The Ocular Surface 18 (2020) 706–712

Contents lists available at ScienceDirect

The Ocular Surface

journal homepage: www.elsevier.com/locate/jtos

Assessing the ocular surface microbiome in severe ocular surface diseases

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ARTICLE INFO

Keywords:
Microbiome
SJS
oGVHD
Dry eye
Floppy eyelid syndrome

ABSTRACT

Purpose: There is growing evidence for a critical role of the microbiome in ocular health and disease. We performed a prospective, observational study to characterize the ocular surface microbiome (OSM) in four chronic ocular surface diseases (OSDs) and healthy controls.

Methods: Sterile swabs were used to collect samples from each eye of 39 patients (78 eyes). Sterile technique and multiple controls were used to assess contamination during DNA extraction, amplification and sequencing. Concurrent use of topical antibiotics, steroids, and bandage contact lenses (BCLs) was documented.

Results: Despite the low biomass of the ocular surface, 47/78 (60%) eyes sampled had positive sequencing reads. We observed that half of patients (8/17, 47%) had distinct microbiomes in each eye. Healthy controls had a *Lactobacillus/Streptococcus* mixture or significant *Corynebacterium*. *Staphylococcus* predominated in 4/7 (57%) patients with Stevens-Johnson Syndrome (SJS) in at least one eye, compared to 0/10 healthy controls. Interestingly, 8/11 (73%) eyes with SJS were using BCLs, including 4/5 (80%) eyes dominated by *Staphylococcus*. Lax eyelid syndrome (LES) and Dry Eye Disease (DED) patients had similar OSMs, with *Corynebacterium* being the most prevalent bacteria. Alpha diversity was higher in controls and ocular graft-vs-host (oGVHD) patients compared to the other OSDs.

Conclusions: Only 50% of the 39 patients had similar microbiomes in each eye. A majority of healthy eyes had a *Lactobacillus/Streptococcus* mix or *Corynebacterium* microbiome. *Staphylococcus* predominated in SJS, *Lactobacillus* in oGVHD, and *Corynebacterium* in DED and LES. There may be an association between different OSDs and the microbiome.

Introduction

The Human Microbiome Project (HMP) started in 2008 and was the first to characterize the microbiota (microorganisms) from 5 different body sites including nasal passages, oral cavity, skin, gastrointestinal tract, and urogenital tract. The second goal was to assess the role of these organisms in human health and disease [1]. For example, increasing evidence suggests a critical role for the gut microbiome in the pathogenesis of a wide variety of systemic diseases, including diabetes, obesity, rheumatoid arthritis, and inflammatory bowel disease [1]. The eye was not included in the HMP study, in part because of the low biomass of the ocular surface.

Prior studies of the ocular surface microbiome (OSM) using traditional culture techniques demonstrated that the most common bacteria observed were coagulase-negative *Staphylococcus* (CNS), *Propionibacterium,* and *Corynebacterium* [2,3]. Recently, genomic techniques have described a more phylogenetically diverse population, but the eye region sampled, culture technique, patient age, contact lens usage and gender may affect the microbiome [4–16]. Ozkan et al. recently demonstrated relative stability of the OSM in a normal population over a 3-month period, yet a highly variable composition between subjects was found, which challenges the idea of a core microbiome [17]. Other studies observed the OSM in various eye diseases, including dry eye disease (DED), blepharitis, and trachomatous disease [6,18,19]. It remains unclear whether the OSM plays a role in the etiology of these diseases, or whether these diseases alter the OSM.

Dry eye disease (DED) is a chronic condition affecting 5–31% of the population in which abnormalities in tear quantity and/or quality result in an unstable tear film, ocular surface inflammation, and associated irritative symptoms [20]. Several studies have examined the microbiota

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https://doi.org/10.1016/j.jtos.2020.07.007
Received 14 October 2019; Received in revised form 5 July 2020; Accepted 9 July 2020

in DED, demonstrating several species of *Staphylococcus*, *micrococcus* and *diptheroids*, which are non-pathogenic corynebacteria [18,21–25]. Less commonly and inconsistently found are *P. acnes*, *Pseudomonas, Bacillus* and *Rhodococcus*.

Stevens Johnson Syndrome/toxic epidermal necrolysis (SJS/TEN) is a rare but severe cutaneous adverse reaction (SCAR) associated with up to 30% mortality. It is usually triggered by either medication or less commonly, infection, in a genetically predisposed population [26]. It can result in severe inflammation of the mucus membranes, including the ocular surface (cornea and conjunctiva, eyelids). Patients who survive the acute disease often suffer from lifelong eye complications, including chronic ocular inflammation and severe dry eye, symblepharon, corneal scarring, ulceration, perforation and blindness [3,19,27–29]. Studies using traditional culture methods demonstrated that the ocular surfaces of SJS patients were more likely to grow CNS and *S. aureus* compared to healthy controls [30,31]. No studies using genomic techniques have been reported in this population.

