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The Effect of Face Mask Wear on the Ocular Surface and Contact Lens Microbiome

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Objectives: As face mask wear can result in the redirection of nasal and oral exhalation toward the ocular region, this study investigated the impact of face mask wear on the conjunctiva, eyelid margin, and contact lens (CL) surface microbiome.
Methods: In this prospective, cross-over study, experienced CL wearers (N=20) were randomized to wear a face mask for 6 hr/day (minimum) for a week or no mask for a week. The conjunctiva, eyelid, and CLs were then sampled. After a 1-week washout period, participants were crossed over into the alternate treatment for 1 week and sampling was repeated. Sampling was bilateral and randomly assigned to be processed for culturing or 16S ribosomal(r) RNA gene sequencing.
Results: Culturing showed no effect of mask wear on the average number of bacterial colonies isolated on the conjunctiva, eyelid, or CL, but there was increased isolation of *Staphylococcus capitis* on CL samples with mask wear (P=0.040). Culture-independent sequencing found differences in the taxonomic complexity and bacterial composition between the three sites (P<0.001), but there was no effect of bacterial diversity within and between sites. Mask wear did not impact dry eye or CL discomfort, but increased ocular surface staining was reported (P=0.035).
Conclusions: Mask wear did not substantially alter the microbiome of the conjunctiva, eyelid margin, or CL surfaces in uncompromised healthy eyes.

Key Words: Microbiome—Culture—16S rRNA gene sequencing—Facemask—Contact lens—Bacteria.

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The dataset supporting the conclusion of this article is publicly available at https://github.com/jozkan/Facemask-effect-Ocular-CL-micro.

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During the COVID-19 pandemic of the severe acute respiratory syndrome coronavirus 2, face mask wear was mandated in most parts of the world. Wearing a face mask results in the creation of an upward thermal air plume toward the eye from nasal and oral exhalation.[1] This has been reported to cause elevation of the ocular surface temperature[2] and result in a condition called mask-associated dry eye, which is associated with increased dry eye symptoms, damage to the ocular surface, reduced tear production, and greater tear evaporation.[3–5]

The microbial load and diversity of the oral cavity[6] and nares[7] are higher than the ocular surface[8] and skin.[9] Of concern, bacteria that are found in the oral cavity and nares, including *Staphylococcus aureus*, *Streptococcus pneumonia*, *Corynebacterium diphtheriae*, and *Pseudomonas aeruginosa*,[6] can be pathogenic to the eye.[10] Six hours of wear of an N95 mask in an office environment has been found not to alter the diversity and composition of the skin microbiome[11] but did not adversely alter the oral microbiome.[12] However, a recent study that compared the incidence of postoperative endophthalmitis before, and during, the COVID-19 period in Japan found that postvitrectomy endophthalmitis increased during the COVID-19 mask wear period. The authors speculated that the increase was likely caused by exposure of the eye to an increase of oral bacteria before and after vitrectomy surgery, perhaps related to mask use by patients during the COVID-19 pandemic.

Before the pandemic, mask use was primarily by medical personnel and by individuals for hygiene/general health protection in some societies.[13] The COVID-19 pandemic resulted in an unprecedented, worldwide uptake of mask wear, including many contact lens (CL) wearers. No study to date has investigated what effect a prolonged redirection of oral and nasal exhalation has on the microbiome of the ocular surface, lid margins, and CL surface, with the potential consequence of increased incidence of inflammatory and infectious CL-related ocular adverse events. This study hypothesized that the wear of face masks results in an alteration in the ocular microbiome and on worn contact lenses compared with no-mask wear. To test this hypothesis, this study used conventional culture and 16S rRNA gene sequencing to investigate what effect face mask wear has on the microbiome of the ocular surface, eyelid margin, and CL surface.

MATERIALS AND METHODS

Study Design

This was a prospective, single-center, cross-over study and was reviewed and approved by the Human Research Ethics Committee of The University of New South Wales (H220006) and followed the tenets of the Declaration of Helsinki. Informed consent was obtained before the enrollment of subjects into this study.

Participants were required to be over 18 years of age, be experienced CL wearers, have ocular health findings considered to be "normal" (e.g., no ocular diseases or history of eye surgery), have no active ocular inflammation or infection, and have no use of topical or systemic antibiotics, anti-inflammatories, or immunosuppressants 3 months prior and during the study period.

