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Ocular Surface Microbiome in Health and Disease

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Abstract: The ocular surface is exposed continuously to the environment and, as a consequence, to a variety of different microbes. After the results of the Human Microbiome Project became publicly available, international research groups started to focus interest on exploring the ocular surface microbiome and its physiopathological relationship to the eye. For example, numerous research studies the existence of the ocular surface's bacterial flora, typically gathering cultures from healthy patients and finding few variations in the bacterial species. More recently, culture-independent methods, including 16S ribosomal ribonucleic acid (rRNA) gene sequencing, are being used to define the ocular microbiome. These newer methods suggest that the microbial communities have a greater diversity than previously reported. These communities seem to serve an immune-modulating function and maintain relationships with other microbes and organs, even distant ones. This review summarizes the literature exploring the ocular microbiome, both in health and in different diseases.

Key Words: 16S rRNA gene, disease, health, human, microbiology, microbiome, ocular surface

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INTRODUCTION

Humans and our microbiomes have most likely coevolved to form a physiological community that is composed of distinct body site niches. The convergence and divergence of these niches (made up of collective germs like bacteria, fungi, and viruses) result in metabolically and antigenically vibrant diverse communities of microbes. These microbes include *mutualists* (symbiotically beneficial microbes), *commensals* (microbes neither harmful nor beneficial to the host), and *pathogens* (microbes detrimental to the host).[1,2] Our current knowledge of the human microbiome has arisen through complex analyses carried out with genome technology (Tables 1 and 2).

The Human Microbiome Project has analyzed the largest cohort and set of 5 major areas of our bodies: the oral cavity, airways, the skin, the gastrointestinal tract, and the vagina.[1,3,4] Each mucosal region represents a distinct microbial niche within the human body, where the microbiomes are determined by host site factors that include pH and oxygen levels. The microbiome is further refined through interactions with its environment, including diet and antibiotic exposure. Some mucosal sites, such as the gastrointestinal tract, have a bacterial genome >100 times the size of the human genome.[3] Other surfaces, such as the ocular surface, are paucibacterial, with approximately 0.06 bacterium per human cell.[3,5,6] Although the human genome contains approximately 20,000 genes, the microbiome, however, contains approximately 8 million genes. It is important to note that the microbiome is not composed of only bacteria; rather, viruses and fungi are also present in the microbiome of healthy people.[3,5,6] In the future, describing the nonbacterial components of the microbiome will be an increasingly important avenue of research.[5,7,8]

Humans require commensal microbes for vital functions, which include developmental, defensive, nutritional, and physiologic processes. For example, bacteria help us extract nutrients from food, defend against pathogens, and maintain the immune system in check.[5,9] Indeed, preliminary studies of the gut have shown that these commensal bacteria contribute to maintaining homeostasis and shaping immunity.[5,7,8] For example, *Clostridium difficile* colitis, irritable bowel syndrome, and inflammatory bowel disease are all believed to involve dysregulation of the gut microbiome. Therefore, the successful treatment of *C. difficile* colitis with fecal microbiota transplantation provides strong evidence to support the therapeutic manipulation of gut microbes.[10] Importantly, additional studies have also linked the microbiota to risks of a variety of other diseases that include colon cancer, diabetes, obesity, and most recently, inflammatory diseases such as rheumatoid arthritis. It is thought that these diseases arise through the alteration of large-scale host immune relationships.[2,5,11]

The gut-eye axis is especially relevant to the field of ophthalmology, as gut bacteria seem to influence immunity at distant sites, which includes the eye.[12] A variety of ocular conditions, such as Sjögren-associated dry eye, glaucoma, diabetic retinopathy, infectious keratitis, and macular degeneration, have all been associated with gut microbiome abnormalities.[5,10]

Although it is true that the tear film is a critical physical barrier between the eye and its environment, it should be noted that the commensal bacteria on the ocular surface also play a role in both adaptive and innate immunity. Indeed, the eye has fostered a harmonious relationship with commensal bacteria on the ocular surface. For example, in a cell-culture model, the healthy epithelial cells of the cornea and conjunctiva did not produce an inflammatory response to commensal bacteria such as *Staphylococcus epidermidis* or *Propionibacterium acnes*. Conversely, epithelial cells of the eye produced inflammatory cytokines (IL-6 and IL-8) when they were exposed to pathogens, such as *Pseudomonas aeruginosa*.[5,13]