Ocular graft vs. host disease (oGVHD) is a common complication of hematopoietic stem cell transplantation (SCT). Systemic GVHD can result in lacrimal gland fibrosis and associated aqueous tear deficiency as well as severe OSD associated with corneal stem cell deficiency [32]. Chronic GVHD generally presents within 3 years of the transplant and has systemic inflammatory and autoimmune effects [33]. A recent paper by Shimizu et al. using culture techniques demonstrated that the OSM in GVHD patients was more diverse compared to non-GVHD patients and controls [34]. *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Propionibacterium*, aerobic gram-positive cocci, *Haemophilus influenzae*, and aerobic gram-positive rods were observed in the GVHD patients, whereas only a few species were detected in the other groups.

Floppy eyelid syndrome (FES), first described by Culbertson et al., in 1981, is characterized by the combination of eyelid laxity and papillary conjunctivitis in young overweight men [35–37]. In 1994, Van den Bosch presented lax eyelid condition (LEC, lax eyelids only without conjunctivitis), and lax eyelid syndrome (LES, lax eyelids with conjunctivitis) as subsets of FES in patients of any age and weight [38]. More severe cases of FES can result in corneal inflammation, neovascularization and ulceration [39]. Eyelid hyperlaxity has a well-known association with obstructive sleep apnea (OSA), which occurs in up to 33% men and 17% women, making this a common but overlooked condition [40]. Finally, matrix metalloproteinase-9 has also been detected in the tear film of patients with (LES) and OSA [41], supporting an inflammatory component of LES. FES microbiome studies have also not been reported.

The exact relationship between ocular surface inflammation and the OSM is unclear. Although low microfloral diversity appears to correlate with ocular surface disease, some studies have demonstrated differences between patients with OSDs and controls, while others have not [6,18,22,24,25,31]. Due to the morbidity and prevalence of DED and LES, as well as the more severe OSD forms caused by chronic SJS and GVHD, we sought a better understanding of the OSM of these patients compared to healthy controls using uniform genomic techniques.

Materials and methods

This study adhered to the tenets of the Declaration of Helsinki. Approval for this project was obtained from the Loyola University Medical Center Institutional Review Board. Written informed consent was obtained from all study subjects and participation was voluntary. Healthy controls and patients with the following diseases were recruited: i) chronic SJS, ii) oGVHD, iii) LES, and iv) DED. Patient selection was determined by established criteria: biopsy proven SJS/TEN, ocular oGVHD according to Chronic Ocular Graft-vs-Host-Disease (GVHD) Consensus Group, LES according to van den Bosch, and DED according to DEWS II criteria [37,38,42,43]. Healthy controls consisted of patients without OSD complaints and an Ocular Surface Disease Index (OSDI) screening questionnaire score of 5 or less. Normal eyes were excluded if they used any eye drops or contact lenses, had any ocular surface inflammation, any type of conjunctivitis, or if they had ocular surgery in the past 3 months. The use of topical antibiotics and contact lenses in the experimental groups was recorded. This is a descriptive study of rare ocular surface diseases without a formal null hypothesis to test, and so no sample size calculation was conducted *a priori*.

Methods used in this study were modeled after Dong et al. [12]. Two different sterile cotton swabs (1) Dacron, (Medical Packaging Corp., Camarillo, CA) and (2) Puritan, (Medical Packaging Corp., Camarillo, CA) were used to collect 3 samples from each eye of 39 patients (78 eyes): 1) superior tarsal conjunctiva, 2) inferior tarsal conjunctiva and 3) inferior conjunctival fornix. Tetracaine hydrochloride 0.5% drops were used to anesthetize each eye and sterile technique was used in the collection of all samples. Because of prior reports of the differentiation between the superficial and deep microbiome, strong pressure was used in swabbing the conjunctiva while trying to avoid significant discomfort [12]. The Dacron swabs were used to swab the bulbar conjunctiva/fornix, while the Puritan swabs were used to swab the superior and inferior tarsal conjunctiva. Each sample was assigned a unique participant number and stored at -80° C.