The sample size was calculated based on the application of a species accumulation curve from our previous ocular microbiome studies,[8] in which greater than 85% of microbial species on the ocular surface were detected after taking 20 samples per timepoint. The study cohort therefore consisted of 20 experienced CL wearers who were required to wear daily disposable contact lenses (MyDay, stenoficon A; Coopervision, Pleasanton, CA) each day for a minimum of 6 hr for 1 week. Participants were randomized to wear face masks (disposable, nonwoven, three-ply, medical grade mask; Purist International, Lane Cove West, NSW, Australia) for a minimum of 6 hr a day for 1 week or no mask for 1 week. No specific instructions were provided to participants as to how the mask should be worn to simulate "real-world" conditions. Swabs were taken off the bulbar conjunctiva and inferior eyelid margin (FLOQSwabs, Copan, Brescia, Italy), and contact lenses were collected aseptically at the end of each week of mask or no-mask wear. There was a washout period of 1 week between sampling, after which participants were crossed over into the alternate intervention (Fig. 1). Participants were required to complete the Ocular Surface Disease Index (OSDI) questionnaire[14] and the Contact Lens Dry Eye Questionnaire (CLDEQ-8)[15] at baseline and at the end of each week of mask or no-mask wear. Ocular surface staining, to assess surface damage, was also performed at these visits using the modified Oxford Scheme.[16]

On the day of sampling/lens collection, participants were required to have worn lenses for a minimum of 6 hr. Each participant had a separate swab taken, using standard techniques,[17,18] of the inferior bulbar conjunctiva and another swab of the lower eyelid margin. Samples (swabs and contact lenses) were collected bilaterally from each participant and were then randomly allocated to be processed for culture or 16S rRNA gene sequencing (i.e., if the right eye samples were cultured, then left eye samples were processed for sequencing, and vice versa; Fig. 1).

Swabs and contact lenses assigned to microbial culturing were placed in sterile vials containing 2 mL of phosphate-buffered saline and were processed within 1 hour of collection. Swabs and lenses allocated for 16S rRNA gene analysis were placed inside sterile, DNA-free, microcentrifuge tubes and immediately frozen in the clinic room to −20°C with a portable LabTop cooler (Thermo Fisher Scientific, Coralville, MA) and then transferred to a −80° C freezer within 1 hour. To identify contaminating taxa from the environment or reagents, 12 negative controls (nine unused blank swabs, three new unworn contact lenses) were collected at regular intervals throughout this study.

Microbial Culturing

The vials containing the swabs or contact lenses were vortexed at maximum speed for 30 s. Two chocolate blood agar (CBA) (Oxoid, Basingstoke, United Kingdom) plates were inoculated with 400 μL aliquots. The CBA plates were incubated at 37°C in 5% CO_2 for 48 hr (CO_2 sachet, Thermo Fisher) or under anaerobic conditions (AnaeroGen sachet, Thermo Fisher Scientific) for 96 hr. Each macroscopically different microbial colony was then stored and processed for microbial identification using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (Ultraflextreme, Bruker Daltonics, Bremen, Germany) and a direct colony extraction technique.[19] For this, individual pure cultures were prepared using the procedure described in the MALDI Biotyper Protocol Guide (Edition 5, 2017; Bruker Daltonics). In brief, each culture was washed with 100% ethanol and then mixed with formic acid (70%) and acetonitrile. The mixture was laid on the MALDI-TOF target plate and left to dry. Once dry, an α-cyano-4-hydroxycinnamic acid matrix was laid on each culture and left to dry. The MALDI-TOF target plate was run on the mass spectrometer to obtain microbial peptide mass fingerprints (PMFs). Bacterial test standard and matrix were also included on the plate for each run. The PMFs were run against the MALDI Biotyper OC database Version 4.0.19 (Bruker Daltonics) for identification.

Microbial Community Analysis

The characterization of bacterial communities in the samples involved amplification and sequencing of the 16S rRNA gene, following a previously established protocol.[20] Polymerase chain reaction (PCR) amplifications were performed, targeting the V3 to V4 region, and the resulting amplicons were sequenced using the Illumina MiSeq platform (2 × 300 bp sequencing run) at the Ramaciotti Centre for Genomics, University of New South Wales. Raw sequencing reads were quality filtered, merged, denoised, and clustered into amplicon sequencing variants (ASVs) using Usearch version 11.0.667[21,22] and the UNOISE algorithm.[23] Chimeric sequences were removed de novo using UCHIME[24] with the SILVA v138 database as a reference (https://www.arb-silva.de/documentation/release-138/). Taxonomic classification of ASVs was achieved using the Bayesian Last Common Ancestor Algorithm[25] against the Genome Taxonomy Database (GTDB) r207 database (https://data.gtdb.ecogenomic.org/releases/release207/). Nonbacterial, nonaligned, and singleton ASVs were excluded from further analysis. To identify contaminant taxa, a linear regression filter was applied, utilizing the relative abundances of ASVs in the negative controls as explanatory variables and ASVs in the actual samples as dependent variables.[8] The identified contaminant ASVs were then removed from the dataset.

Statistical Analysis

Differences in sex and age between the sampled regions were evaluated using Pearson chi-square and Kruskal–Wallis tests, respectively. A paired *t* test was used to assess differences in CL discomfort (CLDEQ-8 questionnaire, OSDI) and clinical assessment (ocular surface staining) related to mask wear.