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TABLE 1. Distribution of the Typical Microbiome of the Ocular Surface, According to Culture-Based and 16S rRNA Sequencing Studies

	Culture-Based Studies[20–24]	16S rRNA Sequencing Studies[17,18]
Commonly detected genera	Coagulase-negative staphylococci *Propionibacterium* *Corynebacterium* *Staphylococcus aureus* *Streptococcus*	*Corynebacterium* *Acinetobacter* *Pseudomonas* *Staphylococcus* *Propionibacterium* *Streptococcus* Phylum level: -Actinobacteria (53%): *Corynebacterium* and *Propionibacterium* -Proteobacteria (39%): *Pseudomonas* and *Acinetobacter* -Firmicutes (8%): *Staphylococcus* and *Streptococcus* -Bacteriodetes
Sporadically detected genera	*Pseudomonas aeruginosa* *Haemophilus influenzae* *Megasphaera elsdenii* *Bacteroides ureolyticus* *Bacteroides pneumosintes* *Stomatococcus* sp.[17,18,20]	*Bacillus*

rRNA indicates ribosomal ribonucleic acid.

Previous studies describing surface microbes used the only available technique at the time, the conventional culture-based approach. After specimens are collected, they are seeded on some type of solid media (typically blood or chocolate agar) or liquid medium (thioglycolate broth or brain heart infusion). The specimens are then generally incubated at 37°C, examined daily for bacterial growth for 1 week, and subjected to weekly examinations for up to 1 month. Cultures that showcase growth of at least 2 strains of bacteria on any plate, or changes in the color or turbidity of the broth medium, are considered to be positive. Liquid broth specimens considered positive are subcultured on blood agar plates to identify the microbe. The microbes' phenotypes are then arrived through observing the isolated colonies and their morphotinctorial characteristics through a bacterioscope after Gram staining, and under optical microscopy.[14,15] Although this approach identifies viable bacteria, the bacteria that it does detect are only those that grow in these standardized laboratory conditions. Bacterial growth is highly dependent on the incubation conditions that are used, which include the type of media, the atmospheric conditions (ie, aerobic, microaerophilic, and anaerobic), and the duration of the incubation period. It has been estimated that the vast majority of bacteria have not been cultivated within the laboratory.[15,16]

By contrast, however, culture-independent methods are used to target specific genes present only within bacteria. The 16S ribosomal ribonucleic acid (rRNA) gene, for example, is the standard technique in prokaryotic community sampling. After collecting the specimens, deoxyribonucleic acid (DNA) is extracted from the complex microbial communities through an established process; subsequently, primers are utilized to target the conserved parts. After the targeting of the conserved parts, variable regions of these genes can be amplified simultaneously into taxonomically different bacteria or fungi. The amplified DNA is further sequenced, quality filtered, and clustered into

TABLE 2. Distribution of Microbiome in Selected Diseases

Disease	Microbiota-Associated
Diabetes	No difference between diabetes and controls[50,51] Increased *Staphylococcus aureus*[52] More diversity and increased Proteobacteria[53] Increased Bacteriodetes, decreased Proteobacteria[54]
Alcoholism	Increased *Staphylococcus*[55]
Dry eye and meibomian gland dysfunction	Blepharitis: Increased *Staphylococcus*, Streptophyta, *Corynebacterium*, *Enhydrobacter*, and a decrease of *Propionibacterium*[56] Meibomian gland dysfunction: Increased frequency of *Propionibacterium acnes,* decreased *Staphylococcus*[57] Dry eye: Increased *Staphylococcus aureus*, *Corynebacterium*, and *Propionibacterium*[58] Increased *Rhodococcus* and *Klebsiella oxytoca*[5,59]
Sjögren syndrome	No difference between Sjögren and controls[10]
Trachoma	Increased *Corynebacterium* and *Streptococcus,* lower diversity[40]
Stevens-Johnson syndrome	Increased coagulase-negative staphylococci and *Corynebacterium,* more pathogenic species[61] Coagulase-negative *Staphylococcus*[62] *Corynebacterium*, *Streptococcus,* more pathogenic species[24]
Chronic limbal stem cell deficiency	*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*[63]
Chronic ocular graft-versus-host disease	*Staphylococcus*, alpha-haemo *Streptococcus*, *Corynebacterium*, *Propionibacterium acnes*, aerobic gram-positive cocci, *Haemophilus influenzae*, and aerobic gram-positive rod[64]
Allergic conjunctivitis	Lower alpha diversity[65]
Behçet disease	*Staphylococcus aureus*, *Moraxella*, *Streptococcus*[66]

