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Investigation of effects of orthokeratology and povidone iodine disinfecting solution on the conjunctival microbiome using MALDI-TOF mass spectrometry

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ABSTRACT

Purpose: To determine organisms present in the conjunctiva of children before and after orthokeratology lens wear, using MALDI-TOF mass spectrometry.

Methods: Conjunctival samples were collected from children aged 8–12 years (inclusive) at baseline and on three occasions over the first six months of orthokeratology treatment. All lenses were disinfected using the povidone iodine-based solution every day after use. Specimens were cultured and all isolated colonies were identified using MALDI-TOF mass spectrometry. Numbers of organisms and diversity were compared over the study period and the presence of any ocular pathogens noted and participants informed, where appropriate, to enhance their compliance with lens care routine.

Results: Organisms isolated from 76 children were generally similar to other studies employing culture methods. However, MALDI-TOF results yielded a wider range of species of *micrococci* and *corynebacteria*, as well as a few less frequently reported organisms. Only one culture yielded fungi. Ocular pathogens were only isolated from 9 subjects (4 before lens wear and 5 after lens wear), each on one occasion only. Diversity and numbers of organisms fell slowly over the period of the study, but the changes were not significant.

Conclusions: Lens wear did not affect the overall content of the ocular microbiome, but the diversity was somewhat reduced. The incidence of ocular pathogens was low, suggesting that risk of ocular infection was not substantially increased by orthokeratology treatment using a povidone-iodine disinfecting solution.

1. Introduction

The human microbiome project showed that many areas of the body harboured an extremely diverse community of microorganisms.[1] The ocular microbiome was not included in the original project, as it was considered to be less diverse than other colonized sites.[1] However, research using 16S rRNA techniques revealed a wider range of organisms than culture techniques.[2] As the eye is exposed, organisms can contaminate the surface from adjacent skin, fingers, and the environment,[3] but defence mechanisms, including blinking, antibacterial substances in the tears, and tightly packed epithelial cells,[4] help reduce contamination.

Whilst traditional culture methods reported few organisms in the conjunctiva, 16S rRNA revealed a wider range of organisms.[5,6] A major limitation of nucleic acid detection methods is their inability to distinguish viable from non-viable organisms. Additionally, as the conjunctiva is exposed, air-borne organisms may contaminate the surface. Also, it was determined that many isolates, supposedly from the sampled sites, were actually contaminants from swabs.[7] Incorporation of negative controls, now a standard requirement of 16S rRNA studies, reduced the number of genera reported in the ocular microbiome,[8] with those commonly detected being largely similar to those previously reported by culture.[2]

Culture-based methodology may not lead to accurate identification if only routine biochemical testing is employed. The introduction of matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry greatly enhanced identification, especially of less frequently encountered species.[9,10] This technique has been widely adopted in diagnostic laboratories and frequently used in research. However, its use to identify organisms in the ocular microbiome has not been previously reported.

Contact lens use can affect the conjunctival microbiome due to introduction of organisms originating from the fingers, tap water, or lens storage cases and other accessories.[11–13] As overnight wear of orthokeratology

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(ortho-k) lenses, primarily used for myopia control in children,[14,15] increases the risk of ocular infection due to reduction in tear exchange, debris removal, and oxygen tension, particular care is required for lens cleaning and disinfection. Transfer of residual disinfecting solution into the eye may affect organisms present in the conjunctiva,[16] as lens wear appeared to decrease incidence of sporadically detected genera using 16S rRNA.[7,8] In addition, disinfecting solutions, particularly those using quaternary ammonium compounds or biguanides, may select for organisms carrying disinfectant-resistant genes.[17]

Recently, disinfecting solutions incorporating povidone iodine as the active agent have been introduced, which abnegates this problem. Although their effectiveness in vitro against both planktonic and biofilm organisms has been demonstrated,[18,19] effects on the ocular microbiome remain unknown. The current study, which forms part of a larger investigation to evaluate a povidone iodine-based disinfection care system for ortho-k lenses,[18–20] aimed to investigate changes in the ocular microbiome. A culture-based approach was adopted, supplemented by the use of MALDI-TOF, to enhance correct identification at species level, which was not possible using routine laboratory test.

