Alterations of conjunctival microbiota associated with orthokeratology lens wearing in myopic children

Ju Zhang[1,2,3†], Xiuhai Lu[2†], Zhiwei Cheng[2], Dulei Zou[2,3,4], Weiyun Shi[2,3*] and Ting Wang[2,3*]

Abstract

Background Orthokeratology (OK) lens wear increases the risk of bacterial infection, but little is known about the microbiota of the conjunctival sac in myopic children wearing OK lenses. This study aimed to investigate the changes of conjunctival microbiota in children after treatment with OK lenses using 16 S rDNA sequencing.

Methods Twenty-eight myopic children who had been continuously wearing OK lenses for 12 to 13 months were enrolled in this prospective study. Twenty-two gender- and age-matched myopic children who had not worn OK lenses or discontinued OK lens wear at least 1 year ago were recruited as controls. Conjunctival swabs from each participant were collected for exploration of the microbiota profiles, targeting the V3–V4 regions of the 16 S rRNA gene by MiSeq sequencing. The differences in the microbial community structure and diversity were also compared between groups.

Results The bacterial alpha diversity indices in the OK lens group were not different from those in the non-wearer group ($P > 0.05$, Wilcoxon test), while beta diversity examined using principle coordinate analysis of unweighted UniFrac divided the two groups into different clusters. *Proteobacteria*, *Bacteroidetes*, and *Firmicutes* were the abundant phyla in the conjunctival sac microbiota in both groups ($P < 0.05$, Mann–Whitney U test). Among children in the OK lens group, the Linear discriminant analysis Effect Size identified the compositional changes in OK lens-associated bacteria. Key functional genera such as *Blautia*, *Parasutterella*, and *Muribaculum* were enriched, whereas *Brevundimonas*, *Acinetobacter*, *Proteus*, and *Agathobacter* decreased significantly ($P < 0.05$, Mann–Whitney U test). Phylogenetic investigation of communities by reconstruction of unobserved states also showed altered bacterial metabolic pathways in OK lens-associated microbiota. Moreover, using receiver operating characteristic curves, *Brevundimonas*, *Acinetobacter*, *Proteus*, and *Agathobacter* alone (the area under the curve was all > 0.7500) or in combination (the area under the curve was 0.9058) were revealed to discriminate OK lens wearers from controls.

†Ju Zhang and Xiuhai Lu contributed equally to this work.

*Correspondence:
Weiyun Shi
weiyunshi@163.com
Ting Wang
wt-ting@163.com

Full list of author information is available at the end of the article

Conclusions The relative abundance of the microbial community in the conjunctival sac of myopic children can alter after OK lens wear. *Brevundimonas*, *Acinetobacter*, *Proteus*, and *Agathobacter* may be candidate biomarkers to distinguish between OK lens wearers and non-wearers.

Keywords Orthokeratology lenses, Conjunctiva sac, Microbiome, *Brevundimonas*, *Acinetobacter*, *Proteus*, Genomics, 16S rDNA gene sequencing

Background

Myopia is predicted to affect half of the world's population and become one of the leading causes of irreversible blindness by 2050 [1]. Orthokeratology (OK) is the use of rigid gas-permeable contact lenses with an inverse geometric design which can temporarily flatten the central corneal curvature to reduce the degree of myopia and improve uncorrected visual acuity (UCVA) in children and adolescents [2–5]. The growing incidence of myopia in the young population enables OK lenses increasingly used nowadays [6–8]. The sale volume of OK lenses in China was about 3 million pairs in 2020. However, OK lens wear enhances the risk of bacterial infection because lenses directly touch the surface of the cornea and are worn overnight [9]. Moreover, wearing contact lenses changes the microbiota of the conjunctival sac, which has been identified as a risk factor of ocular infections, like keratitis and macropapillary conjunctivitis, and corneal infiltrative events [10–12]. Recent studies have shown that the incidence of bacterial keratitis, one of the vision-threatening complications, [13–16]. was 13.9/10,000 in adolescents wearing OK lenses [17, 18]. Daily and extended contact lens wear has been found to cause an increase of *Staphylococci*, mainly *S. epidermidis* [19] and *S. aureus* [20]. Contact lens wearers might have more variable and skin-like bacterial community structures, with higher abundance of *Methylobacterium*, *Lactobacillus*, *Acinetobacter*, and *Pseudomonas* [10]. Zhang et al. [21] found that the abundance of *Bacillus, Tatumella*, and *Lactobacillus* was less in OK lens wearers than in non-wearers. These changes in the ocular microbiome have been suggested to affect the infection development in individuals wearing contact lenses. Nonetheless, the effect of contact lens wear on ocular microbiota has been inconclusive. It is necessary to further elucidate the interplay between microbiome and contact lens usage for better control of the complications [22, 23]

Although previous studies using the conventional culture technique have proved that applications of soft contact lenses can cause bacterial contamination, [19, 24] little is known about the impact of OK lenses on the structure and function of the microbiota on the surface of eyes, especially the microbiota of the conjunctival sac in children wearing OK lenses. In this study, 16 S rDNA gene high-throughput sequencing-based bacterial detection and identification [25] were performed, aiming to explore the true diversity of conjunctival microbiota in pediatric OK lens wearers and thus to guide clinical management of associated ocular surface inflammation.

Subjects and methods

Subjects

Myopic patients who had been wearing OK lenses for 12–13 months and myopic patients who had not worn OK lenses or discontinued OK lens wear at least 1 year ago, aged 8–15 years, were prospectively enrolled at the Eye Hospital of Shandong First Medical University from September 2020 to December 2020. Among the participants, there were 14 males and 14 females in the OK lens group (L group), averaged 12.5 ± 2.6 years old, and 11 males and 11 females in the non-wearer group (N group), averaged 10.0 ± 2.2 years old. No significant differences in gender ($P>0.05$, Chi-square test) and age ($P>0.05$, Student's T-test) existed between the two groups. Patients who had a history of eye surgery or trauma, other ocular or systemic diseases, antibiotic, anti-inflammatory or immunosuppressive medications within 6 months were excluded from the study.

This study was approved by the Ethics Committee of the Eye Hospital of Shandong First Medical University (SDSYKYY20200713) and registered on the Chinese Clinical Trial Registry (ChiCTR2000037230, 27/08/2020). All the procedures adhered to the tenets of Declaration of Helsinki. Written informed consent was obtained from legal guardians of each participant. A schematic diagram of the research procedure is shown in Fig. 1.