operational taxonomic units. The operational taxonomic units are then annotated using reference databases that include the Ribosomal Database Project, SILVA, Greengenes, and the National Center for Biotechnology Information GenBank database.[16–18] This method not only allows an entire microbial community to be described, but it further defines the relative abundance of these communities spatially and temporally. However, it should be specified that 16S rRNA gene sequencing does not distinguish between viable and nonviable microbes. Less frequently employed methods for characterizing microbial communities include whole genome sequencing (more commonly used for analyzing microbial metabolic and functional pathways) and in silico karyotyping.[17]

Recently, ophthalmologists have become interested in quantifying the composition of the ocular surface microbiome and its role in ocular surface physiology. Here we summarize the literature about ocular surface microbiome by conventional culture-based and 16S rRNA sequencing.

NORMAL OCULAR SURFACE MICROBIOME

The first description in the literature of the ocular surface microbiota dates back to the 1930s. At this time, researchers used culture-dependent techniques to identify microbial species. At that time, coagulase-negative staphylococci, *Propionibacterium* spp., *Corynebacterium* spp., *Staphylococcus aureus,* and *Streptococcus* were thought to make up the bulk of the ocular surface microbiome.[18,19] The microbiome composition, measured through culture-dependent methods, varied little between the 1930s and the present day, and between regions, as reported in studies conducted in Brazil, Japan, Korea, United States, Finland, Uganda, and rural populations of Sierra Leone.[20–24] Some variations described as commensal microbes of ocular microbiome included *P. aeruginosa, Haemophilus influenzae*,[20] *Megasphaera elsdenii, Bacteroides ureolyticus, Bacteroides pneumosintes,* and *Stomatococcus* sp.[25]

A recent systematic review of 11 published controlled cohorts used 16S rRNA sequencing to define, at a phylum level, the most commonly present bacteria or the core genera. The review found that *Corynebacterium* was present in all publications (11/11), comprising a median proportion of 10% of the species found in the eye microbiome. *Corynebacterium* was followed by *Acinetobacter* (9/11; 6%), *Pseudomonas* (8/11; 19%), *Staphylococcus* (7/11; 6%), *Propionibacterium* (7%), and *Streptococcus* (3%) (both 5/11). *Bacillus*, although not considered to be part of the core microbiome (4/11), was fairly abundant (8%) when present.[18] The major phyla were found to be Proteobacteria (*Pseudomonas*), Actinobacteria (*Corynebacterium* and *Propionibacterium*), Firmicutes (*Staphylococcus* and *Streptococcus*), and Bacteroidetes, with the first 2 being the most abundant.[2,5,7,17,18,26–36]

IMPACT OF AGE, SEX, AND GEOGRAPHICAL LOCATIONS ON THE OCULAR SURFACE MICROBIOME

Culture-based studies have highlighted changes in the microbiota isolated during life. Immediately after birth, the neonatal conjunctiva has a higher level of positive cultures and a greater diversity of species compared with other moments in the human lifecycle. Although coagulase-negative *Staphylococcus* and *Propionibacterium* dominate it, the conjunctiva also contains microbiota similar to that of the cervix, and after 2 days, fewer microbes (including pathogenic bacteria) are isolated from the conjunctiva.[17,37,38] From 3 to 90 years of age, aerobic cocci and *Propionibacterium* are the most common in the younger age group, decreasing with age. However, *Corynebacterium* and an increased proportion of anaerobic cocci among anaerobes become more frequent with age.[17,25]

Using 16S rRNA sequencing, 1 study found differences in the microbiome of adults over the age of 18 years, with a mean of 38 ± 10 years.[39] However, another research study indicated that although there was no impact of sex on the microbial diversity, there was a significantly higher richness and Shannon index exhibited in children.[40]