Multivariate statistical tools were employed to analyze the taxonomic tables of ASVs and to compare the effects of the treatment (mask wear).[18] ASV data were normalized using DESeq2,[26] and alpha-diversity was compared using linear mixed models (R package lme4) with treatment, site, sex, and age as fixed effects and individuals as random factors.[27] Significance was assessed using analysis of variance (ANOVA), and pairwise comparisons were conducted using the post hoc Tukey (honestly significant difference) HSD test. The microbial beta-diversity was assessed using Bray–Curtis dissimilarity for relative abundance enabling comparisons between the mask and no-mask groups through permutational ANOVA.[28]

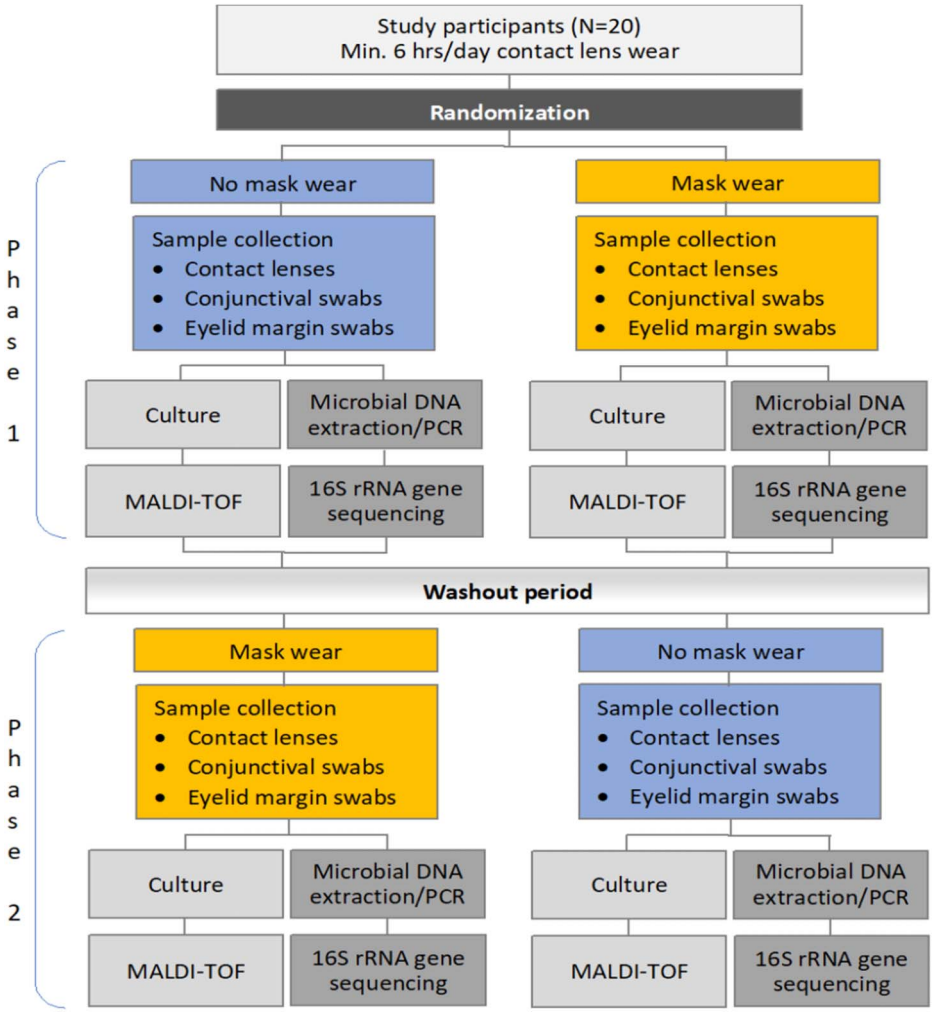

FIG. 1. Cross-over study design and sample allocation and processing.

Colony forming unit (CFU) data were natural log transformed (Log [CFU+1]). A generalized estimating equation was used to determine the cross-over effect of wearing a mask while accounting for the order of the treatment. The within-subject correlation was accounted for using subject random intercepts and exchangeable correlation structure. Normal distribution with identity link function was used for log-transformed CFU data, and binomial distribution with logit link function was used for the binary variables. The level of statistical significance was set at 5%.

RESULTS

All participants (N=20, mean age 26±12 years [min 18.5–max 61 years], 75/25% women/men) completed both phases (mask and no-mask wear during CL wear) of this cross-over study. A total of 240 samples were collected during this study, which consists of 120 samples (40 lenses, 40 conjunctiva, and 40 eyelid margin swabs) for culture-independent microbial community analysis and 120 samples (40 lenses, 40 conjunctiva, and 40 eyelid margin swabs) for culture-based analysis to isolate viable bacteria present in the samples.

As mask wear was legally required to be worn in some settings during the period this study was conducted, there was some mask wear by participants during the no-wear phase. As such, we assessed the duration of wear during both phases and found there remained a significant difference in the duration of mask wear between the two phases of this study (mask wear phase=6.7±1.3 hr compared with no-wear phase=1.6±0.9 hr, $P<0.001$). Mask wear resulted in marginally significant higher corneal staining of left eye samples (0.4±0.6 vs. 0.2±0.4, mask vs. no-mask wear, respectively, $P=0.035$), but otherwise there was no statistical support for differences between mask and no-mask wear for dry eye and CL symptoms with the OSDI and CLDEQ-8 questionnaires, respectively, or for conjunctival staining in both eyes (Table 1).