Conjunctival swab collection

Conjunctival swab samples were taken from the right eye of each patient by the same clinician to ensure consistency. To eliminate the influence of anesthetics on the conjunctival sac microbiota, anesthetic eye drops were not used in the sample collection process [10]. The lower palpebral and fornix conjunctiva was swabbed three times using a sterile cotton swab (CLASSIQSwabs; Copan, Brescia, Italy), which avoided any contact with the eyelid margin and was rotated toward the opposite direction of the conjunctiva to maximize effective collection. Then the samples were immediately placed in a sterile, RNase-free cryopreservation tube, frozen with liquid nitrogen, and preserved in a refrigerator at minus 80 ℃. 16 S rDNA gene high-throughput sequencing was performed within 1 month after sample collection.
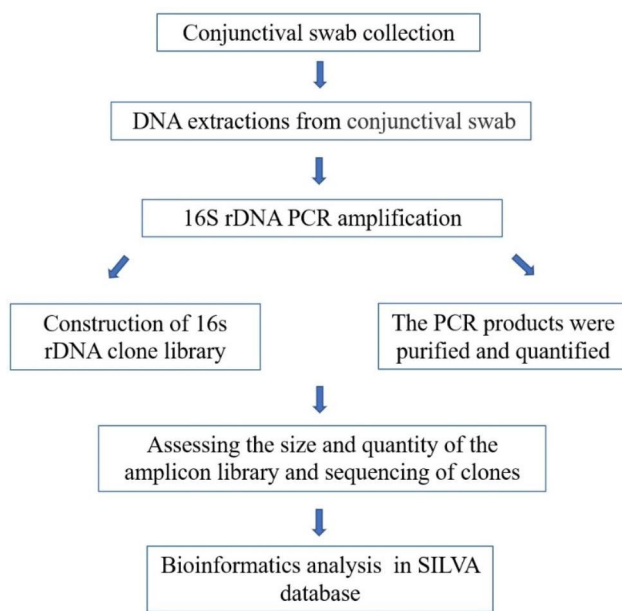
Fig. 1 A schematic diagram of the research procedure beginning from conjunctival swab collection, followed by DNA extractions, PCR amplification, construction of 16s rDNA clone library, sequencing of clones, and bioinformatics analysis

DNA extraction
According to the instructions of the PowerMax® Soil DNA Isolation Kit, the DNA extraction of all the samples was completed in a biosafety cabinet. The DNA purity and concentration were checked by ultraviolet spectrophotometer reading. The quality of the extracted DNA was verified using agarose gel electrophoresis, and the quantification of DNA was achieved by ultraviolet spectrophotometry. Throughout the DNA extraction process, ultrapure water instead of a sample solution was used to exclude the possibility of false-positive polymerase chain reaction (PCR) results.

PCR amplification and 16 S rDNA sequencing
The primers were designed according to the conserved regions in the ribosomal RNA of microorganisms as 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GAC-TACHVGGGTATCTAATCC-3'), and universal adapters and barcode sequences were added for PCR amplification of the V3-V4 variable regions [26]. The 5' ends of the primers were tagged with specific barcodes per sample and sequencing universal primers. PCR amplification was performed in a 25-µL reaction mixture containing 25 ng of template DNA, 12.5 µL of PCR premix, 2.5 µL of each primer, and PCR-grade water to adjust the volume. The PCR conditions to amplify the prokaryotic 16 S fragments consisted of an initial denaturation at 98 ℃ for 30 s, 32 cycles of denaturation at 98 ℃ for 10 s, annealing at 54 ℃ for 30 s, an extension at 72 ℃ for 45 s, and a final extension at 72 ℃ for 10 min [27]. The PCR products were purified using AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA) and quantified using the Qubit fluorometer (Invitrogen, Carlsbad, CA, USA). The amplicon pools were prepared for sequencing, and the size and quantity of the amplicon library were assessed on the Agilent 2100 Bioanalyzer (Agilent, CA, USA) and with the Library Quantification Kit for Illumina (Kapa Biosciences, Woburn, MA, USA), respectively. The libraries were sequenced on the NovaSeq PE250 platform.

Bioinformatics analysis
Samples were sequenced on an Illumina NovaSeq platform according to the manufacturer's recommendations at the LC-Bio (Hangzhou, China). After dereplication using Divisive Amplicon Denoising Algorithm 2, [28] we obtained the feature tables and feature sequences. Alpha diversity and beta diversity were calculated by random normalization to the same sequences. Alpha diversity was applied in analyzing complexity of species diversity for each sample with indices of Chao1, Observed species, Goods coverage, Shannon, and Simpson calculated using quantitative insights into microbial ecology 2 [29]. Beta diversity calculated using principle coordinate analysis derived from unweighted UniFrac distances was used to indicate the difference in the overall composition and distribution of the microbial community between groups [30]. Blast was used for sequence alignment, and the feature sequences were annotated with the SILVA database for each representative sequence.

Statistical analysis
All data were statistically analyzed by SPSS20.0 software. The t-test and Mann-Whitney U test were applied for continuous variables. The Chi-square was used for categorical variables between groups. The R package (v3.5.2) was used for preparation of graphs. All tests of significance were two sided, and $P < 0.05$ or corrected $P < 0.05$ was considered statistically significant. The sample size was determined from G*Power [31].

Accession number
The sequence data from this study are deposited in the GenBank Sequence Read Archive with the accession number PRJNA819236.

Results
Changed overall structure of conjunctival microbiota in children wearing OK lenses
In total, 3,920,380 high-quality reads (2,187,649 for the OK lens group and 1,732,731 for the non-wearer group), with an average of 78,407 reads per sample, were obtained for the subsequent microbiota analysis. The value of Good's coverage was 99.83%, indicating that a majority of bacterial phylotypes [7,177 operational taxonomic

units (OTUs)] in the conjunctival microbiota were identified. It was observed that bacterial alpha diversity indices of Shannon, Simpson, Chao1, and observed OTUs were not significantly different between groups (Wilcoxon test, $P > 0.05$; Fig. 2A-D). The rarefaction curve of each sample tended to be flat, indicating that most of the bacteria were detected (supplementary 1). Beta diversity also divided the two groups into different clusters (ANOSIM test, $P < 0.05$; Fig. 2E and F). The Venn diagram illustrates the overlap of OTUs in the conjunctival microbiota of the two microhabitats (Fig. 2G).

Altered conjunctival microbiota composition in children wearing OK lenses

The compositions of conjunctival microbiota in the OK lens wearers and the controls were assessed at different taxonomic levels. Using the Ribosomal Database Project (RDP) classifier, the sequences were classified into 37 phyla, 304 families, and 778 genera. At the phylum level, the abundance of *Actinobacteria* was significantly reduced in the OK lens group (1.47% vs. 2.95%, $P = 0.00$, Wilcoxon test). The abundant bacterial genera in both groups were *Pseudomonas* (61.18% vs. 64.26%, $P = 0.56$, Wilcoxon test), *Ralstonia* (10.48% vs. 12.04%, $P = 0.92$, Wilcoxon test), and Muribaculaceae-unclassified (6.18% vs. 0.03%, $P = 0.00$, Wilcoxon test). The abundance of Muribaculaceae-unclassified was significantly increased in the OK lens group (Fig. 3). In terms of bacterial phenotypes, the abundance of facultative anaerobe decreased significantly in the OK lens group (5.07% vs. 8.23%, $P = 0.009$, Wilcoxon test; supplementary 2). The Linear discriminant analysis (LDA) Effect Size (LEfSe) identified the differential bacteria at different taxonomic levels between the two groups (LDA score > 3, $P < 0.05$, Mann-Whitney U test). Among the key functional bacteria identified in the conjunctival microbiota, *Muribaculaceae-unclassified*, *Blautia*, *parasutterella*, and *Muribaculum* had higher abundance at the genus level in the OK lens group, while the abundance of *Brevundimonas*, *Acinetobacter*, *Proteus*, and *Agathobacter* was higher in the non-wearer group (Figs. 4 and 5). These differential genera were candidate biomarkers to discriminate the two groups of patients.

Discrimination with conjunctival microbiota-based signatures

We used each single differential bacterial genus as predictor to generate the area under the receiver-operating characteristic (ROC) curves (AUC) and observed the values of four abundant genera as biomarkers: *Proteus* (AUC = 0.8490), *Brevundimonas* (AUC = 0.8157), *Acinetobacter* (AUC = 0.7532), and *Agathobacter* (AUC = 0.7500). We also applied multivariable stepwise logistic regression analysis to further distinguish the OK lens wearers

from the non-wearers, using the four abundant genera. The predictive performance was significantly improved (AUC = 0.9058) (Fig. 6).