2. Materials and methods

Eighty healthy myopic children undergoing ortho-k treatment, aged 6–10 years, were recruited. Informed consent and assent were obtained from the parents and the subjects, respectively, before participation of the study. This study followed the Tenets of the Declaration of Helsinki revised in 2013, was approved by the Departmental Research Committee of the School of Optometry (HSEARS20170430002), and was registered at ClinicalTrials.gov (NCT03193255). Subjects with ocular pathogens identified were informed, where appropriate, to enhance their compliance with lens care routine and reduce the risk of ocular infection.

Subjects were prescribed with the Katt BE free ortho-k lenses (Precision Technology Services, Vancouver, B.C., Canada) made from HDS 100 material (paflufocon D (fluorosilicone acrylate), Dk 100 units), and were instructed on correct lens wear and care. All lens procedures, including insertion, removal, and cleaning were performed by parents. Before use, lenses were rinsed with saline (cleadew® rinsing solution, Ophtecs, Japan) and cushioned with a drop of unpreserved artificial tears (Teare®, Ophtecs) on the back surface of the lenses before insertion. On removal, lenses were disinfected with povidone iodine-based solution (cleadewGP®, Ophtecs). All solutions and accessories were replaced monthly. All subjects were randomly assigned into four cleaning methods before disinfection, with respect to rubbing the lenses before disinfection, use of a separate daily cleaner, and bi-weekly protein removal.

Samples from the left conjunctiva were collected on two occasions before and 1-, 3-, and 6-month after commencement of lens wear by a trained examiner wearing fresh gloves for each subject. For the post-ortho-k wear visit, all subjects were required to wear their lenses the night before the scheduled visit or the appointment would be postponed to the following week. Subjects were asked to look up and the lower eyelid was gently pulled down to expose the palpebral conjunctiva and avoid touching the cornea or the eyelashes during sampling. A Remel BactiSwabs (Thermo-Fisher Scientific, Massachusetts, US), moistened with sterile phosphate buffered saline, was gently rolled across the conjunctiva, placed in transport media refrigerated at 0–4 °C, and transported to the laboratory for culture, within 24 h.

2.1. Microbial assessment

Swabs, cultured overnight in freshly prepared brain heart infusion broth after vortexing to release organisms, were sub-cultured onto two blood agar plates, one incubated at 37 °C aerobically and the second, which had been pre-reduced, anaerobically, and a chocolate agar plate incubated at 37 °C in 5% CO_2. Plates were examined at 24 h and 72 h. Organisms from each colony type present on either agar were identified using MALDI-TOF spectrometry (Bruker Microflex LT/SH system; Bruker Corp, Billerica, MA), and the Bruker Biotyper database (version 4.1.80), following the manufacturer's instructions.

2.2. Statistical analysis

All statistical analysis were performed using SPSS software version 26 (IBM corporation, NY, USA). As no differences were observed among the groups using various cleaning regimes with respect to frequency and organisms,[20] results were pooled for further analysis. Change in colonization with time was evaluated using the Friedman test. Binary logistic regression was used to evaluate the effect of age, gender, use of solutions, and baseline colonization on colonization after lens wear. Further analysis on association of frequency of colonization after lens wear with factors determined in the logistic regression was performed using the Chi-square test.