Microbial Community Analysis

All amplicons of the test samples and negative controls (which had no visible PCR band) were sequenced. After quality filtering, there were 10,323,328 sequences with an average of 81,931 sequences per sample, clustering into 2,817 ASVs. After contaminant filtering, there were 4,290,811 sequences with an average of 35,757 sequences per sample, clustering into 2,728 ASVs.

TABLE 1. *Questionnaire and Clinical Data With and Without Mask Wear*

	Mask Wear (Mean±SD)	No Wear (Mean±SD)	*P*
Duration of face mask wear during contact lens wear (hr)	6.7±1.3	1.6±0.9	**<0.001**
CLDEQ-8	9.0±5.5	8.0±5.7	0.186
OSDI	9.5±14.6	9.7±13.0	0.874
Conjunctival staining—right eye	0.6±0.5	0.8±0.4	0.234
Conjunctival staining—left eye	0.6±0.6	0.6±0.5	1.000
Corneal staining—right eye	0.4±0.6	0.4±0.5	0.505
Corneal staining—left eye	0.4±0.6	0.2±0.4	**0.035**

Bold value indicates statistical significance $P < 0.05$.

Ocular Site, But Not Mask Wear, Influenced the Alpha-Diversity of Microbiome

For Chao1 richness, there was a difference for site ($P<0.001$) and for individuals ($P<0.001$), but there was no effect of mask wear overall ($P=0.527$) or at each site, that is, for CL (37±18 vs. 35±19 ASVs, $P=0.574$, no-mask vs. mask wear), conjunctiva (61±31 vs. 48±15 ASVs, $P=0.342$, no-mask vs. mask wear), and eyelid margin (140±89 vs. 121±71, $P=0.990$, no-mask vs. mask wear) (Fig. 2). Overall, Chao1 richness showed a lower level of complexity on the CL and conjunctival surface compared with the eyelid margin.

For the Shannon diversity index, there was a significant difference between sites ($P<0.001$) and individuals ($P=0.001$), but there was no effect of mask wear overall ($P=0.240$) or at each site, that is, for CL (2.1±0.6 vs. 2.2±0.4, $P=0.615$, no-mask vs. mask wear), conjunctiva (2.8±0.6 vs. 2.5±0.5, $P=0.230$, no-mask vs. mask wear), and eyelid margin (3.2±0.8 vs. 2.9±0.9, $P=0.409$, no-mask vs. mask wear) (Fig. 2). Shannon diversity index was lowest on the CL and conjunctiva compared with the eyelid margin.

Beta-Diversity

Analysis of the bacterial community structure using Bray–Curtis dissimilarity between samples showed support for a significant difference between the three sites (conjunctiva, eyelid margin, and CL) ($P>0.001$), but there was no overall effect of mask wear ($P=0.114$) or at each site, including CL ($P=1.000$, no-mask vs. mask wear), conjunctiva ($P=0.735$, no-mask vs. mask wear), and eyelid margin ($P=1.000$, no-mask vs. mask wear) (Fig. 3).

Despite the lack of statistical support for differences in the ASV-level microbiome as a whole with mask wear, analysis at the levels of taxonomic groups showed some subtle differences. Overall, the dominant phyla across all samples were Firmicutes (16.2%), Proteobacteria (19.7%), and Actinobacteriota (13.6%), with Firmicutes dominant on the contact lenses (25.0%), Proteobacteria on the conjunctiva (23.7%), and Actinobacteriota (21.7%) on the eyelid margins (see Figure, Supplemental Digital Content 1, http://links.lww.com/ICL/A300). With mask wear, there was a significantly lower relative abundance of Firmicutes on contact lenses (32.3±6.7% vs. 53.9±7.3%, $P=0.0006$, mask wear vs. no-mask wear, respectively). The dominant genera across the three sites were *Corynebacterium, Staphylococcus, Bacillus, Acinetobacter,* and *Pseudomonas*_E (Fig. 4). With mask wear, there was a significantly lower relative abundance of *Aeribacillus* (5.3±2.3 vs. 16.3±5.9, $P=0.044$, mask wear vs. no-mask wear, respectively) and *Bacillus* (6.7±4.0 vs. 15.7±6.2, $P=0.0416$, mask wear vs. no-mask wear, respectively) on contact lenses and of *Pseudomonas*_E (2.7±0.9 vs. 12.1±4.5, $P=0.0264$, mask wear vs. no-mask wear, respectively) on the conjunctiva.

