Next-Generation Sequencing of the Ocular Surface Microbiome

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Eye & Contact Lens

Next Generation Sequencing of the Ocular Surface Microbiome: in Health, Contact Lens Wear, Diabetes, Trachoma and Dry Eye

--Manuscript Draft--

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Abstract:	Objectives To assess publications examining the occurrence, composition and clinical significance of a microbiome at the ocular surface.
	Methods MEDLINE, EMBASE and Google Scholar were searched. Reference lists of included articles were also searched for relevant citations. All publications up to 1 st June 2019 were analysed.
	Results Eleven articles and 1 abstract were included, analysing 661 patients. Articles generally report bacteria to genus level. The presence of DNA associated with diverse bacterial species was reported including pathogenic species such as <i>Pseudomonas</i> and <i>Neisseria</i> species. Bacterial DNA that make up the microbiome in other parts of the body such as <i>Acinetobacter</i> , <i>Actinomyces</i> , <i>Aquabacterium</i> , <i>Bradyrhizobium</i> , <i>Corynebacterium</i> , <i>Sphigomonas</i> , <i>Staphylococcus</i> and <i>Streptococcus</i> were found. The putative ocular microbiome is consistent between right and left eye and is affected by contact lens use (higher <i>Pseudomonas</i> levels) and blepharitis (higher <i>Staphylococcus</i> levels).

Conclusions

There is significant likelihood that there is an ocular surface microbiome, with *Acinetobacter* , *Corynebacterium* , *Propionibacterium* , *Staphylococcus* and *Streptococcus* detected in at least 7/11 studies. However, further investigation attempting to control for environmental and methodological contaminants (*Aquabacterium* and *Bradyrhizobium* are commonly identified as a contaminate in DNA extraction kits) is required. Bacteria capable of causing sight threatening infection such as *Propionibacterium* , *Staphylococcus* and *Streptococcus* may reside on a healthy ocular surface. With greater understanding, we can establish if elements of the ocular surface microbiome are harmful or protective (despite their small quantities); furthermore, new therapeutic agents can be identified to treat and prevent ocular surface infection and inflammation.

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Editor
Eye & Contact Lens

14th October 2019

Dear Editorial Board

I am pleased to submit a Review Article entitled "The Ocular Surface Microbiome: in Sickness and in Health". We believe this study summarises the literature and identifies methodological gaps that need filling to help bring the area closer to clinical practice.

In this manuscript, we show that the ocular surface microbiome exists and comprises of many bacteria previously thought to be pathogenic only.

We believe that this manuscript is appropriate for publication by Eye & Contact Lens because it represents a comprehensive review of literature in a topical area that is applicable to the readership. This manuscript is an original review, has not been published and is not under consideration for publication elsewhere. We have no conflicts of interest to disclose.

Thank you for your consideration.

Yours Sincerely,

Dr Arthur Okonkwo MRes MB BS PgCert

Line 63: Pflugfelder has shown that the tear film is not 3 distinct layers, but rather a milieu of components. Please eliminate the words "(from superficial to deep)".	This has been amended, Line 66
Line 179: add "with next generation sequencing" to the end of the sentence for clarity.	This has been added, Lines 195-196
Line 230: "(although what aspects of this were not reported)" is confusing. Please clarify.	This has been clarified, Line 247
Line 262: did the study in trachoma patients also look at the microbiome after treatment?	This has been clarified, patients with clinical signs of trachoma but no active infection were included, Lines 275-277
However, the authors failed to acknowledge some publications. A typical examples is de Paiva 2016, which evaluated both ocular, oral and tongue microbiome (in healthy and in SS patients) but it is not cited related to the ocular microbiome section. Work of other scientists in the ocular microbiome were not properly cited either.	<p>The search/inclusion/exclusion criteria used has been better explained. Lines 153-164</p> <p>The reference below uses a mouse model to investigate the gut microbiome in sjogrens syndrome which is beyond the scope of the review.</p> <p>de Paiva CS, Jones DB, Stern ME, et al. Altered Mucosal Microbiome Diversity and Disease Severity in Sjögren Syndrome. Sci Rep. 2016;6:23561.</p>
The title remains misleading, since the bulk of the work relates to contact-lenses induced alterations in the microbiome. One could argue that contact lenses are an intermediate step between health and sickness, as many effects are induced by their presence.	The title of the manuscript has been changed to specifically reflect the content of the article. Lines 1-5