3. Results

Of the initial 80 subjects, analysis was performed on 76 (mean age: 9.1 ± 1.1 years; 58% female), as baseline samples of four subjects were discarded due to a delay in delivery to the laboratory. Fig. 1 shows the conjunctival colonization of the subjects over time, of whom 42 had positive colonization on at least one occasion. Although 18 subjects had positive cultures only after ortho-k, overall frequency of colonization reduced over the period of lens wear. Of the 24 subjects (32%) colonized at baseline, only 15 had one or more positive cultures after commencing lens wear, most commonly at 1-month (9/24), with only five and six subjects positive at 3- and 6-month, respectively. Only one subject yielded positive cultures on all occasions. Of the 52 subjects not colonized at baseline, seven were colonized at 1-, nine at 3-, and five at 6-month visits. Although there was a downward trend for colonization, this did not reach significance (Friedman test, $P = 0.079$). Binary logistic regression showed that colonization after lens wear was associated with baseline colonization (OR 3.69; 95% CI: 1.3–10.8), but not with age or gender ($P > 0.672$). Those with baseline colonization were also likely to be associated with a greater number of colonized visits after lens wear (chi-square test, $p = 0.018$).

As expected, a fairly limited number of species were identified from conjunctival colonization cultures. Most cultures also only yielded relatively small number of colonies. Only a minority of cultures (9/288, 3.1%) yielded ocular pathogens, with four isolations each of *Staphylococcus aureus* and *Streptococcus pneumoniae*, and one of *Acinetobacter junii*. These isolates were from nine different subjects, each on a single occasion: four occurring before lens wear and five after lens wear. The presence of opportunistic and non-pathogenic organisms did not increase the risk of ocular pathogens before or after lens wear (chi-squared tests, $P = 0.363–0.695$). No obligate anaerobic species were isolated despite extended incubation.

The most common isolates were *Micrococcus luteus* (28%) and *S. epidermidis* (13%) (Table 1). Other species of micrococci were also identified, including *S. equorum, S. warneri*, and *Kocuria marina*. Several species of coryneform bacteria were present with four isolations of *Corynebacterium macginleyi* and one each of *C. accolens* and *Microbacterium paraoxydans*. Cultures from four subjects yielded *Bacillus* species, with three isolates of *B. cereus* and one of *B. subtilis*. Isolation of gram-negative organisms was rare, being present in cultures of only four subjects. Other than the *Acinetobacter* mentioned above, there was one isolation each of *Moraxella osloensis, Moraxella catarrhalis*, and *Stenotrophomonas maltophilia*. There was only one fungal isolate, *Aspergillus terreus*, from a subject after 3-month of lens wear. Overall, a total of 17 species were cultured, but the variety reduced together with the percentage of positive colonizations over time (Table 1). The four isolations of *S. aureus* were all from different subjects: one at baseline; one after 1-month of lens wear; and two after 6-month.

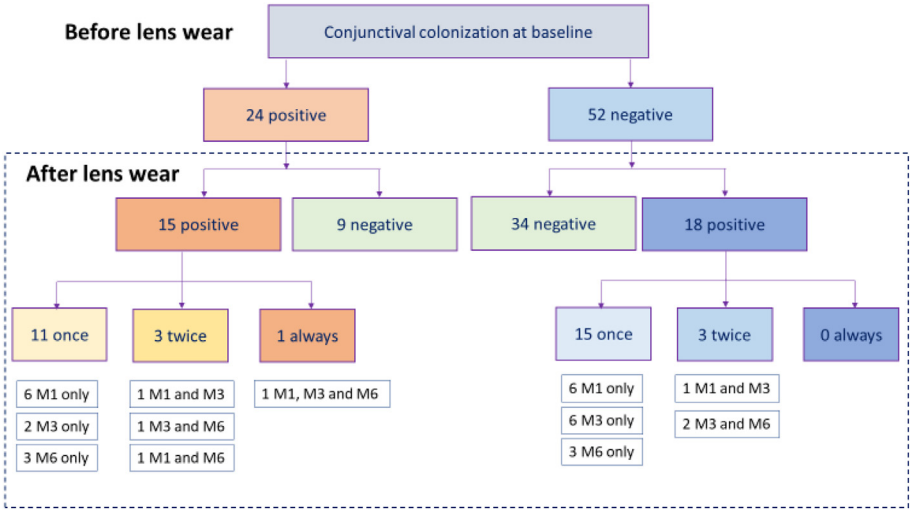

Fig. 1. Presence of organisms in the conjunctiva of subjects before and after wearing orthokeratology lenses at the three post lens wear visits (M1: 1-month; M3: 3-month; M6: 6-month).