Microbial DNA was extracted using a protocol adapted from Yuan et al. [28]. Next, whole genome amplification was performed using multiple displacement amplification (MDA) technology, with the REPLI-g Mini Kit (Qiagen, Hilden, Germany). We verified that MDA did not significantly alter the relative abundance of positive or negative controls (Supplemental Fig. 1). After MDA, sequencing ready libraries were generated using a 2-step polymerase chain reaction as previously described [12,44]. Briefly, in the first reaction, the variable 4 region (V4) of the 16S rRNA gene was amplified using the universal primers 515F and 806R, which were modified to encode the Illumina MiSeq sequencing primer sequence at the 5' end. Reaction mixtures were incubated at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 90 s. To ensure complete amplification, samples were incubated at 72 °C for an additional 10 min. Ten-microliter aliquots of each reaction mixture were run on a 1% agarose gel. Samples containing a band of approximately 360 bp were considered PCR positive and processed further, while those with no visible product were not. The PCR-positive reaction mixtures were diluted 1:50 and amplified for an additional 10 cycles using primers encoding the required adaptor sequences for the Illumina MiSeq and an 8-nucleotide sample index, using the PCR conditions described above [44]. Extraction controls were included to assess contamination (Supplemental Fig. 2). First, it was verified that the controls produced few reads compared to patient samples, demonstrating increased DNA levels in the samples (Supplemental Fig. 2a). Second, the microbiome profile was compared between patient samples and healthy controls to verify that similar patterns were not observed, as would be the case if significant contamination were present (Supplemental Fig. 2b). The final PCR products were then purified using the Ampure XP System (Beckman-Coulter, Brea, CA), normalized to equimolar concentrations, and pooled. The Qubit 2.0 Fluorometer (Thermo-Fisher, Waltham, MA) and Bioanalyzer 2100 (Agilent, Santa Clara, CA) were used to analyze the quantity and quality of the final pooled library. The samples were sequenced on the Illumina MiSeq, rendering 250 bp paired-end reads.

Raw sequencing reads were processed using Mothur software v. 1.31.2 [45]. Samples were subsampled at a read depth of 1000 and samples with < 1000 reads were eliminated. Operational taxonomic units (OTUs) were assigned using a 0.03 cutoff level, resulting in 911 OTUs. Relative abundance graphs were generated using the R package Phyloseq (v1.19.1) and dendrograms were constructed by measuring the Bray-Curtis dissimilarity using the R package vegan (v. 2.4–2). The Shannon Index, Inverse Simpson Index and Species Observed were calculated using Mothur software. Samples from the three sites were combined and positive samples, with > 1000 reads, constituted the OSM in each eye. For patients with sufficient reads in both eyes, OSMs were considered dissimilar for Bray-Curtis dissimilarity scores ≥ 0.30.

Table 1
Patient characteristics by disease.

	Healthy	SJS	GVHD	FES	DED
Total Patients with reads, n	6	7	4	5	8
Age, median (range)	44 (28–65)	46 (10–61)	60 (55–72)	73 (40–86)	57.5 (30–77)
Female, n (%)	3 (50.0)	2 (28.6)	1 (25.0)	4 (80.0)	6 (75.0)
Eyes, n	10	12	6	8	11
Topical Antibiotics, n (%)	0 (0.0)	5 (41.7)	3 (50.0)	2 (25.0)	0 (0.0)
Topical Steroids, n (%)	0 (0.0)	6 (50.0)	0 (0.0)	1 (12.5)	2 (18.2)
Contact Lenses, n (%)	0 (0.0)	9 (75.0)	1 (16.7)	1 (12.5)	0 (0.0)

As 16S rRNA sequencing is a semi-quantitative technique, relative abundances of each bacterium were compared descriptively. Patient characteristics were presented by disease group, as medians and ranges for continuous variables or counts and percentages for nominal variables.

Results

Patient median age in each group ranged from 44 to 73 years and over half were females (53.3%, Table 1). Among those with ocular disease, treatments included topical antibiotics (29.2%) or topical steroids (20.8%). 47 of 78 (60%) eyes produced sufficient reads to be analyzed. This resulted in positive samples for 10/16 (63%) healthy eyes, 12/16 (75%) SJS eyes, 6/14 (43%) oGVHD eyes, 8/16 (50%) LES eyes and 11/16 (69%) DED eyes. In total, the 47 positive samples had 16 different genera present at levels greater than 1% of reads, though many more were detected at sub-threshold levels.

We first plotted the results for each patient to see if healthy controls and OSD patients had similar microbiomes in each eye (Fig. 1a and b). Of the 17 patients and controls where sequencing reads were detected in both eyes, 8 (47%) had the same microbiome in each eye (Fig. 1a, Bray-Curtis dissimilarity score < 0.3). Surprisingly, 9 (53%) had unique microbiomes in each eye (Fig. 1b, Bray-Curtis dissimilarity score ≥ 0.3). The distribution of the 9 patients with unique microbiomes included 1/4 healthy, 3/5 SJS, 1/2 GVHD, 2/3 FES and 2/3 DED. The differences were substantial, with one patient having mostly *Corynebacterium* in one eye and *Streptococcus* in the second eye (Fig. 1b, Patient 1). A second patient had mostly *Corynebacterium* in one eye and a diverse population, including *Actinomyces, Streptococcus, Rothia, Prevotella* and *Corynebacterium* in the second eye (Fig. 1b, Patient 6). Despite having different genera detected in each eye, we found that both eyes had similar alpha diversity values for three different calculations, including Fig. 1c) species observed, 1d) Shannon Index, and 1e) Inverse Simpson Index. This suggests that although different bacteria may inhabit the eye, the overall community structure is not changing. Due to these results, all further analyses were conducted on the individual eye level, rather than combined for each patient.

