [25] model found *Blautia* to be most associated with DED followed by several different species of *Corynebacterium*. Our model produced a mean AUC value of 0.845 suggesting 84% chance [34] to discriminate severe DED from presumably healthy individuals based on the ASV abundance, age and sex. While this only suggests an association with and not causation of DED, *Blautia* and *Corynebacterium* are interesting candidates to investigate further in terms of their role on the ocular surface in health and disease. Although *Blautia* was identified to be associated with DED based on the NGS dataset and the logistic regression model, we did not find members of the genus in the culture-dependent analysis. A possible explanation for this is that the culture conditions used in this study may not have been suitable for *Blautia* growth. A recent study of microbiota in patients with refractive allergic conjunctival diseases [35] found that *Blautia* was the only genus that increased significantly in abundance between mild and severe cases of the condition. There is thus mounting evidence that *Blautia* are of significant health relevance and are deserving of future characterization.

The most striking examples of single-species domination in the NGS data are those of *Corynebacterium* species. Although most *Corynebacterium* are recognized as innocuous commensals which may even play important roles in modulating the immune system [36], some are or are becoming regarded as ocular pathogens [37]. Only *Corynebacterium* reached % relative abundances over 70% in subjects at genus and ASV levels. Five putative species dominate the relative abundance data for individuals – i.e., represent alone 50% or more of the sequence reads (Supplementary Fig. 2). Given the high abundance of *Corynebacterium* detected on the ocular surface in this study and previous work [2,4,31,38], future studies could investigate how this genus interacts with the immune system and its potential effects on ocular health.

A challenge in NGS studies is contaminating DNA originating from laboratory personnel and reagents, which can be wrongfully reported to be a part of microbiomes [39]. Although many studies include negative extraction controls, fewer report the use of blank swabs to examine potential contaminants on the materials used for sampling [6]. Only a handful of studies report how they identify and treat contaminating sequences in their dataset [6,26]. We included three unused swabs, blank extraction controls and mock community to control for extraction efficiency of gram-positive and gram-negative bacteria. Our dataset was then decontaminated using the Decontam package from R which identifies contaminating sequences based on their frequency in a sample and their prevalence in the unused swabs. This resulted in over 15 ASVs belonging to *Pseudomonas*, a common laboratory contaminant [40], being removed from the dataset. No gram-negative bacteria were among the 190 cultured isolates, suggesting that easily culturable gram-negative bacteria are not common on the eye. During sample collection and transportation, the Floq swabs were kept on ice without the use of transport medium. This decision was taken to minimize the possibility of post-sampling growth. Exclusion of transport medium could possibly result in loss of bacterial cells, and the culture-dependent analysis would then be an underestimation of viable microorganisms present on the ocular surface. A possible weakness in the study was the pooling of samples from both eyes, as each eye may have different microbiomes. However, the expected low bioburden on each eye especially with regards to generation of NGS libraries guided our choice. Cost restrictions was another factor. A number of participants used artificial tears to alleviate symptoms of pain and discomfort. This is a possible source of bias which should be controlled for in future studies. Lastly, a possible limitation to this study is the higher prevalence of women in the NDEC group as well as the severe DED group compared to the OsloMet group. Some studies have not found any differences between sex and ocular microbiomes, whereas other report differences in Shannon diversity and abundance of some genera [41].

The present study confirms previous findings regarding the taxonomic composition of the ocular microbiome. We found the most abundant genera to be *Staphylococcus*, *Corynebacterium* and *Cutibacterium,* which was in agreement with culturing. Our logistic regression analysis indicated that *Blautia* and *Corynebacterium* are associated with severe DED, this should be investigated further in future studies. One approach could be to tailor culture conditions explicitly to detect these genera. Another possible approach would be the use of qPCR with genus-specific primers. The present study reports the results of an analysis performed on a single day for each participant. We placed emphasis on describing a relatively large number of subjects, rather than following a smaller group over time. We are currently planning whole genome sequencing of many of the isolates with special focus on *Corynebacterium* which were recovered in abundance from agar cultures. Future studies could include more clinical parameters such as goblet cell density, ocular surface staining, meibomian gland expression and tear meniscus height to determine if dry eye severity is correlated to changes in the microbiome.

Data availability

The NGS dataset used in this study is publicly available at: https://github.com/marnaq/Ocular-microbiome.

Declaration of competing interest

Maria Naqvi and Colin Charnock declare that they have no conflicts of interest.

Fredrik Fineide: co-owner of the Norwegian Dry Eye Clinic.

Tor Utheim: Irrespective of any potential conflicts of interest, for the sake of transparency, Tor Paaske Utheim is co-founder and co-owner of The Norwegian dry eye clinic and the Clinic of eye health, Oslo, Norway,

which delivers talks for and/or receives financial support from the following: ABIGO, Alcon, Allergan, AMWO, Bausch&Lomb, Bayer, European school for advanced studies in ophthalmology, InnZ Medical, Medilens Nordic, Medistim, Novartis, Santen, Specsavers, Shire Pharmaceuticals and Thea Laboratories. He has served on the global scientific advisory board for Novartis and Alcon as well as the European advisory board for Shire Pharmaceuticals. Utheim is the Norwegian Global Ambassador for Tear Film and Ocular Surface Society (TFOS), a Board Member of the International Ocular Surface Society, an International Member of the Japanese Lid and Meibomian gland working group (LIME), a Consultant at the Norwegian Association for the Blind and Partially Sighted, the President of the Oslo Society of ophthalmology, and the Editor-in-Chief of Oftalmolog, an eye journal distributed to all eye doctors in the Nordic region since 1980. Besides publishing articles of presumed interest to our readers, Oftalmolog publishes advertisements from pharmaceutical companies, companies selling ophthalmological equipment, and associations organizing conferences and events in ophthalmology. For more information, visit: oftalmolog.com.

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CRediT authorship contribution statement

Maria Naqvi: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Fredrik Fineide:** Investigation, Validation, Writing – review & editing. **Tor Paaske Utheim:** Conceptualization, Funding acquisition, Investigation, Supervision, Writing – review & editing. **Colin Charnock:** Conceptualization, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtos.2024.02.002.

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