Table 1
Micro-organisms isolated from the lower conjunctiva before and after orthokeratology lens wear.

	Number of subjects	Baseline	1-month	3-month	6-month
Number of valid samples	–	76	71	73	68
Positive colonization	–	24 (32%)	16 (23%)	14 (19%)	11 (16%)
Pathogenic					
Staphylococcus aureus	4	1	1	0	2
Streptococcus pneumoniae	4	2	0	2	0
Acinetobacter	1	1	0	0	0
Opportunistic					
Staphylococcus epidermidis	10	4	0	2	4
Corynebacterium macginleyi	4	1	2	1	0
Staphylococcus warneri	4	0	2	2	0
Bacillus cereus	3	1	0	0	2
Staphylococcus equorum	2	0	2	0	0
Aspergillus terreus	1	0	0	1	0
Bacillus subtilis	1	0	1	0	0
Corynebacterium accolens	1	0	1	0	0
Moraxella osloensis	1	0	0	1	0
Moraxella catarrhalis	1	1	0	0	0
Stenotrophomonas maltophilia	1	0	1	0	0
Non-Pathogenic					
Micrococcus luteus	21	15	7	5	3
Kocuria marina	1	0	0	1	0
Microbacterium paraoxydans	1	1	0	0	0
Number of species	17	9	8	8	4

4. Discussion

This study adopted a novel approach to identify organisms present in the conjunctivas of children commencing ortho-k treatment. Unlike most previous studies of the ocular microbiome, the use of MALDI-TOF allowed for accurate speciation, assessment of frequency of isolation over a sustained period, and a relatively large sample size. It was not possible to sample all subjects on every occasion, but about 85%–95% of subjects attended each sampling session. A large number of conjunctival samples yielded no positive culture, with less than a third positive at baseline. Similar lack of growth in a high percentage of samples has been reported in a review of normal ocular microbiota.[21] Overall frequency of colonization reduced consistently over the 6-month period of monitoring, suggesting use of povidone iodine reduces colonization. Studies have shown that contact lens wearers have reduced levels of colonization, possibly due to residual disinfecting solution transferred to the eye on lens insertion.[12] Although RGP material would not absorb disinfecting solution,[22] any deposits on the lens surface, not removed during cleaning, may take up solutions during soaking, which would leach out into the eye during lens wear. Such deposits can increase discomfort of lens wear and possibly ocular trauma. Thus, lens wearers are encouraged to rub their lenses during cleaning. Zhang et al.[8] used 16s rRNA to investigate conjunctival organisms of wearers of various types of contact lenses, including ortho-k lenses, and reported changes in the microbiome. They reported that organisms in ortho-k lens wearers differed from those of soft contact lens wearers, with fewer *Bacillus*, *Tatumella*, and *Lactobacillus*. However, their study only included 11 ortho-k wearers of average age of 12 years. Only one sample was collected from each participant and wearing time of ortho-k lenses ranged from two months to four years. They compared the microbiota with those of adults aged 20–30, who were either spectacle or soft contact lens wearers. The current study, using a considerably larger sample size, with baseline samples from 76 subjects and all three post-wear samples from 87% of the subjects, allowing a better assessment of changes due to ortho-k lens wear.

Although somewhat limited compared to the numbers of organisms identified using 16s rRNA techniques, the range of organisms isolated was wider than usual reports from conjunctival culture. This may be partly due to misclassification of all *micrococci* as *S. epidermidis*, as speciation of coagulase negative species is rarely performed in medical laboratories if isolated from a healthy patient. Interestingly, the most frequently isolation species in the current study was *M. luteus*, which is less commonly reported than *S. epidermidis* in reports of conjunctival culture, probably being labelled as coagulase negative staphylococci. An increasing proportion of *micrococci* in the conjunctiva of contact lens wearers has previously been reported in 16s rRNA-based studies.[12,23] This may be due to a transfer of skin flora, possibly from the fingers into the eye. However, Shin et al.[7] found no difference in proportions of *micrococci* associated with lens wear.