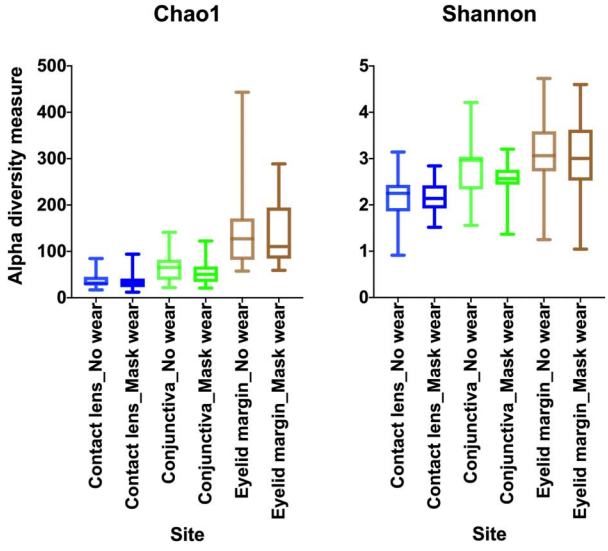

FIG. 2. α-Diversity based on Chao1 and Shannon diversity indices of the contact lens, conjunctiva, and eyelid margin, with and without mask wear.

Culture Analysis

Bacteria were isolated from all the eyelid margin samples from both the mask and no-mask wear groups and from the majority of the conjunctiva (80% vs. 75%, no wear vs. mask wear) and CL (95% vs. 85%, no wear vs. mask wear) samples (Fig. 5). There was, however, no difference in the rates of sterility (no recovery of viable microorganisms) between mask wear or no-mask wear from the CL ($P=0.160$) or conjunctiva ($P=0.705$) samples (Fig. 5).

A total of 16 species from six genera were isolated across the conjunctiva (8 vs. 7 species, no wear vs. mask wear), CL (11 vs. 6 species, no wear vs. mask wear), and eyelid margin (10 vs. 9 species, no wear vs. mask wear) (Fig. 6). The predominant genus recovered from all three sites was *Staphylococcus*. Other genera recovered from the ocular region included *Pseudomonas*, *Micrococcus*, *Bacillus*, *Curtobacterium*, and *Kytococcus* (Fig. 6). A total of nine *Staphylococcus* spp. were isolated with *Staphylococcus epidermidis* being the dominant isolated species (Fig. 6). *S. epidermidis* was the most frequently isolated species at the three sites in both groups (Fig. 6). There was no effect of mask wear on the frequency of any specific bacterial species recovered from the three sites ($P>0.05$).