Microbial functional dysbiosis in OK lens wearers

To identify the metabolic and functional disparities in the conjunctival microbiota between the OK lens wearers and the non-wearers, PiCRUSt was used to analyze the functional potential of the microbiota based on closed-reference OTU picking. We compared 64 KEGG pathways and identified several KEGG categories with clearly differential abundance between the two groups, finding that carbohydrate metabolism, xenobiotics' biodegradation and metabolism, and transport and catabolism significantly increased after OK lens wear, while transcription, immune system, and environmental adaptation significantly decreased ($P < 0.05$, t- test; Fig. 7).

Discussion

Contact lens wear is a known risk factor that may cause microbial keratitis and other ocular inflammation [10, 32–37]. Previous studies have disclosed that the microbiota of the conjunctival sac has a protective effect against foreign bacterial invasion [38]. However, wearing contact lenses can alter the microbiome of the ocular surface, which may reduce the restoration of the conjunctival microenvironment [10, 39, 40]. Moreover, the positive detection rate of the conventional bacterial culture method was reported to be only 34-65% [41–44]. Such culture results are not efficient to reflect the micro-environment status of the bacterial community in the conjunctival sac [11, 45]. In this study, we comprehensively examined changes in the microbial community structure of the conjunctival sac in children wearing OK lenses. Using 16 S rDNA gene sequencing can accurately detect and identify slowly growing, non-culturable or culture-resistant bacteria and those with special growth requirements, such as *Pseudomonas*, *Acinetobacter*, *Brevundomonas*, *Sphingomonas*, and *Streptococcus*, [10, 25] besides bacteria which have been detectable using the conventional bacterial culture, like *coagulase-negative Staphylococcus*, *Propionibacterium*, and *Corynebacterium* [46].

Graham et al. [25] measured the conjunctival sac microbiome of healthy people by 16 S rDNA sequencing, finding the high abundance of bacteria belonged to *S. epidermidis*, *Coagulase-negative Staphylococcus*, *Corynebacterium sp.*, and *P. acnes.* Dong et al. [39] identified the most abundant bacterial genera in the conjunctival microbiota, namely *Pseudomonas*, *Bradyrhizobium*, *Propionibacterium*, *Acinetobacter*, and *Corynebacterium*. In our series study, the top five bacterial genera with high abundance in the conjunctival sac of children, no matter with or without OK lens wear, included *Pseudomonas*,
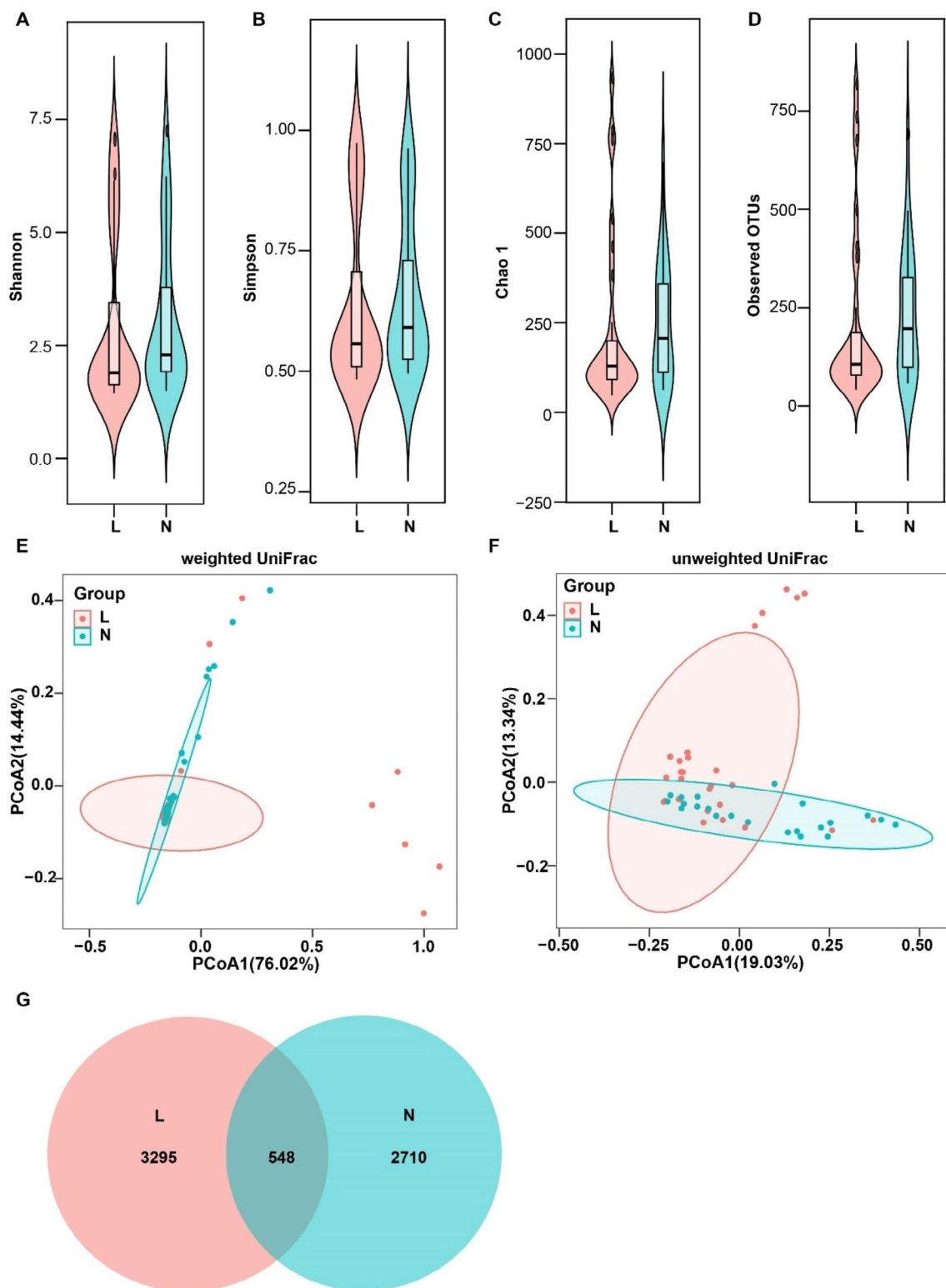
Fig. 2 The bacterial diversity and richness of the conjunctival sac microbiota in two groups. **A-D**: Violin plots representing alpha diversity of different individuals in the two groups (*P* > 0.05, Mann-Whitney U test). **E-F**: Principal coordinates analysis plots. Each point represents a sample, and the points of the same color are from the same group. The closer the distance between two points, the smaller the difference in community composition between the two groups (*P* < 0.05, ANOSIM test). **G**: The Venn diagram illustrates the overlap of OTUs in the conjunctival microbiota among the two microhabitats. L: the OK lens group, N: the non-wearer group
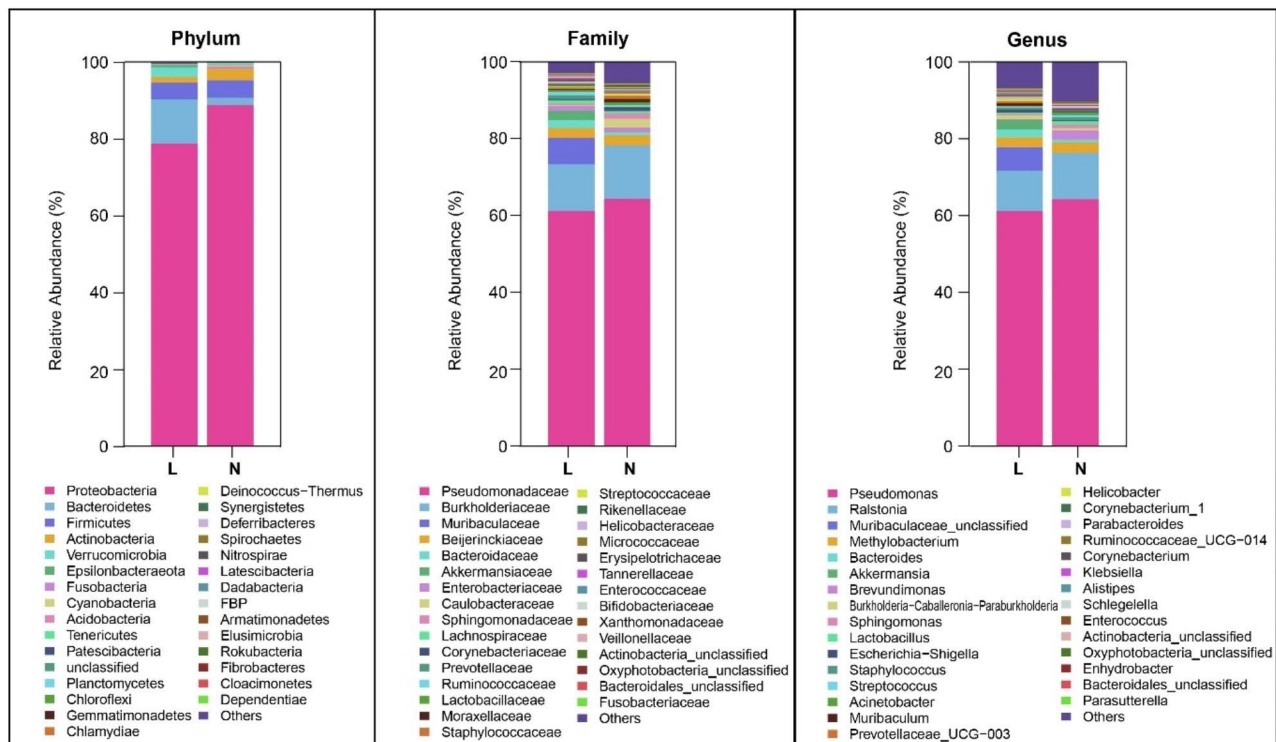
Fig. 3 Histogram of bacterial taxa distribution at the phylum, family, and genus levels in the two groups. The top 30 species with the highest relative abundance in the two groups. The vertical axis represents the relative abundance of each species, whereas the horizontal axis is the group name. The columns of different colors correspond to different bacteria, and the length of the columns represents the proportion of the bacterial taxa. L: the OK lens group, N: the non-wearer group