Use of MALDI-TOF has considerably improved identification of gram-positive cocci, which is difficult if standard biochemical testing is employed, allowing correct speciation of recently defined genera, such as *Kocuria*.[24] Identification using advanced systems, such as MALDI-TOF or 16s rRNA, have increased the reporting of unusual species in ocular infections.[25] These rare isolates are associated with non-ocular habitats, for instance, *M. osloensis* is a pathogen of a nematode that infects slugs.[26] With the exception of *K. marina* and *M. paraoxydans*, all isolates identified in this study have been occasionally reported in ocular infections.[25] However, it is recognized that MALDI-TOF identification is limited by the extent of the database. This can lead to closely related species being misidentified. As such, the database needs to be continuously and regularly updated.[27]

It was encouraging that conventional ocular pathogens were rarely isolated. Four subjects yielded *S. aureus* and another four *S. pneumoniae*, but each on one occasion only *S. aureus* is not usually considered to be part of the ocular microbiome, but to be transferred into the eye via contaminated fingers or lenses, as it is a colonizer of the nasal cavity.[12] As nasal swabs were not collected in the current study, it was not possible to determine if these subjects were nasally colonized.

It was notable that there were few isolates of gram-negative species, with the exception of one isolate each of *A. junii* and *S. maltophilia*. In a review of studies using routine culture and identification methods, only a few reported the presence of gram-negative organisms.[21] The few instances of substantial contamination with potential gram-negative ocular pathogens were thought to be associated with tap water contact.[28] In recent years, there has been increased emphasis on the importance of avoiding contact of lenses and accessories with tap water.[29] The subjects in the current study were recruited from randomized trials involving ortho-k lens wear and would have received frequent reminders about avoidance of tap water. This could explain the paucity of gram-negative isolates. It was previously reported that female lens wearers had more gram-negative organisms than males,[6] but no differences were found in the current study.

It was notable that no anaerobic species were isolated. This may be attributable to delay in culture as samples could not always be transferred to the laboratory within 3 h. Several authors have reported the presence of *Propionibacter acnes*, which is part of the normal skin microbiota, in normal eyes,[8,30] including those of children.[31] This organism is an occasional cause of ocular infections.[32] The external ocular environment is not favourable to anaerobic colonization and numbers of species isolated are small.[8,30] Two studies have shown no differences in diversity or isolations of anaerobes between contact lens and non-lens wearers.[7,8]

5. Conclusions

This study, involving a large sample of children wearing ortho-k lenses, has shown that there was no increase in diversity of microorganisms colonizing the lower conjunctiva compared to baseline levels. The results indicated that the number of species present was generally decreased during the period of wear, which may be associated with use of an effective disinfecting contact lens solution. Use of MALDI-TOF allowed for accurate speciation of *micrococci* and *corynebacteria*, which are not usually fully identified by traditional culture. The absence of ocular pathogens may be associated with the use of povidone iodine-based disinfecting solution. Further studies to compare effects of different disinfecting solutions are warranted.

Study Approval

The authors confirm that any aspect of the work covered in this manuscript that involved human patients or animals was conducted with the ethical approval of all relevant bodies and the study was performed in accordance with the Declaration of Helsinki , and the protocol was approved by the Ethics Committee of the Departmental Research Committee of the School of Optometry (HSEARS20170430002) and registered at ClinicalTrials.gov (NCT03193255).

Informed consent and assent were obtained from both the parents and the children before participation of the study.