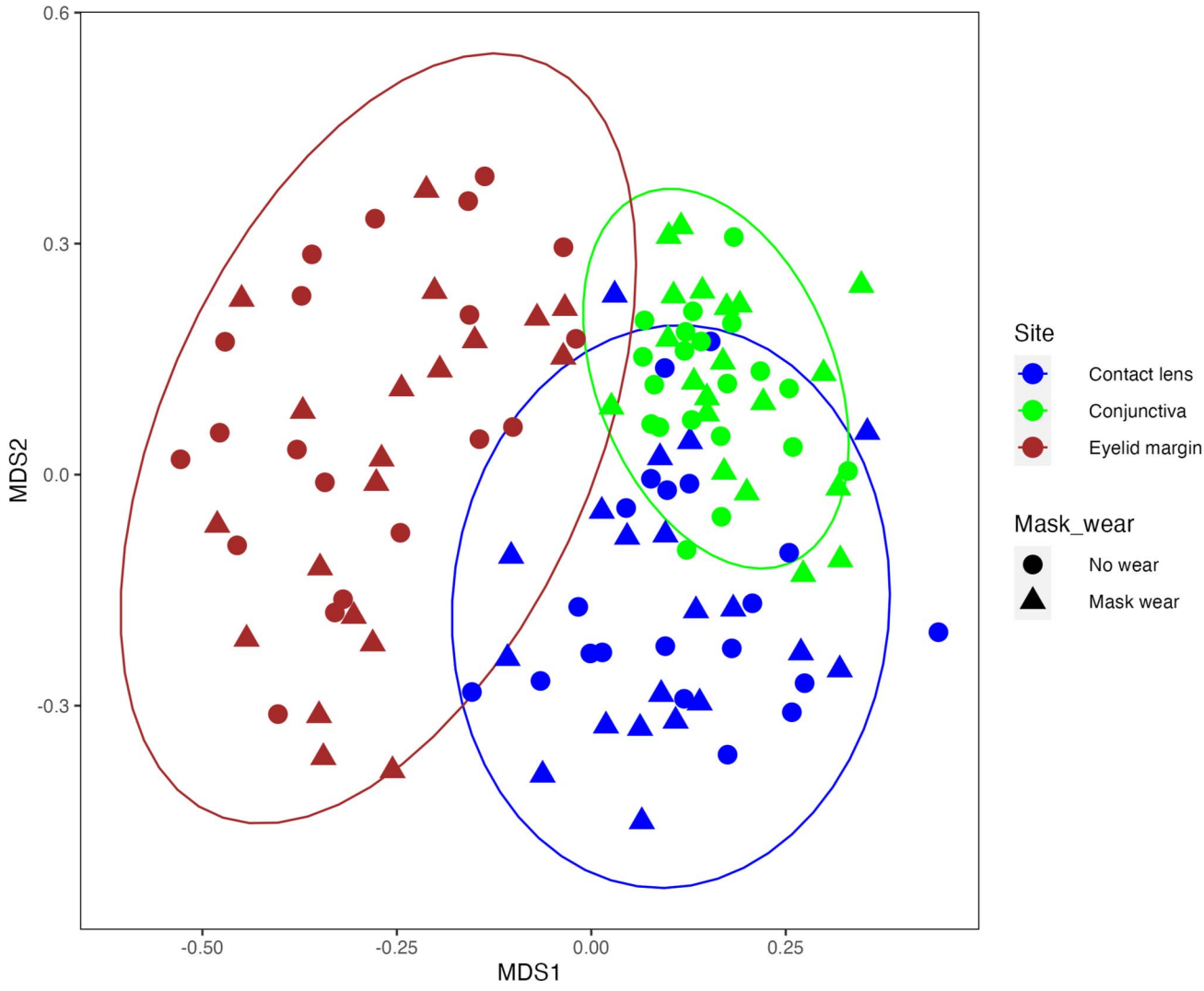

FIG. 3. Nonmetric multidimensional scaling ordination of the microbiomes of the conjunctiva, eyelid margin, and contact lens surface, with and without mask use, using Bray–Curtis dissimilarity of amplicon sequencing variants.

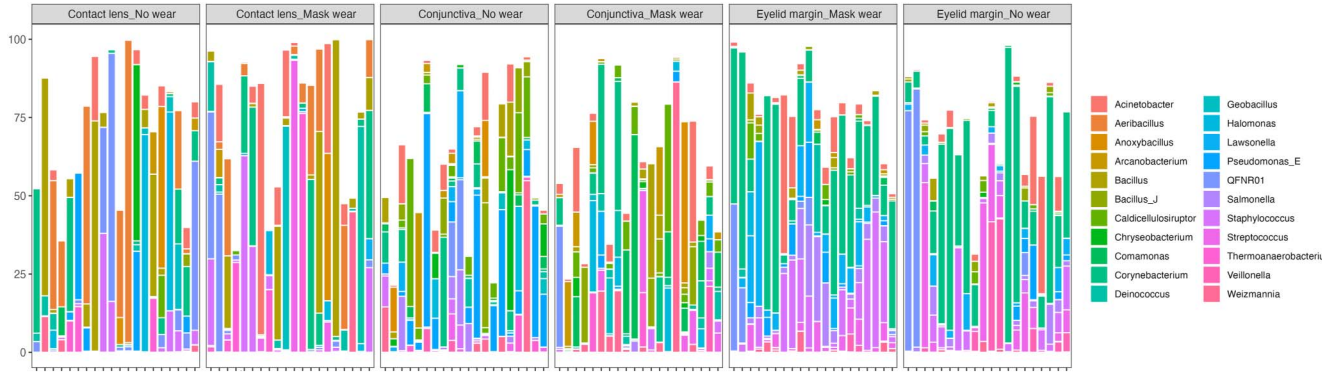

FIG. 4. Genus-level bacterial composition of the conjunctiva, eyelid margin, and contact lenses with and without mask wear.

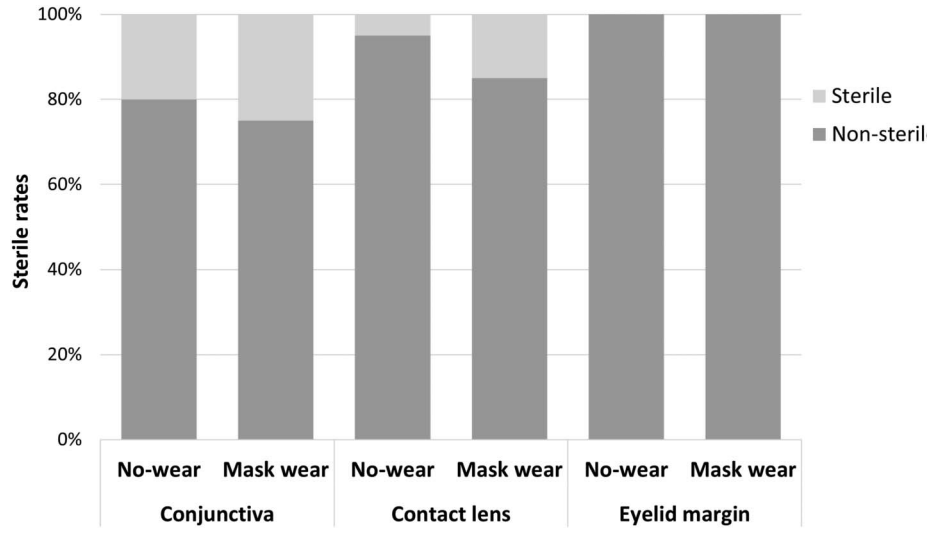

FIG. 5. Rates of sterile samples from contact lens, conjunctiva, and eyelid margin samples with and without face mask wear.