Among the 10 healthy eyes, the most common organisms included a *Lactobacillus* and *Streptococcus* combination (7 eyes) or *Corynebacterium* (3 eyes) (Fig. 2a). Other organisms, including *Enterobacteriaceae*, *Staphylococcus*, *Actinomyces*, *Acinetobacter* and *Pseudomonas* were observed at lower relative abundances. There were significant amounts of low-level bacterial genera detected, with up to 30% of the sequenced reads coming from bacteria which did not reach the 1% threshold (Fig. 2a, light gray, 'other'). Overall, the healthy eyes comprised a mixed population, with only 2 eyes having a predominant organism (> 50% of

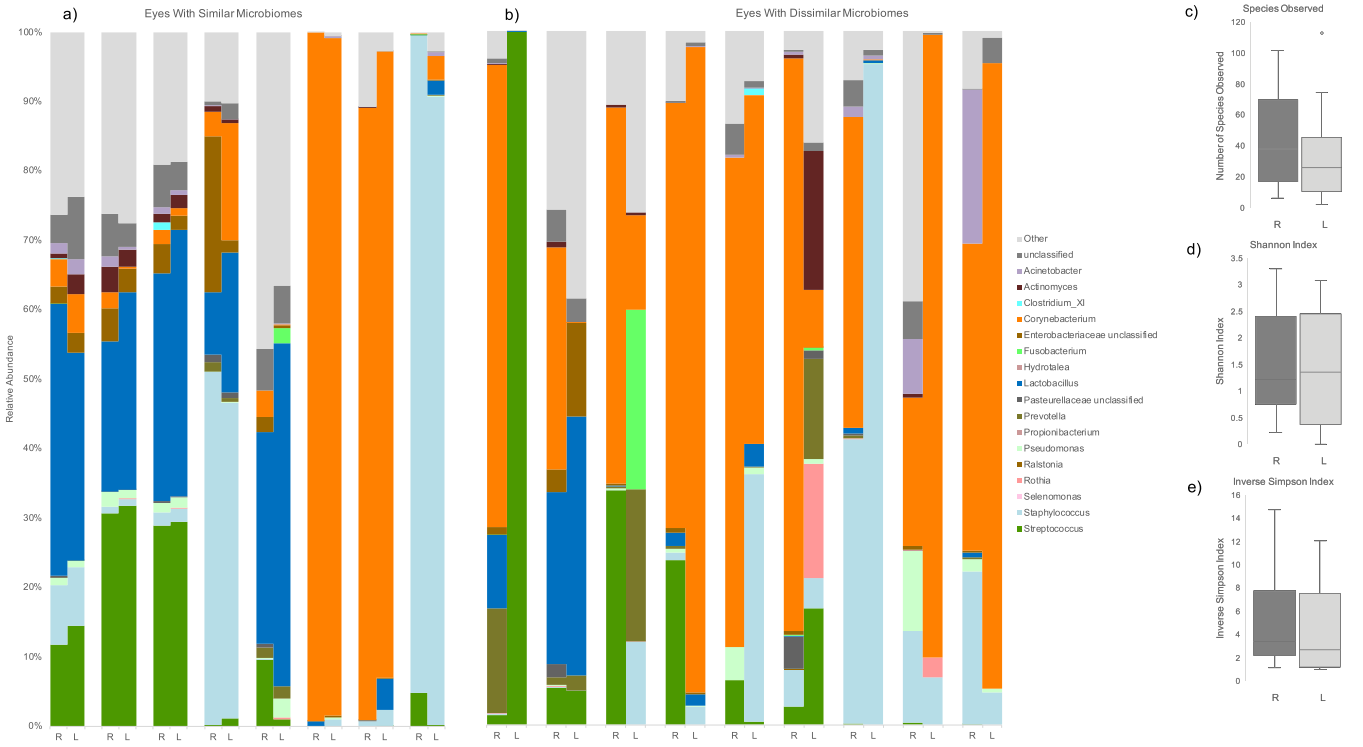

Fig. 1. a) Relative abundance for patient eyes with similar microbiomes (Bray-Curtis dissimilarity < 0.3). b) Relative abundance for patient eyes with different microbiomes (Bray-Curtis dissimilarity ≥ 0.3). c) Species observed, d) Shannon Index and e) Inverse Simpson Index between right (R) and left (L) eyes. The 'Other' group represents a combination of all genera detected at < 1% relative abundance.