Ralstonia, *Muribaculaceae-unclassified*, *Methylobacterium*, and *Bacteroides*. The inconsistency in the predominant species, except *Pseudomonas*, might be due to the age and region differences of the subjects [47–49]. In addition, the possibility that the location and depth of sampling may affect the result cannot be entirely excluded [50, 51]. The current study supports that *Pseudomonas* might be one of the colonizing bacteria in the conjunctival sac, [52] which is different from the traditional culture results [53]. However, once the epithelial barrier of the OK lens wearers is destroyed, [54]*Pseudomonas* can rapidly pass through the barrier, bind to the surface, and cause bacterial aggregates and membrane remodeling. The process may evade the immune surveillance and result in ocular infections [55]. This is consistent with the rapid and violent course of *Pseudomonas aeruginosa* infection in OK lens wearers.

In our study, the number of bacterial species in the OK lens group was reduced compared with that in the non-wearer group, which may be attributed to the following reasons. On one hand, the bacterial residues due to inadequate contact lens care may lead to changes and establish the indigenous microbiome in the conjunctival sac. On the other hand, the increase in the abundance of certain pathogenic bacteria may inhibit other bacteria.

Regarding the abundance distribution of some bacteria, the relative abundance of *Brevundimonas*, *Acinetobacter*, *Proteus*, and *Agathobacter* in the OK lens group decreased, while that of *Muribaculaceae-unclassified*, *Blautia*, *Parasutterella*, and *Muribaculum* increased, breaking the balance of the indigenous microbial environment. Accumulating evidence disclosed that the opportunistic pathogens, such as *Brevundimonas*, *Proteus*, and *Acinetobacter*, increased the potential risk of ocular surface infection [56–58]. Wang et al. [59] discovered that *Blautia* producta was positively correlated with the disease course of lens-associated *Acanthamoeba* keratitis. *Acanthamoeba* is one of the most common etiological agents of infectious keratitis in association with OK lens wear [14]. The intervention of *Blautia* flora seems to be promising for the treatment of lens-associated *Acanthamoeba* keratitis. Yun et al. [60] found that *Actinobacteria* populations at the phylum level and *Muribaculaceae* at the family level of gut bacteria were clearly related to the secretion of tears. We believe that the decreased tear secretion at night after wearing OK lenses may reduce the washing effect of the tear film on bacteria and increase the risk of infection. Meanwhile, it was revealed that the abundance of facultative anaerobe in the OK lens group was significantly lower than that in
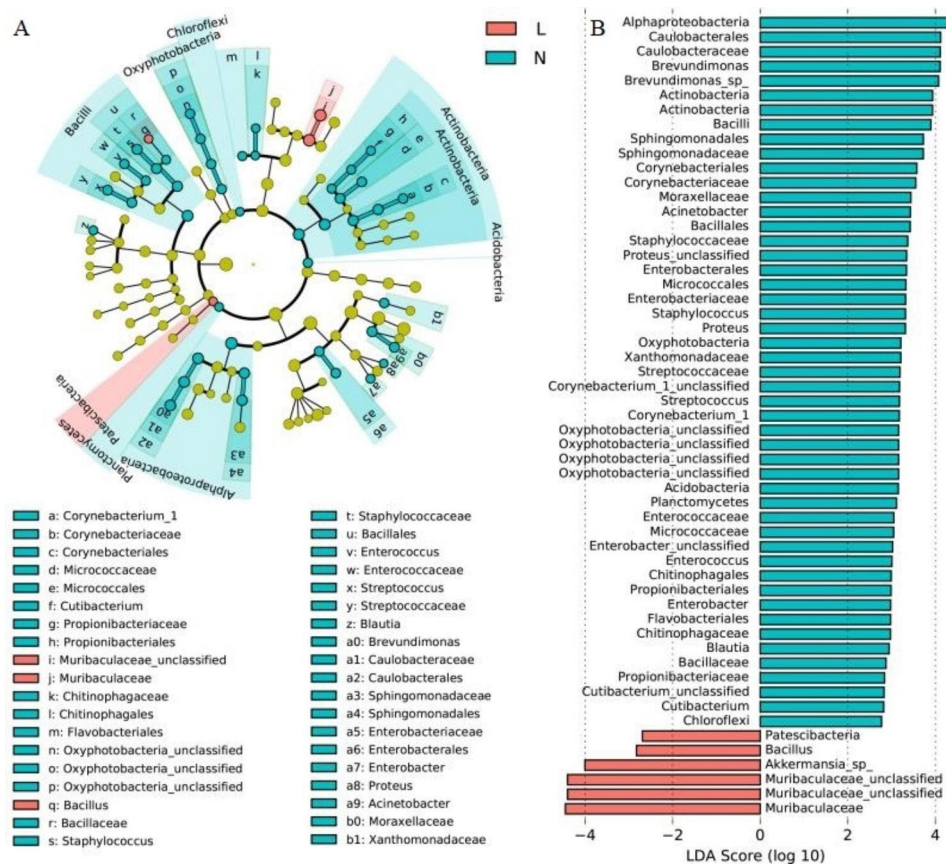
Fig. 4 Linear Discriminant Analysis (LDA) Effect Size (LEfSe) multilevel discriminant analysis of the species differences (LDA > 3, *P* < 0.05, Mann–Whitney U-tests). A: Cladogram. The circle radiating from inside to outside represents the classification level from the kingdom to the genus (or species). Species with no significant differences are uniformly colored in yellow, the red nodes represent the microbial group that plays an important role in the OK group, and the green nodes represent the non-wearer group. B: Histogram of LDA value distribution shows the biomarkers with statistical difference. The LDA value represents the influence of bacterial species, and the longer the length, the higher the degree of influence. L: the OK lens group, N: the non-wearer group