The highest average number of bacterial colonies were isolated from the eyelid margin followed by the CL and conjunctival samples (Fig. 7). There was no effect of mask wear on the average number of bacterial colonies isolated on the conjunctiva (P=0.690), CL (P=0.742), or eyelid margin (P=0.371) (Fig. 7). In the mask-wearing group, there was increased isolation of *Staphylococcus capitis* on CL samples (0.7±1.6 vs. 0.0±0.0 CFU, P=0.040, mask wear vs. no wear). Although *S. epidermidis* was the most commonly isolated bacteria, there was, however, no effect of mask wear on the number of colonies isolated from the contact lenses (2.5±2.9 vs. 3.6±3.0 CFU, P=0.173, mask wear vs. no wear), conjunctiva (1.5±1.9 vs. 2.0±2.0 CFU, P=0.493, mask wear vs. no wear), or the eyelid margin (4.7±2.7 vs. 4.9±2.7 CFU, P=0.757, mask wear vs. no wear).

DISCUSSION

Bacterial species colonize the surfaces and linings of the oral cavity and nasal passage, some of which are potentially pathogenic.[6] It was hypothesized that the elevation of ocular surface temperature[29] and redirection of nasal and oral exhalation toward the eyes caused by the use of face masks[1] would cause an alteration in the ocular region microbiome. To comprehensively describe the ocular bacterial communities with and without mask wear, this

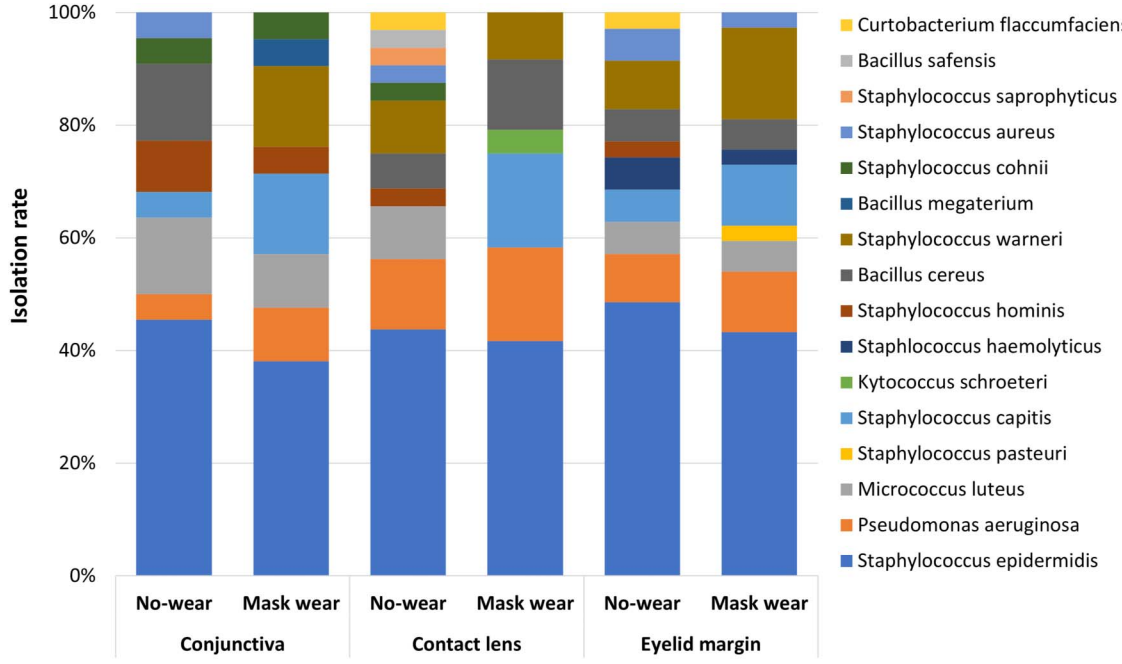

FIG. 6. Bacterial species recovered with and without mask wear from the conjunctiva, contact lens, and eyelid margin.

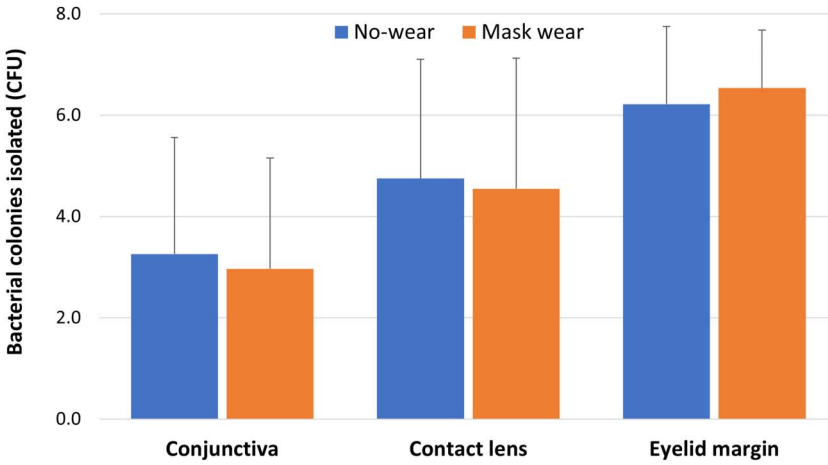

FIG. 7. Colonies isolated (mean±SD) on the conjunctiva, contact lens, and eyelid margin with and without face mask wear.

study used both culturing- and sequencing-based methods across different eye regions and on worn contact lenses. The results showed that using culture, there was a slight increase in the *S. capitis* on contact lenses with mask wear while culture-independent 16S rRNA gene sequencing showed that mask wear did not alter the overall microbiome on the surface of worn contact lenses, the conjunctiva, or eyelid margins for alpha- and beta-diversity. However, mask wear was associated with some subtle changes including fewer specific taxonomic groups and significantly lower relative abundance of the phylum Firmicutes and the genera *Aeribacillus* and *Bacillus* on contact lenses and *Pseudomonas*_E on the conjunctiva.