Next Generation Sequencing of the Ocular Surface Microbiome: in Health, Contact Lens Wear, Diabetes, Trachoma and Dry Eye

Defining the Ocular Surface Microbiome by Next Generation of Healthy Eyes, Contact
Lens Wearers, Diabetics, those with Previous Trachoma and those with Dry Eye: A
Literature Review

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declare.

21 Abstract

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34 *Staphylococcus* and *Streptococcus* were found. The putative ocular microbiome is consistent
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36 and blepharitis (higher *Staphylococcus* levels).

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38 There is significant likelihood that there is at least a transitory ocular surface microbiome,
39 with *Acinetobacter*, *Corynebacterium*, *Propionibacterium*, *Staphylococcus* and *Streptococcus*
40 detected in at least 7/11 studies. However, further investigation attempting to control for
41 environmental and methodological contaminants (*Aquabacterium* and *Bradyrhizobium* are
42 commonly identified as a contaminate in DNA extraction kits) is required. Bacteria capable of
43 causing sight threatening infection such as *Propionibacterium*, *Staphylococcus* and
44 *Streptococcus* may reside on a healthy ocular surface. With greater understanding, we can

45 establish if elements of the ocular surface microbiome are harmful or protective (despite their
46 small quantities); furthermore, new therapeutic agents can be identified to treat and prevent
47 ocular surface infection and inflammation.

48 Keywords

49 Microbiome, microbiota, eye, high-throughput nucleotide sequencing, contact lenses

Exposed mucosal surfaces of the human body are associated with commensal microbiota with a mutualistic/symbiotic relationship with the human host¹. These commensal microbes play a role in preventing infection and in return we provide them with an environment to live¹. Previously they were referred to as the “normal flora”; now commensal microorganisms are often referred to as the “microbiota” (the microbial cells) and the genetic information of the microorganisms is referred to as the “microbiome”².

The “normal” microbiome of the gut, respiratory system and skin are all well described, and researchers have begun to examine how to restore or maintain “normal”¹. Recently the more complex relationship between these microbiomes and their prevention of inflammatory conditions at other sites in the body are being investigated; for example, how changes in the gut microbiome may contribute to uveitis, dry eye or sjögrens syndrome³⁻⁶.

The microbiome of the ocular surface is not as well described as those in other organs and the traditional view is that the ocular surface has only low numbers of transient microbial cells.¹ The ocular surface comprises of the cornea and the conjunctiva both of which have an exposed epithelium. The ocular surface is covered by the tear film which consists of lipid, aqueous and mucin components. The purpose of the ocular surface is to maintain clarity of the cornea, allowing light to enter the eye and be focused onto the fovea to give good vision. The ocular surface, as with other mucous membranes, is constantly exposed to the environment and therefore defensive properties must exist to maintain its homeostasis. Infection and inflammation of the ocular surface can ultimately lead to opacities within the cornea that reduce visual acuity. It has long been reported that there are several non-specific immunological defence mechanisms that exist on the ocular surface. Firstly, mechanical protection is provided by the blinking of the eyelids, drainage of tears and the corneal epithelium. Secondly, ocular surface protection is also provided by components of the tear

film, such as lipids, mucins and antimicrobial proteins (e.g. IgA, lysozyme, lactoferrin and lipocalin)². A resident microbiome may play a role in ocular surface protection.

There is much debate as to whether there is a resident microbiome on the ocular surface due to the effect of antimicrobial tear fluid and the mechanical protection of the eyelids (including blinking). Others have argued that the ocular surface contains small numbers of microbes which may prevent infection and inflammation².