the non-wearer group. Wearing OK lenses at night may lead to different degrees of hypoxia in the conjunctival sac, [61] and facultative anaerobes are capable of switching to fermentation or anaerobic respiration but do not reproduce. Therefore, there was a decrease of abundance, which was also an influencing factor of the microbial abundance in the conjunctival sac.

This study demonstrated that the abundance of some bacterial populations changed obviously after OK lens wear at the levels of phylum and genus. For example, at the genus level, *Proteus*, *Agathobacter*, *Brevundimonas*, and *Acinetobacter* reduced markedly, so they were selected alone or together for the ROC analysis as non-invasive biomarkers to distinguish the OK lens wearers from the non-wearers. This provides a new target for early warning and intervention of OK lens-related flora alterations and possible risks.

Ozkan et al. [53] analyzed the microbial community in the conjunctival sac of healthy people by culture, disclosing that Gram-positive microorganisms accounted for the majority of the isolated microorganisms (94%), and

the most frequently isolated genus was *Staphylococcus* (46.5%), which was not very abundant in gene sequencing in our study. On the contrary, *Pseudomonas* had high gene sequencing abundance, but its positive rate in the traditional culture was low. It seems that a reasonable combination of the two detection methods can better serve clinical needs.

Considering when people put contact lenses into eyes with their fingers, they may touch the eyelashes and eyelids, Costello et al. [62] regarded contact lenses as a medium that can transmit bacteria from the skin of hands or eyelids into the eyes and result in microbial imbalance. Therefore, the factor of daily care should also be involved in the changes of conjunctival sac floras after wearing OK lenses. Thorough and careful hand washing before each insertion and removal of the contact lenses and less contact with the skin are highly recommended.