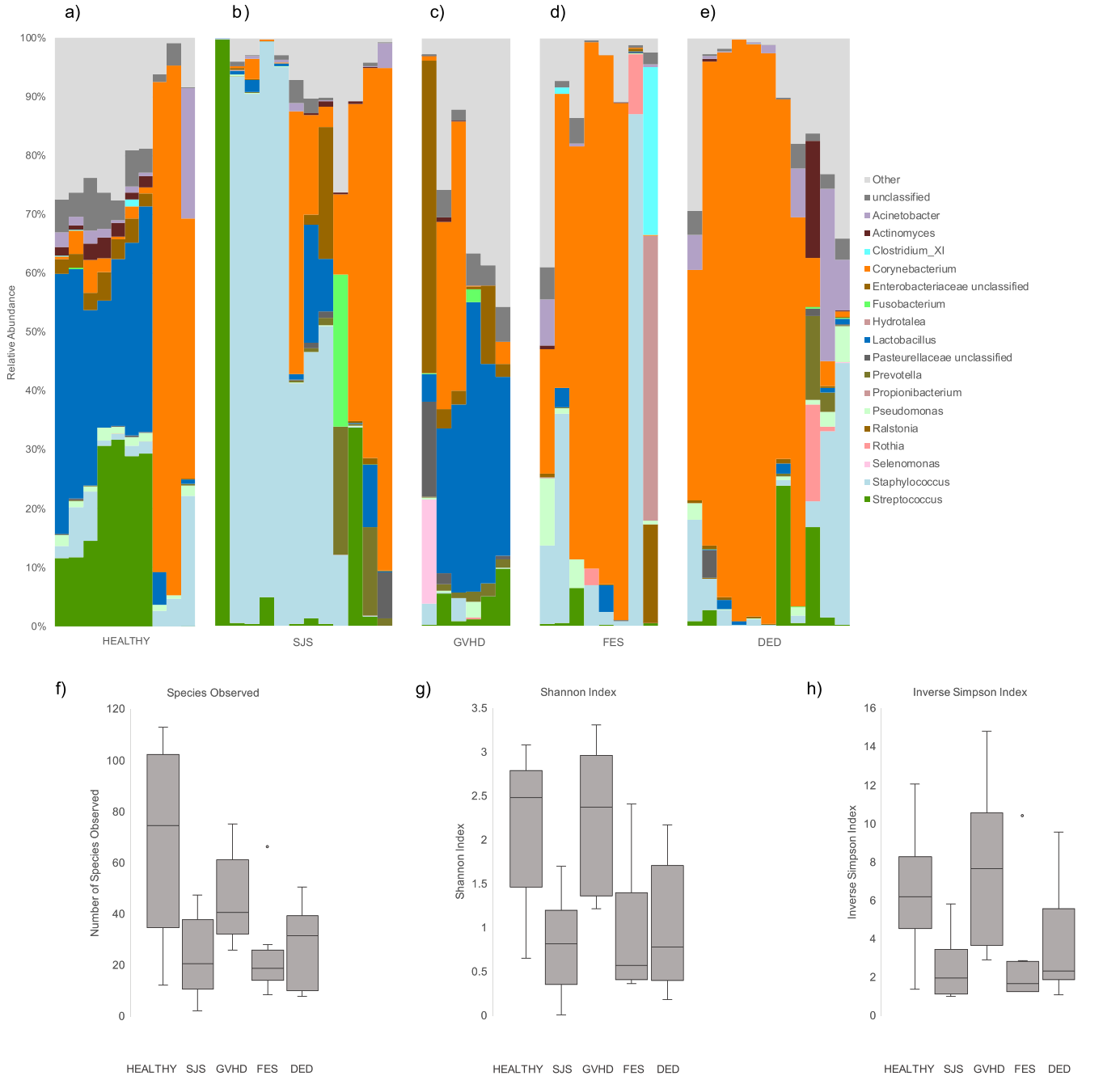

Fig. 2. a) Relative abundance in healthy eyes. b-e) Relative abundance plotted by disease group. f) Species observed, g) Shannon Index and h) Inverse Simpson Index for healthy and diseased eyes. SJS = Steven's-Johnson Syndrome. GVHD = Graft Vs. Host Disease. FES = Floppy Eyelid Syndrome. DED = Dry Eye Disease.

total reads). Finally, higher levels of *Lactobacillus/Streptococcus* were seen in healthy patients versus patients with all forms of OSD (see below).

Secondly, we compared the microbiomes of OSD patients to healthy controls. The relative abundances of bacteria plotted by disease group can be found in Fig. 2b–e. *Staphylococcus* was the predominant bacteria in 5/11 (46%) eyes with SJS, compared with 0/10 controls and 1/24 (4%) for the other OSDs (Fig. 2b). Interestingly, 8/11 (73%) eyes with SJS were actively using BCLs, including 4/5 (80%) eyes where *Staphylococcus* was the dominant bacteria. High levels of *Corynebacterium* were also seen in several SJS patients and one patient's eye was almost entirely *Streptococcus* (Fig. 2b). Smaller amounts of *Lactobacillus*, *Prevotella*, *Fusobacterium* and *Enterobacteriaceae* were also observed.