In 2017, Wen et al found that male and female groups differed only in the beta diversity of bacterial communities. This same study found that younger adults were dramatically different from older adults in terms of beta diversity. Age groups, however, showed significant differences in bacterial composition and metabolic functions. Moreover, several antibiotic resistance genes were enriched in the conjunctival microbiome of old adults. These results suggested that the aging process is a risk factor that changes the immune homeostasis of the ocular surface through alterations of its commensal microbiome.[32] Previous reports have indicated that a sex-steroid imbalance after menopause initiates profound changes in the ocular surface microenvironment, thereby predisposing women to many autoimmune, inflammatory, and allergic ocular diseases, including dry eye syndrome.[32,41]

A publication using 16S rRNA sequencing described the phyla levels of the microbiome core at a pediatric level (younger than 18 years' old). The 3 main phyla were Actinobacteria, which represented 53% of bacteria, Proteobacteria, which represented 39%, and Firmicutes, which represented 8%. No core genera belong to the phylum Bacteroidetes.[18] Cavuoto et al, in 2018, found that samples from children younger than 18 years consistently had a greater overall abundance of bacteria compared with adults.[42]

16S rRNA sequencing studies of the ocular region in healthy cohorts have been conducted globally in countries that have included the United States, China, South Korea, Australia, The Gambia, and Northern Ireland.[17] Even with this climatic and geographic variation, however, the majority of studies showed remarkable similarity at the phylum level with Proteobacteria, Firmicutes, and Actinobacteria predominating. At the genus level, *Corynebacterium*, *Staphylococcus*, *Streptococcus,* and *Propionibacterium* were detected consistently, with variances in the relative abundance, in all studies. Interestingly, *Pseudomonas* and *Acinetobacter* were consistently detected across geographic regions by most studies, albeit with differences in the relative abundance.[17]

IMPACT OF MICROHABITAT ON THE MICROBIOME

Ozkan et al, in 2018, described the microbial communities that reside in various microhabitats on the ocular surface: limbal, fornix conjunctival tissue, eyelid margin tissue, and facial skin swabs.[17,43,44] The microbial community structure differed significantly between sites, with the skin having the most abundant quantity of bacteria and the conjunctiva having the lowest. There were no differences found in the bacterial community between the limbus and fornix. The genus *Pseudomonas* was distributed

differently, however, with a high relative abundance on the conjunctiva (80%) and eyelid tissue (65%), less so on the skin (6%) and the ocular surface (2%).[17,18]

IMPACT OF CONTACT LENS WEARING ON THE OCULAR SURFACE MICROBIOME

Two publications using 16S rRNA sequencing compared the ocular surface swabs of contact lens (CL) wearers versus non-wearers. These publications found some variability in abundance, yet no significant difference in alpha diversity. Another publication (n = 20) reported an ocular surface microbiome in lens wearers that was more similar to that of non–CL wearers' skin under the eye. Furthermore, lens wearers had a higher interindividual variability of their ocular microbiota than non–lens wearers,[45] and Proteobacteria dominated microbiome in CL wearers. Hypotheses that help explain this finding have included that the CL may transfer skin bacteria to the eye, that the lens selects for more skin-like bacterial microbiome composition, or that the lens alters the ocular surface barrier and therefore permits bacteria migration.[45,29]

Retuerto et al analyzed the CL belonging to 42 healthy wearers, correlating the effect of multipurpose solutions versus peroxide disinfection systems on bacterial DNA. Eighty percent of the lenses were negative for bacterial DNA. The lack of bacterial DNA was significantly more common ($P = 0.004$) when a peroxide disinfection system was used. Furthermore, increased diversity and abundance were found when a multipurpose solution was used, with *Corynebacterium*, *Haemophilus,* and *Streptococcus* being more abundant in lenses maintained with multipurpose solution.[18,46]