Further studies are required to overcome the limitations in this research. First, the host response related to the floras should be investigated to better reflect the association with the disease. Second, more samples collected
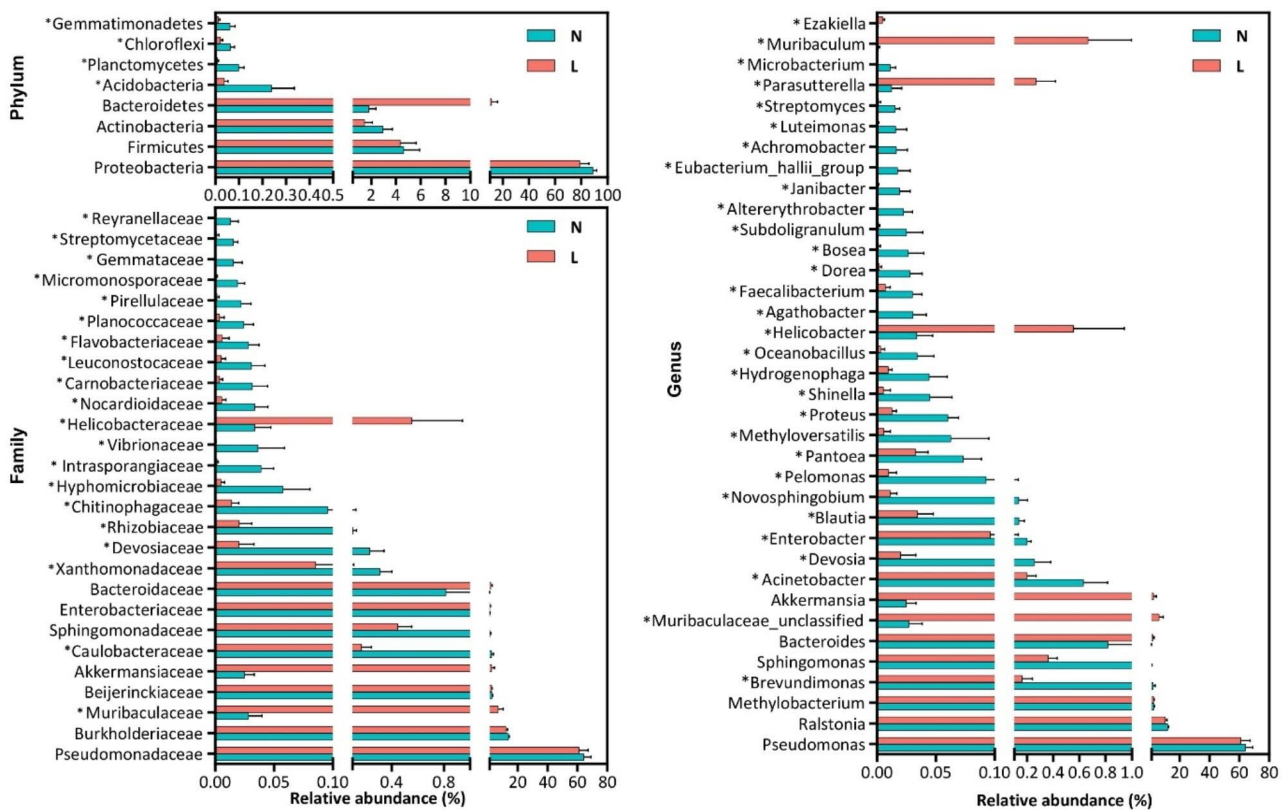
Fig. 5 Comparisons of the relative abundance of the bacterial taxa at the phylum, family, and genus levels. The data are presented as the mean ± standard error. The Mann–Whitney U test is used to analyze variation between the OK lens wearers and the non-wearers. *$P < 0.05$ compared with the non-wearer group. L: the OK lens group, N: the non-wearer group
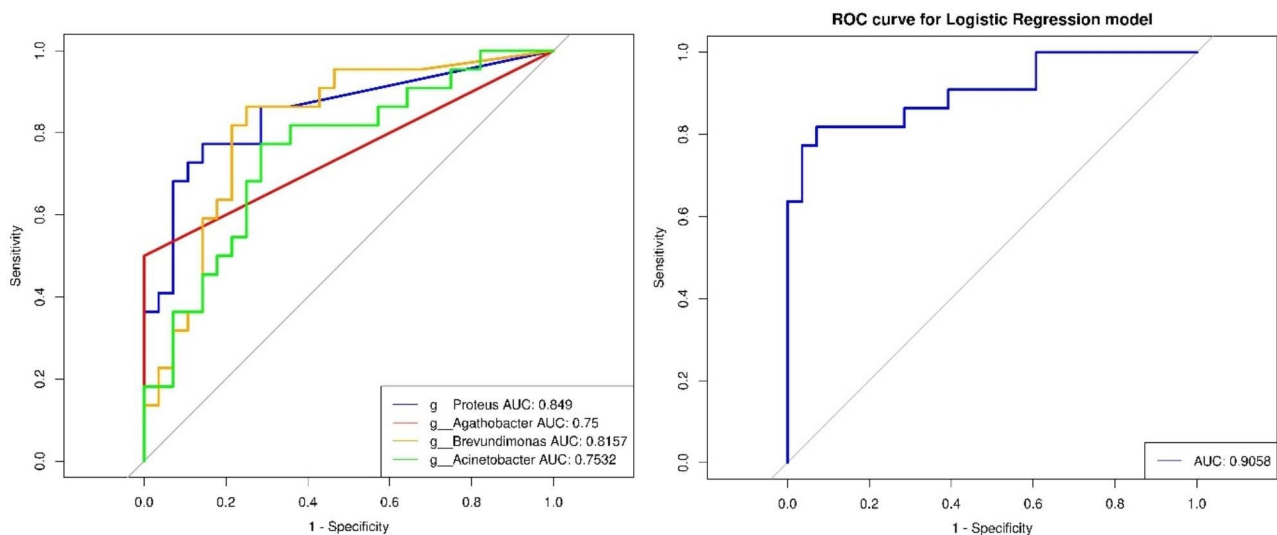
Fig. 6 The differential microbiota as diagnostic markers of the OK lens group. Receiver operating characteristic (ROC) curves for the differential microbiota alone or in combination are used to discriminate the OK lens wearers from the non-wearers. AUC, the area under the receiver-operating characteristic curve. L: the OK lens group, N: the non-wearer group
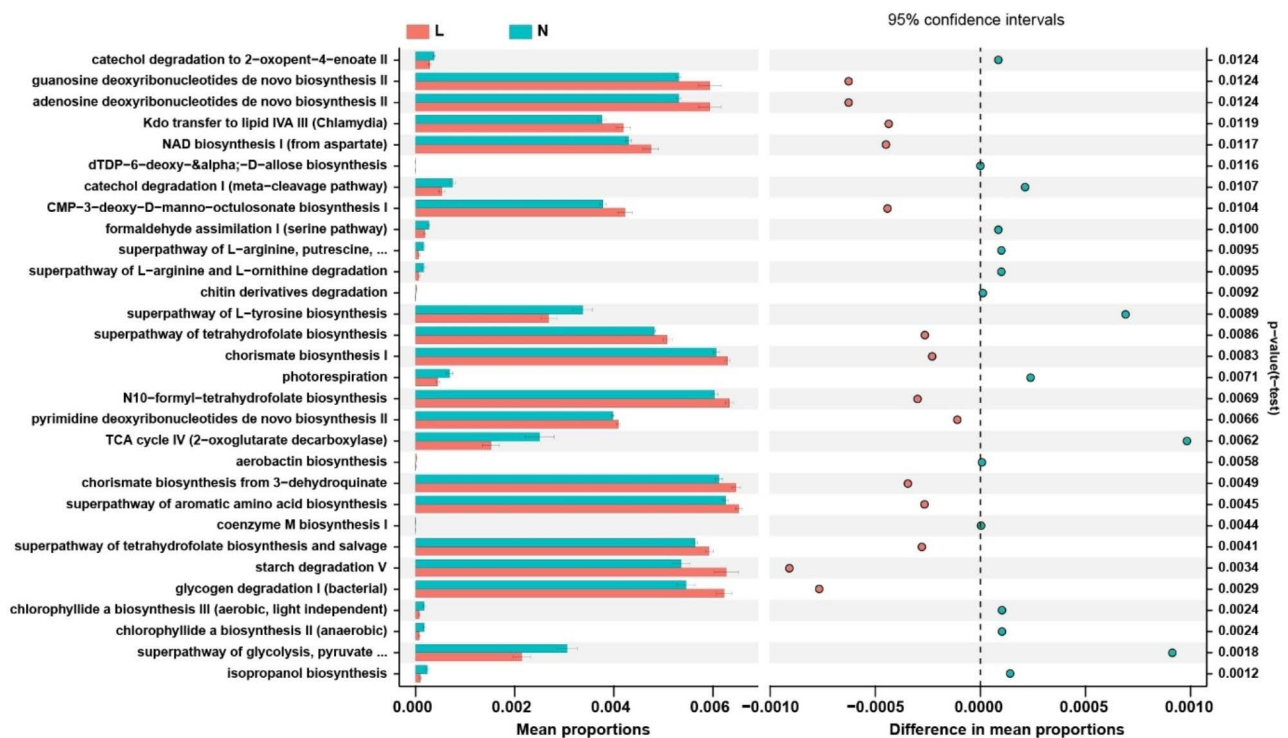
Fig. 7 PiCRUSt-based examination of the conjunctival microbiome of the OK lens wearers and the non-wearers. The different bacterial functions are evaluated between them based on the t-test. Comparisons between the groups for each KEGG functional category are shown by percentage

from a wider range of subjects at different time points are necessary. Third, since a large number of sequences remain unknown, it is difficult to identify strains to the species level using 16 S rDNA sequencing, which is needed to be improved. [63].

Conclusion

In conclusion, using 16 S rDNA gene sequencing, this study confirms that the relative abundance of bacterial taxa can change after OK lens wear in myopic children, affecting the physiological and immune status of the ocular surface. *Brevundimonas, Acinetobacter, Proteus*, and *Agathobacter* may be candidate biomarkers to distinguish between OK lens wearers and non-wearers. The findings are conducive to understanding the role of ocular surface microbiota in the ocular surface inflammation in pediatric OK lens wearers.

Abbreviations

Orthokeratology	OK
LDA	Linear Discriminant Analysis
LEfSe	Linear discriminant analysis effect size
PICRUSt	Phylogenetic investigation of communities by reconstruction of unobserved states
ROC	Receiver-operating characteristic
AUC	Area under the receiving-operating characteristic curves
UCVA	uncorrected visual acuity
OUT	Operational taxonomic unit
RDP	Ribosomal Database Project

Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate
Informed consent was obtained from legal guardians of the participants involved in this study who were less than 16 years of age after the nature and possible consequences of the study had been explained to them. The study had approval from Eye Hospital of Shandong First Medical University Research Ethics Committee (SDSYKYY20200713) and complied with the tenets of the Declaration of Helsinki. Written informed consent was obtained from legal guardians of each participant.

Consent for publication
Not applicable.

Commercial relationships disclosures
None.