Patients with oGVHD had fewer sequencing reads on their ocular surfaces, as only 6/14 (43%) eyes had positive samples (Fig. 2c). However, of the six positive samples, one eye was the only eye in our study predominated by *Enterobacteriaceae*. That eye also had *Selenomonas* and *Pasteurellaceae,* which were not seen in significant amounts in other eyes. Five of the 6 (83%) oGVHD eyes also displayed the *Lactobacillus/Streptococcus* microbiome seen in healthy eyes, albeit at a lower relative abundance. This is consistent with previous reports [34]. Significant amounts of 'Other' were observed in five of six eyes as well.

Eyes with LES were dominated by *Corynebacterium* (5/8, 63%), Fig. 2d). Strikingly, one eye was predominated by *Hydrotalea*, a novel genus with little patient data. Although the patient had a history of corneal erosion, no other eye in our study had significant levels of *Hydrotalea*. That eye also had higher levels of *Ralstonia* and *Clostridium_XI* than what was observed in any other eye in our study.

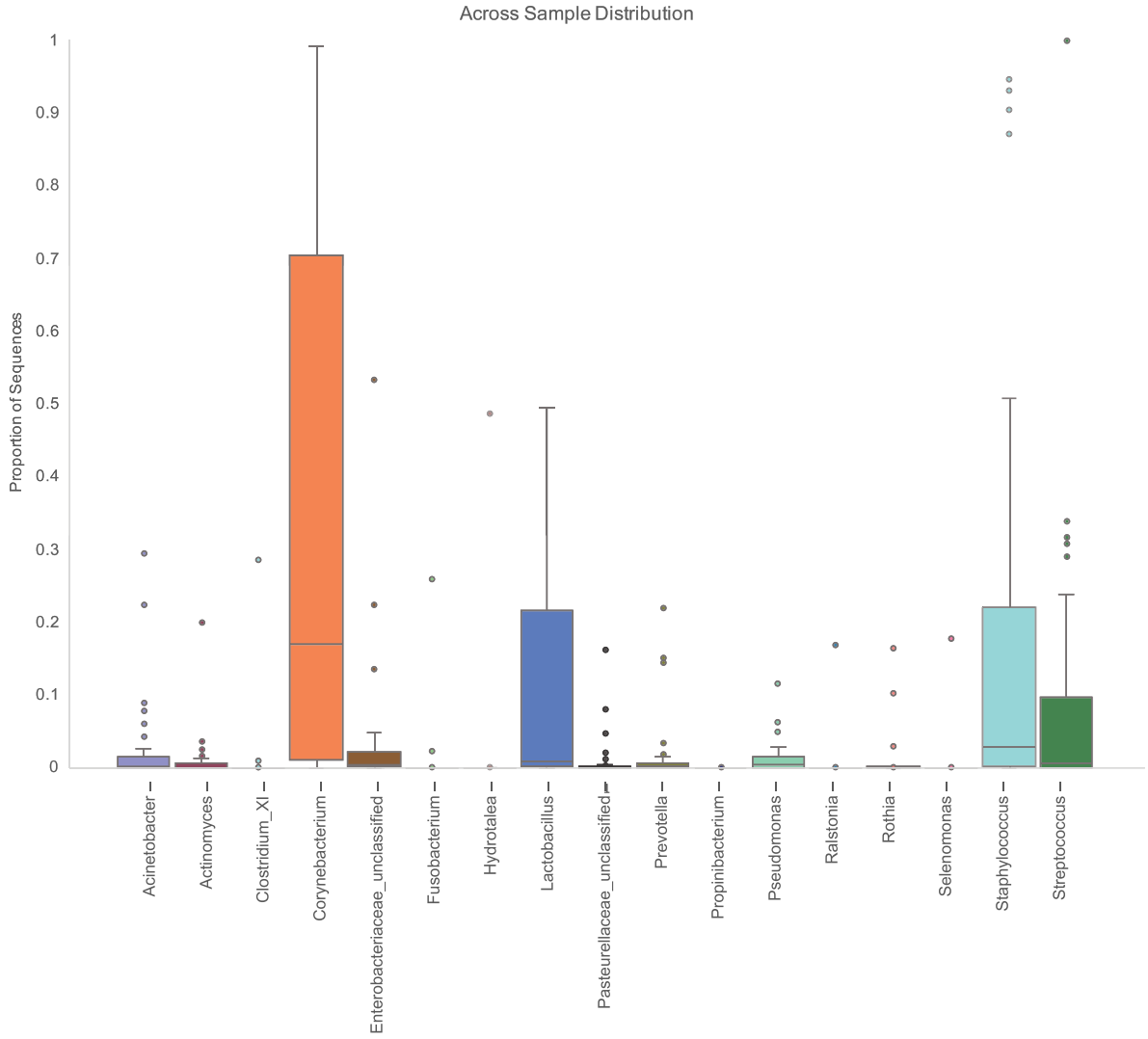

Fig. 3. Across sample distribution for each bacterial genera. The proportion of sequences detected for each genus is plotted for all samples studied.