Author Contributions

The authors confirm contribution to the paper as follows: Conception and design of study: PC, MB; Data collection: SWC; Analysis and interpretation of results: SWC, MB, PC; Drafting the manuscript: PC, MB; All authors reviewed the results and approved the final version of the manuscript.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Turnbaugh PJ, Ley RE, Hamady M, et al. The Human Microbiome Project: exploring the microbial part of ourselves in a changing world. *Nature*. 2007;449:804–810. https://doi.org/10.1038/nature06244.
2. Ozkan J, Willcox MD. The ocular microbiome: molecular characterisation of a unique and low microbial environment. *Curr Eye Res*. 2019;44:685–694. https://doi.org/10.1080/02713683.2019.1570526.
3. Costello EK, Lauber CL, Hamady M, et al. Bacterial community variation in human body habitats across space and time. *Science*. 2009;326:1694–1697. https://doi.org/10.1126/science.1177486.
4. Sack RA, Nunes I, Beaton A, et al. Host-defence mechanism of the ocular surfaces. *Biosci Rep*. 2001;21:463–480. https://doi.org/10.1023/a:1017943826684.
5. Dong Q, Brulc JM, Iovieno A, et al. Diversity of bacteria at healthy human conjunctiva. *Invest Ophthalmol Vis Sci*. 2011;52:5408–5413. https://doi.org/10.1167/iovs.10-6939.
6. Lee SH, Oh DH, Jung JY, et al. Comparative ocular microbial communities in humans with and without blepharitis. *Invest Ophthalmol Vis Sci*. 2012;53:5585–5593. https://doi.org/10.1167/iovs.12-9922.
7. Shin H, Price K, Albert L, et al. Changes in the eye microbiota associated with contact lens wearing. *mBio*. 2016;7(2), e00198. https://doi.org/10.1128/mBio.00198-16.