Topical medication, contact lenses and inflammatory conditions (e.g. blepharitis) all have effects on homeostasis of the ocular surface in both the short and longer term¹. Further investigation of the composition and effects of the microbial communities at the ocular surface may lead to i) a better understanding of the microbiology of the eye in health and ii) a better understanding of the potential involvement of microorganisms in ocular disease. For example, if we can understand the way in which different types of contact lenses and care solutions affect the ocular surface microbiome, developments can be made to reduce their impact on ocular surface homeostasis. This may in turn increase comfort and compliance, whilst simultaneously reducing the risk of complications such as inflammation and infection. Further to this, we understand that systemic antimicrobials can disrupt the gut microbiome and allow *Clostridium difficile* (a component of the gut microbiome) to opportunistically proliferate and cause severe infection⁷. It is conceivable that something similar may happen in the eye, during contact lens wear or following topical antimicrobial treatment.

Next Generation Sequencing

The term next generation sequencing (NGS) refers to modern techniques for rapid DNA sequencing. NGS is quicker and cheaper than older methods; for example, the human genome project took 15 years to sequence the entirety of human DNA at a cost of £4 billion⁸. With the

advent of next generation sequencing, this is possible to complete in one day at a cost of under £1,000⁹.

Microorganisms have a less complex genetic structure than humans, and therefore the time and expense to sequence the constituents of the ocular surface microbiome would be significantly less than for more complex microbiomes. Until recently, investigation of the ocular surface microbiota has largely been performed by culturing swabs, and this approach costs as little as £10¹⁰.

For the purpose of this review we will propose a definition for the normal ocular surface microbiome and factors that may alter it. Previous culture methods were only able to quantify culturable bacteria present above the detection threshold¹¹. Significant numbers of microorganisms present in smaller quantities or do not culture well are often missed; up to 20% of cultured eye swabs may result in no growth¹¹. With the advent of next generation or high throughput sequencing we are now able to quantify paucibacterial communities (bacteria present in relatively low quantity) through presence of its DNA or RNA¹¹.

In 1907 Axenfeld et al. first reported the culture of ocular surface microorganisms in healthy individuals¹². Osato et al. went on to theorise that the ocular surface microbiome changed with age; individuals were thought to pick up *Staphylococcus*, *Streptococci* and *Escherichia coli* from the birth canal¹³. In addition, *Pneumococci* was thought to colonise the ocular surface within the first two decades of life, with *Diphtheroids* colonising the ocular surface in later life¹³.

Sample Collection

Traditional non-invasive sampling of the ocular surface microbiome involves instillation of local anaesthetic, such as 0.5% proxymetacaine or 0.4% oxybuprocaine, followed by sampling of the tear lake from the inferior fornix using a sterile cotton swab.

Next generation sequencing is highly sensitive and can detect small amounts of DNA, whether from living microorganisms, contaminants, or from non-viable microorganisms. On testing their own sterile cotton swabs with next generation sequencing, manufacturers are often able to detect *Pseudomonas*, *Escherichia* and *Bacillus* species, all previously reported to make up part of the ocular surface microbiome¹⁴. This suggests that there is a risk of erroneous results in if blank samples are not used as negative experimental controls, as should be routine.

Local anaesthetic can reduce discomfort when using a swab in the inferior fornix, however, it has been reported that this reduces the quantity of DNA detected on the ocular surface^{1,15}.

Capillary tubes may be used to collect tears in a more comfortable way than with a cotton swab, negating the need for local anaesthetic (Figure 1), and may also reduce the risk of contamination¹⁶. However, use of capillary tubes will sample the ocular surface microbiome as defined in tears only, they are unlikely to sample conjunctival tissue or periocular skin. They may play a role in restricting what is sampled.

Why Tears?

Tears provide us with a non-invasive safe form of tissue sampling to establish a normal microbial environment for the ocular surface, if there indeed is one as defined by tears. Sampling with a cotton bud is safe and non-invasive, however, as well as sampling tears conjunctival epithelium is sloughed into the sample. Furthermore, dependent on technique material from the lid margin and limbal/corneal epithelium may be included at variable rates.

In 2018 Ozkan et al. observed that the ocular surface microbiome within conjunctival tissue taken from surgical samples of individuals with pterygium significantly differed depending on the location that the conjunctiva was sampled from within the eye¹⁷. Limbal and fornix

conjunctival tissue samples were found to have significantly higher levels of *Pseudomonas* species when compared to ocular surface swab samples¹⁷.