An additional study found that gram-positive bacteria were differentially distributed in those who wear CLs, with a higher load exhibited on the lower lid margins when compared with the load on the upper bulbar conjunctiva.[5,47] Additionally, in one prospective longitudinal study that had a sample of 330 new extended-wear CL wearers, contact lens–*H. influenzae* was more frequently isolated from patients who developed contact lens–associated red eye and symptomatic infiltrates compared with those with no ocular surface changes.[48] Cumulatively, the data from these studies suggest that the ocular surface microbiome is altered with CL wear; this alteration could be a risk factor for the development of an infection.[5]

IMPACT OF EYE DROPS ON THE OCULAR SURFACE MICROBIOME

One study, using the culture-dependent method, evaluated the effect that using glaucoma eye drops had on conjunctival bacterial flora against a control group that did not use the drops. The most frequent bacteria that were reported in both of the groups were coagulase-negative staphylococci. There was no difference reported between patients who used eye drops containing a dose of benzalkonium chloride of 0.01% or higher versus those containing a dose <0.01%.[49] Similar culture rates were reported in patients that suffered from dry eye syndrome and were actively using eye drops compared with those that did not.[20] They found that the eye drop group had a lower culture-positive rate of bacteria in the conjunctival sac; this is likely due to the fact that the bacteria were being physically washed out by the eye drops.[20,49]

IMPACT OF DISEASES ON THE OCULAR SURFACE MICROBIOME

Diabetes

Analyzed data from culture-dependent studies have conflicting results regarding the influence of diabetes on ocular flora. For instance, 1 Japanese study found no significant difference in the bacterial detection rate among individuals with variable diabetes statuses, hemoglobin A1c levels, diabetic retinopathy, or glycosuria.[50] Similarly, a study in Turkey, explicitly designed to explore the differences in bacteria cultured from diabetic and nondiabetic patients, reported that there was no difference in culture rates between the 2 groups. However, this study did find a statistically significant ($P = 0.018$) higher rate of gram-negative organisms that were cultured from diabetic patients.[51] Furthermore, a study based in Bangladesh found 64% and 38% culture-positive rates for diabetic (n = 50) and nondiabetic (n = 250) patients respectively, also reporting higher rates of *S. aureus* isolation in diabetic patients.[52]

Ham et al found through 16S rRNA sequencing that the microbial community of diabetic patients was more diverse than that of healthy volunteers. Specifically, Proteobacteria, Firmicutes, Actinobacteria, Cyanobacteria, and Bacteroidetes were found to be the dominant taxa present on the ocular surface; furthermore, there was a significant difference in the relative abundance of the bacterial phyla between the diabetic patients and control subjects. More specifically, Proteobacteria were more abundant in the diabetic group, whereas Firmicutes were more abundant in the control group. Analysis of bacterial taxa at the genus level illustrated that the core microbiome of diabetic patients was comprised of *Acinetobacter*, *Burkholderia*, *Sphingomonas*, and *Ralstonia*, whereas that of the controls was comprised of Bradyrhizobiaceae, *Staphylococcus*, *Corynebacterium*, *Pseudomonas*, *Novosphingobium*, Neisseriaceae, and *Acinetobacter*.[53]

Another study with 16S rRNA sequencing showed more diversity in the ocular surface microbiota of people with type 2 diabetes mellitus than in a control group. Bioinformatic analysis showed that there was a lower abundance of Proteobacteria and a higher abundance of Bacteroidetes; furthermore, there was a significantly increased abundance of *Acinetobacter* and *Pseudomonas* in the group with type 2 diabetes mellitus.[54]

Alcoholism

A 2015 study found changes in the composition of ocular surface flora with a significantly higher incidence of *Staphylococcus* in subjects with chronic alcoholism when compared with the general population.[55]

Dry Eye and Meibomian Gland Dysfunction

One study found an increase in the relative abundance of *Staphylococcus*, Streptophyta, *Corynebacterium*, and *Enhydrobacter*, alongside a decrease of *Propionibacterium* in patients that had blepharitis (this was compared to healthy controls without blepharitis).[56] Specifically, blepharitis was defined by clinical findings on slit-lamp examinations, which included lid margin or tarsal conjunctival erythema, telangiectasia, bulbar conjunctival hyperemia, thickening or irregularity of the eyelid margins, or meibomian gland orifice inclusions. These findings, however, differ from Watters et al, who focused their research on individuals with meibomian gland dysfunction (MGD) (defined as the

presence of terminal duct obstruction, lid margin thickening, bulbar hyperemia, telangiectasia, and/or altered meibomian gland secretions, graded on a composite 3-point severity scale). In the latter study, individuals without MGD had the highest abundance of *Staphylococcus,* whereas cases that exhibited moderate-severe MGD had the lowest abundance. Furthermore, the authors of this research demonstrated that individuals with severe MGD had a higher frequency of *P. acnes* that was recovered from the ocular surface.[57]