Consistent with previous studies, this study was able to detect significant differences in the taxonomic complexity (highest on the eyelid margin and lowest on the conjunctiva and contact lenses) and bacterial community structure between different ocular sites.[18,30,31] The result of this study showed that mask wear did not result in an increased number or abundance of bacterial species of oral and nasal origin, including *Streptococcus, Moraxella, Veillonella, Haemophilus, Prevotella*, and *Lactobacillus,*[32] on the surface of the eyelid margin, conjunctiva, or worn contact lenses. Mask wear was associated with increased isolation of the skin bacteria *S. capitis* and decreased relative abundance of *Aeribacillus* and *Bacillus* (all on contact lenses). *S. capitis* is a skin commensal and a possible reason for its increased isolation on the CL surface, compared with the conjunctiva and eyelid margin, may be due to its primary pathogenic factor which is its ability to form biofilm.[33] The lack of any significant alteration in the ocular region eye microbiome due to mask wear could be due to several factors. Although microbes can be isolated from exhaled breath,[34,35] the overall microbial load is low and may not be sufficient to invade the ocular microbial communities and hence cause a discernible difference. In addition, microbial species from the mouth and nasal region may not be adapted to the antimicrobial factors at play on the ocular surface.[36] A previous study showed that although there were many bacteria shared between the nasal passage and the nasopharynx, there were some bacteria community members that remained site-specific, even with the relative proximity and similarity of the two niches.[37]

Our results showed no effect of mask wear on dry eye symptoms (OSDI) or CL discomfort (CLDEQ-8), but there was a slight increase in ocular surface damage. This latter finding of an increase in ocular surface damage (corneal staining) has been reported previously.[4] The mask-induced, superiorly redirected airflow directed over the eyes may lead to faster tear evaporation leading to ocular surface desiccation and "dry eye" symptoms.[5] Several studies have investigated the effect of mask wear on ocular health and dry eye disease,[4,5] which has been referred to as mask-associated dry eye.[3] This study was not able to replicate changes previously reported for worsening dry eye symptoms (assessed by OSDI questionnaire)[3–5] and did not measure tear production (Schirmer-1 test) or tear break-up time[4] that had previously been reported to change.

Such observed differences could also be related to differences in the study design. For example, this study had a lower duration of mask wear (average of 6.7 hr/day) compared with 8 hr/day in other studies.[4,5] Mask wear duration might be linked to dry eye symptoms, as mask wear greater than 3 hr/day had significantly more dry eye symptoms score compared with those wearing a mask less than 3 hr/day[3] and 8 hr mask wear resulted in increased dry eye symptoms (higher OSDI scores).[4,5] Other factors, such as mask type and design (material, presence of nose wire bridge, tightness of ear loops/head strap, mask shape/geometry) could also explain the difference between studies.

P. aeruginosa is a virulent ocular pathogen and the most commonly isolated pathogen from CL-related keratitis.[38] *P. aeruginosa* was isolated sporadically across all three sites and mask wear did not significantly change the isolation rate. The frequency of *P. aeruginosa* isolation from contact lenses ranged from 5% with no-mask wear and 10% with mask wear, which was in general agreement with the literature, which shows that *P. aeruginosa* was isolated from 4% of worn contact lenses across studies.[39] With sequencing, ASVs associated with the genus *Pseudomonas*_E were consistently detected at low relative abundances across all three sites and were significantly lower on the conjunctiva with mask wear.

Some limitations of this study include the use of only one type of lens wear modality (daily disposable wear). As this type of CL is discarded daily, any potential bacterial contamination from oral and nasal exhalation has a limited time to colonize the surface. The use of a nondaily disposable lens, which would be worn and handled multiple times and be more dependent on compliance with lens care maintenance, could potentially provide more

opportunities for oral and nasal bacteria to colonize. In addition, there was some mask wear by participants during the "no-wear" period as mask wear at the time of this study was legally required in some settings (public transport use, attending lessons at university, work environment). However, this was unlikely to have impacted the final results, as there remained a significant difference in the duration of mask wear during the two phases of this study.

CONCLUSION

The COVID-19 pandemic resulted in an unprecedented, worldwide uptake of mask wear. This study showed for the first time that mask wear does not substantially alter the microbiome of the conjunctiva, eyelid margin, or CL surfaces in otherwise healthy eyes. These findings, particularly as they relate to ocular health and CL use safety, are important as they suggest that mask use is unlikely to pose any ocular health-related issues and will not result in an increased risk of developing inflammatory or infectious CL-related adverse events in otherwise uncompromised eyes.

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