Another LES eye was predominated by *Staphylococcus,* while also having significant amounts of *Rothia* (Fig. 2d). The final eye had a diverse microbiome, with smaller read numbers of several genera present.

Of the eyes with DED, 7/11 (64%) were dominated by *Corynebacterium* (Fig. 2e). The remaining four eyes had diverse microbiomes, but included *Staphylococcus* and *Acinetobacter*. 4/6 (67%) eyes where *Acinetobacter* was > 5% reads were in DED eyes. The 'Other' category was again prevalent, with 5/11 (46%) eyes having > 5% reads belong to 'Other'. Interestingly, higher relative abundances of *Corynebacterium* were seen in FES and DED patients compared to oGVHD.

Thirdly, we calculated the α-diversity for the healthy and diseased eyes. Surprisingly, the number of species observed in healthy subjects and oGVHD patients were higher than for the other three diseases (Fig. 2f). This observation was also seen in the Shannon Index (Fig. 2g) and Inverse Simpson Index (Fig. 2h), suggesting that the lack of diversity in SJS, LES and DED may play a role in disease pathogenesis.

Finally, we conducted an analysis to see the proportion of sequences for each genus across all eyes (Fig. 3). Nearly every eye (46/47 (98%)) in this study contained some *Corynebacterium* and over half had > 20% of their reads map to *Corynebacterium. Lactobacillus*, *Staphylococcus* and *Streptococcus* were also present in a large proportion of eyes, while the remaining genera were infrequently observed. Few eyes (19/47, 40%) had a predominant genus but if it did, it was *Corynebacterium, Staphylococcus* or *Streptococcus*, highlighting the diverse bacterial communities of the ocular microbiome in healthy and diseased patients.

Discussion

Due to the continuous exposure of the ocular surface to the external environment, distinguishing between transient microorganisms and the true "core" microbiome is a significant challenge. In addition, the pressure used to swab the conjunctival surface, tetracaine use in sample collection and the regions sampled have all been shown to influence the OSM [7,8,12]. For example, Ozkan et al. sampled tissue biopsies and found differences between the conjunctival surface and the fornix, the latter being dominated by *Pseudomonas,* which was found in low abundance on conjunctival surfaces. These results and others suggested that the ocular surface likely has a stable paucibacterial microbiome and fungiome, although proper sampling is required [2,12,15,46]. Our goal was to obtain ocular microbiome samples from patients in their current condition including topical antibiotics in order to obtain accurate reflection of the microbiome under actual patient care conditions. We are currently conducting a follow-up study in which one of the exclusion criteria is a history of topical antibiotics within 6 weeks of sampling.

Previous studies using culture methods demonstrated the healthy ocular microbiome to be composed mostly of *Staphylococcus, Propionibacterium* and *Corynebacterium*, with lower levels of other organisms such as *Lactobacillus* and *Escherichia coli* [2,47]. 16S rRNA sequencing, shotgun metagenomics and PCR have identified similar organisms, but have also found *Streptococcus, Acinetobacter, Micrococcus,*

Aeribacillus, Haemophilus and other bacteria [4,5,11,12,16,18,47,48]. We found similar organisms in healthy patients including *Corynebacterium*, *Lactobacillus, Streptococcus* and smaller amounts of *Staphylococcus, Actinomyces, and Acinetobacter*. We also found *Pseudomonas* in samples from healthy and DED patients, which supports the findings of Ozkan et al. and suggests that our sampling technique at least partially captures the true OSM. The ability of more contemporary methods to detect a wider variety of organisms could be due to their increased sensitivity or the limitations of the culture techniques. For example, anaerobic bacteria such as *Lactobacillus* and some species of *Streptococcus* do not grow well under standard culture conditions, which may explain why we see a higher relative abundance of those organisms in our study. It is also possible that biases introduced by MDA alter the relative abundances of certain species found in the microbiome. Further study is required to fully understand these differences.

We also report that half of patients have different microbiomes between eyes in both healthy and OSD patients. Previous studies have found that there is no difference in the α-diversity between a patient's eyes, which we replicated [11,48]. However, we did find genera differences, complicating the interpretation of previous results, as eye-level data were not routinely reported. With α-diversity similar between eyes, this suggests a stable community structure, but that specific bacteria can vary. Longitudinal studies will be needed to understand how bacteria might colonize one eye from the other or from a common source.