Author details
[1]Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan, China
[2]Eye Hospital of Shandong First Medical University (Shandong Eye Hospital), 372 Jingsi Road, Jinan 250021, China
[3]State Key Laboratory Cultivation Base, Shandong Provincial Key Laboratory of Ophthalmology, Shandong Eye Institute, Qingdao, China
[4]Medical College, Qingdao University, Qingdao, China

References
1. Holden BA, Fricke TR, Wilson DA, Jong M, Naidoo KS, Sankaridurg P, Wong TY, Naduvilath TJ, Resnikoff S. Global prevalence of myopia and high myopia and temporal Trends from 2000 through 2050. Ophthalmology. 2016;123(5):1036–42.
2. Helen A, Swarbrick, Ahmed. Alharbi, Kathleen, Watt, Edward, Lum, Pauline: Myopia Control during Orthokeratology Lens wear in children using a Novel Study Design. Ophthalmology. 2015;122(3):620–30.
3. Gifford KL, Richdale K, Kang P, Aller TA, Sankaridurg P. IMI – Clinical Management Guidelines Report. Investig Ophthalmol Vis Sci. 2019;60(3):M184.
4. Pauline C, Sin-Wan C. Protective role of Orthokeratology in reducing risk of Rapid Axial Elongation: a reanalysis of Data from the ROMIO and TO-SEE studies. Investigative ophthalmology & visual science; 2017.
5. Pauline C, Sin-Wan C. Retardation of myopia in Orthokeratology (ROMIO) study: a 2-Year randomized clinical trial. Invest Opthalmology Visual Sci. 2012;53(11):7077–85.
6. Jsw A, Ac B, Pc C, Kg D, Lj E, Dj E, Sg E, Ming LF, Cl G, Nsl A. Global trends in myopia management attitudes and strategies in clinical practice – 2019 Update. Contact Lens and Anterior Eye. 2020;43(1):9–17.
7. Spillmann L. Stopping the rise of myopia in Asia. Graefes Arch Clin Exp Ophthalmol. 2020;258(5):943–59.
8. Dolgin E. THE MYOPIA BOOM. Nature 2015.
9. Stapleton F, Naduvilath T, Keay L, Radford C, Dart J, Edwards K, Carnt N, Minassian D, Holden B. Risk factors and causative organisms in microbial keratitis in daily disposable contact lens wear. PLoS ONE. 2017;12(8):e0181343.
10. Shin H, Price K, Albert L, Dodick J, Park L, Dominguez-Bello MG. Changes in the Eye Microbiota Associated with Contact Lens wearing. mBio. 2016;7(2):e00198.
11. Eguchi H, Hotta F, Kuwahara T, Imaohji H, Miyazaki C, Hirose M, Kusaka S, Fukuda M, Shimomura Y. Diagnostic Approach to Ocular Infections using various techniques from Conventional Culture to Next-Generation sequencing analysis. Cornea. 2017;36(Suppl 1):46–s52.
12. Chao C, Akileswaran L, Cooke Bailey JN, Willcox M, Van Gelder R, Lakkis C, Stapleton F, Richdale K. Potential role of ocular microbiome, host genotype, tear cytokines, and environmental factors in corneal infiltrative events in contact Lens Wearers. Investig Ophthalmol Vis Sci. 2018;59(15):5752–61.
13. Zada M, Cabrera-Aguas M, Branley M, Azar D, Watson S. Microbial keratitis associated with long-term orthokeratology. Clin Exp Ophthalmol. 2019;47(2):292–4.
14. Kam KW, Yung W, Li GKH, Chen LJ, Young AL. Infectious keratitis and orthokeratology lens use: a systematic review. Infection. 2017;45(6):727–35.
15. Obrubov AS, Slonimskii AY. [Contact lens-related keratitis and purulent corneal ulcers]. Vestn Oftalmol. 2018;134(4):17–24.
16. Hiraoka T, Sekine Y, Okamoto F, Mihashi T, Oshika T. Safety and efficacy following 10-years of overnight orthokeratology for myopia control. Ophthalmic Physiol Opt. 2018;38(3):281–9.
17. Lee YS, Tan HY, Yeh LK, Lin HC, Ma DH, Chen HC, Chen SY, Chen PY, Hsiao CH. Pediatric microbial keratitis in Taiwan: clinical and microbiological profiles, 1998–2002 versus 2008–2012. Am J Ophthalmol. 2014;157(5):1090–6.
18. Bullimore MA, Sinnott LT, Jones-Jordan LA. The risk of microbial keratitis with overnight corneal reshaping lenses. Optometry and Vision Science: Official Publication of the American Academy of Optometry. 2013;90(9):937–44.
19. Sankaridurg PR, Markoulli M, de la Jara PL, Harmis N, Varghese T, Willcox MD, Holden BA. Lid and conjunctival micro biota during contact lens wear in children. Optometry and Vision Science: Official Publication of the American Academy of Optometry. 2009;86(4):312–7.
20. Willcox M, Sharma S, Naduvilath TJ, Sankaridurg PR, Gopinathan U, Holden BA. External ocular surface and lens microbiota in contact lens wearers with corneal infiltrates during extended wear of hydrogel lenses. Eye Contact Lens. 2011;37(2):90–5.
21. Zhang H, Zhao F, Hutchinson DS, Sun W, Ajami NJ, Lai S, Wong MC, Petrosino JF, Fang J, Jiang J, et al. Conjunctival Microbiome Changes Associated with Soft Contact Lens and Orthokeratology Lens wearing. Investig Ophthalmol Vis Sci. 2017;58(1):128–36.
22. Garza A, Diaz G, Hamdan M, Shetty A, Hong BY, Cervantes J. Homeostasis and defense at the Surface of the Eye. The Conjunctival Microbiota. Curr Eye Res. 2021;46(1):1–6.
23. Aragona P, Baudouin C, Benitez Del Castillo JM, Messmer E, Barabino S, Merayo-Lloves J, Brignole-Baudouin F, Inferrera L, Rolando M, Mencucci R, et al. The ocular microbiome and microbiota and their effects on ocular surface pathophysiology and disorders. Surv Ophthalmol. 2021;66(6):907–25.
24. Iskeleli G, Bahar H, Eroglu E, Torun MM, Ozkan S. Microbial changes in conjunctival flora with 30-day continuous-wear silicone hydrogel contact lenses. Eye Contact Lens. 2005;31(3):124–6.
25. Graham JE, Moore JE, Jiru X, Moore JE, Goodall EA, Dooley JS, Hayes VE, Dartt DA, Downes CS, Moore TC. Ocular pathogen or commensal: a PCR-based study of surface bacterial flora in normal and dry eyes. Investig Ophthalmol Vis Sci. 2007;48(12):5616–23.
26. Fadrosh DW, Ma B, Gajer P, Sengamalay N, Ott S, Brotman RM, Ravel J. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. Microbiome. 2014;2(1):6.
27. Wang H, Shou Y, Zhu X, Xu Y, Shi L, Xiang S, Feng X, Han J. Stability of vitamin B12 with the protection of whey proteins and their effects on the gut microbiome. Food Chem. 2019;276:298–306.
28. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13(7):581–3.
29. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37(8):852–7.
30. Lozupone C, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol. 2005;71(12):8228–35.
31. Bennett PN, Parsons T, Ben-Moshe R, Neal M, Weinberg MK, Gilbert K, Ockerby C, Rawson H, Herbu C, Hutchinson AM. Intradialytic laughter yoga therapy for haemodialysis patients: a pre-post intervention feasibility study. BMC Complement Altern Med. 2015;15:176.
32. Keay L, Stapleton F, Schein O. Epidemiology of contact lens-related inflammation and microbial keratitis: a 20-year perspective. Eye Contact Lens. 2007;33(6 Pt 2):346–53. discussion 362 – 343.
33. Szczotka-Flynn LB, Pearlman E, Ghannoum M. Microbial contamination of contact lenses, lens care solutions, and their accessories: a literature review. Eye Contact Lens. 2010;36(2):116–29.
34. Chen KJ, Hou CH, Sun MH, Lai CC, Sun CC, Hsiao CH. Endophthalmitis caused by Acinetobacter baumannii: report of two cases. J Clin Microbiol. 2008;46(3):1148–50.
35. Willcox MD. Pseudomonas aeruginosa infection and inflammation during contact lens wear: a review. Optometry and Vision Science: Official Publication of the American Academy of Optometry. 2007;84(4):273–8.
36. Lai CC, Cheng A, Liu WL, Tan CK, Huang YT, Chung KP, Lee MR, Hsueh PR. Infections caused by unusual Methylobacterium species. J Clin Microbiol. 2011;49(9):3329–31.