8. Zhang H, Zhao F, Hutchinson DS, et al. Conjunctival microbiome changes associated with soft contact lens and orthokeratology lens wearing. *Invest Ophthalmol Vis Sci*. 2017;58:128–136. https://doi.org/10.1167/iovs.16-20231.
9. van Belkum A, Chatellier S, Girard V, et al. Progress in proteomics for clinical microbiology: MALDI-TOF MS for microbial species identification and more. *Expert Rev Proteomics*. 2015;12:595–605. https://doi.org/10.1586/14789450.2015.1091731.
10. Kostrzewa M, Nagy E, Schröttner P, et al. How MALDI-TOF mass spectrometry can aid the diagnosis of hard-to-identify pathogenic bacteria - the rare and the unknown. *Expert Rev Mol Diagn*. 2019;19:667–682. https://doi.org/10.1080/14737159.2019.1643238.
11. Wu YT, Willcox M, Zhu H, et al. Contact lens hygiene compliance and lens case contamination: a review. *Contact Lens Anterior Eye*. 2015;38:307–316. https://doi.org/10.1016/j.clae.2015.04.007.
12. Cheung SW, Boost M, Shi GS, et al. Microbial contamination of periorbital tissues and accessories of children. *Optom Vis Sci*. 2016;93:612–618. https://doi.org/10.1097/OPX.0000000000000843.
13. Fonn D, Jones J. Hand hygiene is linked to microbial keratitis and corneal inflammatory events. *Contact Lens Anterior Eye*. 2019;42:132–135. https://doi.org/10.1016/j.clae.2018.10.022.
14. Cho P, Cheung SW. Retardation of myopia in Orthokeratology (ROMIO) study: a 2-year randomized clinical trial. *Invest Ophthalmol Vis Sci*. 2013;53:7077–7085. https://doi.org/10.1167/iovs.12-10565.
15. Santodomingo-Rubido J, Villa-Collar C, Gilmartin B, et al. Myopia control with orthokeratology contact lenses in Spain: refractive and biometric changes. *Invest Ophthalmol Vis Sci*. 2012;53:5060–5065. https://doi.org/10.1167/iovs.11-8005.
16. Boost M, Cho P, Wang Z. Disturbing the balance: effect of contact lens use on the ocular proteome and microbiome. *Clin Exp Optom*. 2017;100:459–472. https://doi.org/10.1111/cxo.12582.
17. Shi GS, Boost MV, Cho P. Prevalence of antiseptic resistance genes increases in staphylococcal isolates from orthokeratology lens wearers over initial six-month period of use. *Eur J Clin Microbiol Infect Dis*. 2016;35:955–962. https://doi.org/10.1007/s10096-016-2622-z.
18. Cho P, Reyes S, Boost MV. Microbiocidal characterization of a novel povidone-iodine based rigid contact lens disinfecting solution. *Contact Lens Anterior Eye*. 2018;41:542–546. https://doi.org/10.1016/j.clae.2018.08.001.
19. Cho P, Boost MV. Evaluation of prevention and disruption of biofilm in contact lens cases. *Ophthalmic Physiol Opt*. 2019;39:337–349. https://doi.org/10.1111/opo.12635.
20. Cheung SW, Boost MV, Cho P. Effect of povidone iodine contact lens disinfecting solution on orthokeratology lens and lens case contamination and organisms in the microbiome of the conjunctiva. *Contact Lens Anterior Eye*. 2021;44:101412. https://doi.org/10.1016/j.clae.2021.01.007.
21. Willcox MDP. Characterization of the normal microbiota of the ocular surface. *Exp Eye Res*. 2013;117:99–105. https://doi.org/10.1016/j.exer.2013.06.003.
22. Phillips AJ. 9 - rigid Gas permeable corneal and corneoscleral lens fitting. In: Phillips AJ, Speedwell L, eds. *Contact Lenses*. sixth ed. London: Elsevier; 2019:175–206.
23. Iskeleli G, Bahar H, Eroglu E, et al. Microbial changes in conjunctival flora with 30-day continuous-wear silicone hydrogel contact lenses. *Eye Contact Lens*. 2005;31:124–126. https://doi.org/10.1097/01.icl.0000141923.63458.df.
24. Florio W, Tavanti A, Barnini S, et al. Recent advances and ongoing challenges in the diagnosis of microbial infections by MALDI-TOF mass spectrometry. *Front Microbiol*. 2018;9:1097. https://doi.org/10.3389/fmicb.2018.01097.
25. Sahay P, Goel S, Nagpal R, et al. Infectious keratitis caused by rare and emerging micro-organisms. *Curr Eye Res*. 2020;45:761–773. https://doi.org/10.1080/02713683.2019.1708407.
26. Tan L, Grewal PS. Pathogenicity of Moraxella osloensis, a bacterium associated with the nematode Phasmarhabditis hermaphrodita, to the slug Deroceras reticulatum. *Appl Environ Microbiol*. 2001;67:5010–5016. https://doi.org/10.1128/AEM.67.11.5010-5016.2001.
27. Rychert J. Benefits and limitations of MALDI-TOF mass spectrometry for the identification of microorganisms. *J Infect*. 2019;2(4):1–5. https://doi.org/10.29245/2689-9981/2019/4.1142.
28. Willcox MD, Power KN, Stapleton F, et al. Potential sources of bacteria that are isolated from contact lenses during wear. *Optom Vis Sci*. 1997;74:1030–1038. https://doi.org/10.1097/00006324-199712000-00025.
29. Arshad M, Carnt N, Tan J, et al. Water exposure and the risk of contact lens-related disease. *Cornea*. 2019;38:791–797. https://doi.org/10.1097/ICO.0000000000001898.
30. Zhou Y, Holland MJ, Makalo P, et al. The conjunctival microbiome in health and trachomatous disease: a case control study. *Genome Med*. 2014;6:99.
31. Cavuoto KM, Banerjee S, Miller D, et al. Composition and comparison of the ocular surface microbiome in infants and older children. *Transl Vis Sci Technol*. 2018;7(6):16. https://doi.org/10.1167/tvst.7.6.16, 10.1186/s13073-014-0099-x.
32. Brook I. Ocular infections due to anaerobic bacteria in children. *J Pediatr Ophthalmol Strabismus*. 2008;45:78–84. https://doi.org/10.3928/01913913-20080301-02.