Frizon et al. reported on 41 eyes with chronic SJS using culture techniques [30]. The most common bacteria grown included CNS, *Staphylococcus aureus* and *Corynebacterium,* which were also found in another study [31]. Our study supported these findings, as SJS patients had *Staphylococcus* in 8/12 (67%) eyes and *Corynebacterium* in 8/12 (67%) eyes. In addition, we found a significant number of eyes with *Streptococcus* (5/12, 42%), *Prevotella* (4/12, 33%) and *Lactobacillus* (4/12, 33%), including one SJS eye that was 99.9% *Streptococcus*. The α-diversity of SJS patients was lower compared to healthy and oGVHD eyes by three diversity measures, but similar to LES and DED eyes. As in our study, Frizon et al. reported bandage contact lenses and topical antibiotics were frequently used for managing tarsal conjunctival scarring [30]. This is a confounding variable, which cannot be controlled due to small sample sizes. Shin et al. found that contact lens-wearers had OSMs more similar to skin flora and lower *Staphylococcus* abundance than non-wearers [8]. Further study is warranted to determine the cause of higher levels of *Staphylococcus* in SJS patients.

Using culture techniques, Shimizu et al. found that patients with oGVHD had more diversity compared to non-GVHD patients, a finding we replicated [34]. They identified *Staphylococcus, Streptococcus, Corynebacterium, Propionibacterium* and aerobic gram-positive cocci and rods in their oGVHD eyes. We found similar genera and also found that oGVHD eyes were more diverse than the other disease groups. One eye was the only eye to be dominated by *Enterobacteriaceae,* which was not previously reported. In our study, only 6/14 (43%) oGVHD eyes had positive samples, likely due to the use of antibiotics in this population.

To our knowledge, this is the first study of the OSM in patients with LES. The predominant organism detected was *Corynebacterium* (5/8 eyes, 63%), which was similar to the DED group (see below). This is an interesting finding in that most patients with lax eyelids are probably undiagnosed and managed as DED. Uniquely, one eye was predominated by *Hydrotalea*, a novel organism first isolated from water in Sweden and described in 2011 [49]. It is gram negative, rod-shaped, aerobic and generally non-motile [50]. It has also been found in rat guts, scallops and is a predominant genus in canine vaginal samples, which may be relevant for human exposure [51–53].

Several previous studies have examined the ocular microbiota of DED patients [18,21–23,25]. In general, the studies mostly found CNS, *Staphylococcus epidermidis*, *Corynebacterium* and *Propionibacterium acnes,* as have been described in other disease states [18,21–23,25]. We also found a high prevalence of *Corynebacterium* and *Staphylococcus* (4/11 eyes, 36%), but we did not find *Propionibacterium*. As in another study, we also found *Streptococcus* (4/11, 36%) and *Pseudomonas* (4/11, 36%) [21]. Intriguingly, de Paiva et al. also reported decreased diversity in both patients with Sjogren's Syndrome as well as in an animal model of DED [54]. In our study, patients with both LES and DED had a lower α-diversity compared to healthy and oGVHD patients and had similar diversity to SJS patients, which has not been reported. St Leger et al. demonstrated an immunoregulatory role of resident *Corynebacterium* in the host response to *Pseudomonas* and candida [55]. Our finding of *Corynebacterium* as one of the most frequent organisms in our diseased eyes may reflect this role in ocular surface disease. Future therapeutic approaches to the management of DED and possibly other OSD might include improving conditions for *Corynebacterium* growth, enhancing regulatory T cells (Tregs) or other methods of altering the ocular surface microbiome to reduce inflammation [56,57].

We found general agreement with previous studies that typically found CNS and *Corynebacterium* to be the major inhabitants of ocular surfaces. Like other reports, our results show large variations in the OSM and the predominant species in these OSDs as compared to healthy patients. Particularly, patients with OSDs are less likely to have OSMs predominated by *Lactobacillus*. Patients with SJS have higher relative abundance of *Staphylococcus* present on their ocular surfaces. LES and DED patients had OSMs dominated mostly by *Corynebacterium*. We also documented reduced microbiome diversity in OSD patients compared to healthy controls, which also have been shown to have greater fungal diversity [58]. Further study is warranted to uncover the role of the OSM in these diseases.

Disclosure of funding

Illinois Society for the Prevention of Blindness Grant; Department of Ophthalmology, Geoffrey Gunnar Memorial Scholarship, Loyola University Chicago Institutional Funds, Richard A. Perritt Charitable Foundation.

Declaration of competing interest

The authors have no conflicts of interest to disclose.

Acknowledgements

The authors would like to thank the Loyola Clinical Research Office, Illinois Society for the Prevention of Blindness, the Geoffrey Gunnar Memorial Scholarship, and the Perritt Charitable Foundation for their support of this research project.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtos.2020.07.007.

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