37. Mattila JS, Holopainen J. [Keratitis associated with contact lens wear]. Duo-decim. 2013;129(18):1901–7.

38. Lyczak JB, Cannon CL, Pier GB. Establishment of Pseudomonas aerugi-nosa infection: lessons from a versatile opportunist. Microbes Infect. 2000;2(9):1051–60.

39. Dong Q, Brulc JM, Iovieno A, Bates B, Garoutte A, Miller D, Revanna KV, Gao X, Antonopoulos DA, Slepak VZ, et al. Diversity of bacteria at healthy human conjunctiva. Investig Ophthalmol Vis Sci. 2011;52(8):5408–13.

40. Lee SH, Oh DH, Jung JY, Kim JC, Jeon CO. Comparative ocular microbial com-munities in humans with and without blepharitis. Investig Ophthalmol Vis Sci. 2012;53(9):5585–93.

41. Lee CS, Hong B, Kasi SK, Aderman C, Talcott KE, Adam MK, Yue B, Akileswaran L, Nakamichi K, Wu Y, et al. Prognostic utility of whole-genome sequenc-ing and polymerase chain reaction tests of ocular fluids in Postprocedural Endophthalmitis. Am J Ophthalmol. 2020;217:325–34.

42. Ren Z, Liu Q, Li W, Wu X, Dong Y, Huang Y. Profiling of Diagnostic Information of and latent susceptibility to bacterial keratitis from the perspective of ocular bacterial microbiota. Front Cell Infect Microbiol. 2021;11:645907.

43. Ferreira CS, Figueira L, Moreira-Gonçalves N, Moreira R, Torrão L, Falcão-Reis F. Clinical and Microbiological Profile of Bacterial Microbial Keratitis in a portuguese Tertiary Referral Center-Where are we in 2015? Eye Contact Lens. 2018;44(1):15–20.

44. Willcox MD. Characterization of the normal microbiota of the ocular surface. Exp Eye Res. 2013;117:99–105.

45. Abayasekara LM, Perera J, Chandrasekharan V, Gnanam VS, Udunuwara NA, Liyanage DS, Bulathsinhala NE, Adikary S, Aluthmuhandiram JVS, Thanaseelan CS, et al. Detection of bacterial pathogens from clinical specimens using conventional microbial culture and 16S metagenomics: a comparative study. BMC Infect Dis. 2017;17(1):631.

46. Watters GA, Turnbull PR, Swift S, Petty A, Craig JP. Ocular surface microbiome in meibomian gland dysfunction. Clin Exp Ophthalmol. 2017;45(2):105–11.

47. Zhou Y, Holland MJ, Makalo P, Joof H, Roberts CH, Mabey DC, Bailey RL, Bur-ton MJ, Weinstock GM, Burr SE. The conjunctival microbiome in health and trachomatous disease: a case control study. Genome Med. 2014;6(11):99.

48. Ozkan J, Willcox M, Wemheuer B, Wilcsek G, Coroneo M, Thomas T. Biogeogra-phy of the human ocular microbiota. Ocul Surf. 2019;17(1):111–8.

49. Zhang SD, He JN, Niu TT, Chan CY, Ren CY, Liu SS, Qu Y, Chong KL, Wang HL, Tao J, et al. Bacteriological profile of ocular surface flora in meibomian gland dysfunction. Ocul Surf. 2017;15(2):242–7.

50. Ozkan J, Willcox MD. The ocular microbiome: Molecular Characterisation of a unique and low microbial environment. Curr Eye Res. 2019;44(7):685–94.

51. Salter SJ, Cox MJ, Turek EM, Calus ST, Cookson WO, Moffatt MF, Turner P, Parkhill J, Loman NJ, Walker AW. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biol. 2014;12:87.

52. Ham B, Hwang HB, Jung SH, Chang S, Kang KD, Kwon MJ. Distribution and diversity of Ocular Microbial Communities in Diabetic Patients compared with healthy subjects. Curr Eye Res. 2018;43(3):314–24.

53. Ozkan J, Nielsen S, Diez-Vives C, Coroneo M, Thomas T, Willcox M. Temporal Stability and Composition of the ocular surface Microbiome. Sci Rep. 2017;7(1):9880.

54. Wei C, Zhu M, Petroll WM, Robertson DM. Pseudomonas aeruginosa infec-tious keratitis in a high oxygen transmissible rigid contact lens rabbit model. Investig Ophthalmol Vis Sci. 2014;55(9):5890–9.

55. Ruch TR, Engel JN. Targeting the Mucosal Barrier: how pathogens modulate the Cellular Polarity Network. Cold Spring Harb Perspect Biol 2017, 9(6).

56. Ji X, Dong K, Pu J, Yang J, Zhang Z, Ning X, Ma Q, Kang Z, Xu J, Sun B. Comparison of the ocular surface microbiota between thyroid-associated ophthalmopathy patients and healthy subjects. Front Cell Infect Microbiol. 2022;12:914749.

57. Ayehubizu Z, Mulu W, Biadglegne F. Common bacterial causes of external ocular infections, associated risk factors and antibiotic resistance among patients at ophthalmology unit of Felege Hiwot Referral Hospital, Northwest Ethiopia: a cross-sectional study. J Ophthalmic Inflamm Infect. 2021;11(1):7.

58. An N, Wang C, Dou X, Liu X, Wu J, Cheng Y. Comparison of 16S rDNA amplicon sequencing with the culture method for diagnosing causative pathogens in bacterial corneal infections. Transl Vis Sci Technol. 2022;11(2):29.

59. Wang YJ, Li SC, Lin WC, Huang FC. Intracellular microbiome profiling of the Acanthamoeba Clinical isolates from Lens Associated Keratitis. Pathogens 2021, 10(3).

60. Yun SW, Son YH, Lee DY, Shin YJ, Han MJ, Kim DH. Lactobacillus plantarum and Bifidobacterium bifidum alleviate dry eye in mice with exorbital lacrimal gland excision by modulating gut inflammation and microbiota. Food Funct. 2021;12(6):2489–97.

61. Ladage PM, Yamamoto K, Ren DH, Li L, Jester JV, Petroll WM, Bergmanson JP, Cavanagh HD. Proliferation rate of rabbit corneal epithelium during overnight rigid contact lens wear. Investig Ophthalmol Vis Sci. 2001;42(12):2804–12.

62. Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. Science. 2009;326(5960):1694–7.

63. Franzosa EA, Huang K, Meadow JF, Gevers D, Lemon KP, Bohannan BJ, Hut-tenhower C. Identifying personal microbiomes using metagenomic codes. Proc Natl Acad Sci U S A. 2015;112(22):E2930–2938.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.