It has been found that dry eye has additionally been associated with ocular surface microbiome alterations. For instance, 1 report related higher frequencies of *S. aureus*, coagulase-negative *Staphylococcus*, and *Corynebacterium,* in individuals with dry eye (defined by symptomatology, tear break-up time <10 seconds, and combined fluorescein and lissamine green staining ≥ 3) than in control groups.[58] Furthermore, in another study, individuals who exhibited dry eye were found to have a higher relative abundance of the potential pathogens, *Rhodococcus* and *Klebsiella oxytoca*, compared with control groups. Additionally, the individuals with dry eye showcased an upward trend of increased bacterial counts and decreased goblet cell numbers.[5,59]

Sjögren Syndrome

One study collected microbiome samples from the inferior conjunctiva of 8 healthy volunteer controls that included 6 rosacea patients and 15 Sjögren syndrome (SS) patients. Subsequently, these patients were analyzed through 16S rRNA sequencing. A core microbiome consisting predominantly of Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes phyla was found from all 3 groups without significant differences in overall composition, richness, or structure of ocular surface microbiome between the 3 groups.[10]

This same study induced a mouse model of SS by subjecting mice to 10 days of desiccating stress and antibiotics. As part of the study, they also characterized the microbiota fecal by evaluating the effects of intestinal dysbiosis on the dry eye phenotype. In this mouse model, worse dry eye phenotype was associated with decreased *Clostridium* in stool and a relative increase in *Enterobacter*, *Escherichia/Shigella*, and *Pseudomonas* in stool, in comparison with the controls. The dry eye phenotype was also associated with greater relative abundances of *Pseudobutyrivibrio*, *Escherichia/Shigella*, *Blautia*, and *Streptococcus*, and a lower relative abundance of *Bacteroides*, *Parabacteroides*, *Faecalibacterium*, and *Prevotella* than in the controls. The severity of ocular and systemic SS was found to be inversely correlated with microbial diversity. This correlation suggests that SS is marked by a dysbiotic intestinal microbiome that is driven by the low relative abundance of commensal bacteria and a high relative abundance of potentially pathogenic genera. In turn, the dysbiotic intestinal microbiome is associated with intensified cases of ocular mucosal disease.[10,60]

Trachoma

One report comparing the conjunctival microbiome of 220 residents of The Gambia, some with healthy eyes and some with trachoma, found 610 genera belonging to 22 phyla by 16S rRNA gene sequencing. *Corynebacterium*, *Streptococcus*, *Propionibacterium*, *Staphylococcus*, *Bacillus,* and *Ralstonia* were exhibited by at least 80% of all participants with normal conjunctiva. However, *Pseudomonas* was present at $<1\%$ of the relative abundance. A decreased diversity and an increased abundance of *Corynebacterium* and *Streptococcus* were seen in participants with conjunctival scarring in comparison to the controls.[40]

Stevens-Johnson Syndrome

In a nonrandomized study that included a total of 41 eyes of 22 patients with Stevens-Johnson syndrome (SJS), the frequency of testing positive for conjunctiva culture was higher than that which has been historically reported in healthy patients. Specifically, coagulase-negative staphylococci and *Corynebacterium* were the prominent species that were represented in the positive tests. Half of the patients (54%) tested positive for multiple bacterial species in their flora. Furthermore, it was shown that cultures grew pathogenic bacteria such as *Enterobacter*, *Serratia nonliquefaciens*, *Escherichia coli*, *Morganella morganii*, *Proteus mirabilis*, and *Haemophilus*.[61]

In another study of the ocular microbiome in SJS, it was shown that the frequency of a positive culture was significantly higher in cases than in the control groups without SJS (59% vs 12.9%). Here, coagulase-negative *Staphylococcus* was the most common organism found in both individuals with SJS and the control groups. It is important to note, however, that cultures from individuals with SJS also grew *Enterobacter*, *Streptococcus viridians, and Micrococcus,* which were not found in the controls.[62]

A recent study included 20 chronic SJS patients and 20 healthy subjects for microbiome analysis by conventional cultures and next-generation sequencing methods. Positive-cultured specimens were found in 60% of the SJS group and 10% of the healthy volunteers. *Corynebacterium* and *Streptococcus* were the more frequent bacteria. In the control group, *S. epidermitis* was the most frequent. 16S rRNA sequencing has found that the greater diversity of microbial species, the higher proportion of pathogenic microbes, including *Pseudomonas*, *Staphylococcus*, *Streptococcus*, and *Acinetobacter*, are to be found in the SJS group.[24]

Chronic Limbal Stem Cell Deficiency

One study with 13 patients found a positive conjunctival swab culture in all of them. These patients exhibited both chronic limbal stem cell deficiency due to SJS and ocular chemical injury, and were undergoing cultivated oral mucosal epithelial transplantation. The most common organism isolated was *S. epidermidis*, followed by *S. aureus* and *P. aeruginosa.*[63]

Chronic Ocular Graft-Versus-Host Disease

One study evaluated the conjunctival microbe, using conventional culture-based methods, of 32 eyes of 20 ocular graft-versus-host disease (GVHD) patients and 28 eyes of 20 non-GVHD cases, and found more complex diversity of the ocular surface microbes in the GVHD group. *Staphylococcus*, alpha-haemo *Streptococcus*, *Corynebacterium* species, *P. acnes*, aerobic gram-positive cocci, *H. influenzae*, and aerobic gram-positive rod were all observed in the GVHD patients, whereas few species were detected in the other group.[64]

Allergic Conjunctivitis

One study in allergic rhinoconjunctivitis patients related a negative correlation between ocular surface microbiome diversity and allergy severity. Allergic conjunctivitis patients showed a

lower alpha diversity with increasing disease severity, challenging the results of the nasopharyngeal microbiome. Interestingly, the bacterial community of the nasal mucosa became more similar to that of the conjunctiva in the allergic patients.[65]

Behçet Disease

In a study with Behçet disease with culture had significantly higher rates of colonization with *S. aureus*, *Moraxella,* and *Streptococcus* in Behçet disease patients.[66]

CONCLUSIONS

At first, when culture-dependent methods were the only ones available, the bacteria of our normal ocular microbiome were classified only by the genus level. The genera would be described according to their physiochemical characteristics and by how microbes respired in the microbiological process. In essence, the only identifiable bacteria were coagulase-negative staphylococci, *Propionibacterium*, *Corynebacterium*, *S. aureus,* and *Streptococcus*, with some variations.[17,18,20]

Since the advent of 16S rRNA sequencing, including amplicon and metagenomic sequencing, it has now been possible to discover previously undescribed bacteria, more complex communities, and culture-resistant bacteria. With the new 16S rRNA sequencing technique, the core bacteria (40% of the total ocular surface) of the surface microbiome in healthy humans have been better defined at the phylum level. The core bacteria, including Actinobacteria *(Corynebacterium* and *Propionibacterium*), represent precisely 53% of the ocular surface microbiome, followed by Proteobacteria (*Pseudomonas* and *Acinetobacter*) accounting for 39%, and Firmicutes (*Staphylococcus* and *Streptococcus*) for 8%.[18]

Summarizing the results of this review, normal microbiomes differ by sex, geographic distribution, microhabitat on the eye, eye drop use, and CL use, as discussed in detail previously. The microbiome has been studied in selected systemic diseases, including diabetes and alcoholism. Logically, the eye surface microbiome has also been studied in various eye diseases that have included dry eye and MGD, allergic conjunctivitis, SJS, SS, trachoma, chronic limbal stem cell deficiency, chronic ocular GVHD, and Behçet disease.

In years past, bacteria were primarily studied in relationship to pathologies and with a focus on diagnostics and therapy. However, the research community has steadily been increasing its appreciation of the normal microbiome as we begin to understand the importance of immune-modulating functions and relationships between microbes, the eye, and other organs.

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