There is a possibility that sampling with mechanical methods such as swabs may lead to sampling of both conjunctival epithelium and tears (and maybe even eyelid skin) which may or may not have distinct microbiomes, as suggested by Ozkan.

Search Criteria

MEDLINE, EMBASE and Google Scholar were searched for the keywords:

1. "Next Generation Sequencing" or "High Throughput Sequencing" and
2. "Ocular Surface" or "Eye" or "Cornea" or "Conjunctiva" and
3. "Microbiome" or "Microbiota".

Articles involving humans were included, animal studies were excluded. Articles using Next Generation Sequencing were included those using solely quantitative polymerase chain reactions or solely culture based methods were excluded. Articles investigating bacterial aspects of the ocular surface microbiome were included; those investigating solely viral or fungal elements were excluded.

Reference lists of included articles were also searched for relevant citations. All publications up to 1st June 2019 were analysed.

The Ocular Surface Microbiome in Health

The healthy ocular surface microbiome was originally thought to consist of *Staphylococcal*, *Streptococcal*, *Escherichia* and *Diphtheroid* species¹⁷. In 2011, Dong et al. carried out a pilot study using next generation sequencing to identify the ocular surface microbiome in 4 healthy Caucasian volunteers that were 26-48 years of age with no history of contact lens use¹⁸. Sequencing of tear samples collected by cotton swabs in this study demonstrated that the

167 microbiome of the ocular surface may be significantly more diverse than previously thought¹⁸.
168 59 different types of bacteria were identified from the 4 volunteers, 12 of which were
169 common between all individuals (Figure 2)¹⁸.
170 Five years later Huang et al. analysed the ocular surface microbiome from swabs of 31 eyes
171 of 31 patients¹⁹. Similar bacteria were identified from the eye swabs: *Pseudomonas* (20%),
172 *Propionibacterium* (20%), *Bradyrhizobium* (16%), *Corynebacterium* (15%), *Acinetobacter*
173 (12%), *Brevundimonas* (5%), *Staphylococci* (4%), *Aquabacterium* (2%), *Sphingomonas* (1%)
174 and *Streptococcus* (1%)¹⁹. Huang et al. suggested that there may be both a core ocular surface
175 microbiome that temporally persists in healthy individuals and a variable microbiome that is
176 dependent on environment lifestyle and physiology¹⁹.
177 Doan et al. then used next generation sequencing to attempt to identify the ocular surface
178 microbiome in 89 healthy eyes with no history of contact lens use²⁰. These results were
179 compared to traditional culture methods²⁰. 21.5% of swabs were culture negative, despite
180 next generation sequencing revealing a diverse community of bacteria on the ocular
181 surface²⁰. *Corynebacterium* (14.2%), *Staphylococcus* (13.2%), and *Streptococcus* (4.4%) were
182 again identified. This study sequenced a swab of the environment to control for contaminants
183 identifying *Pseudomonas*, *Elizabethkingia*, *Delftia* and *Propionibacterium* as insignificant²⁰.
184 An essential aspect of a microbiome is the fact that it exists temporally. Ozkan et al. recently
185 investigated the ocular surface microbiome of 43 healthy individuals over 3 months using
186 both culture and next generation sequencing²¹. Cultures indicated that no individuals had the
187 same bacteria present at each time point, suggesting that culture methods may be an
188 unpredictable way to assess the ocular surface microbiome²¹. They were unable to reveal a
189 microbiome common to all 43 individuals, however, they identified that *Corynebacterium*,

Sphingomonas and *Streptococcus* were the most prevalent bacteria with next generation sequencing (Figure 3)²¹.

Ocular Surface Microbiome and Age

Previous culture-based methods have suggested that the ocular surface microbiome may change as we age. A study by Cavuoto et al. compared infants (6 months old) with older children (6 months old to 18 years old)²². The study demonstrated that there was no difference between the right and left eye of an individual²². *Staphylococcus* (56.5%), *Streptococcus* (16.9%), *Corynebacterium* (6.2%) and *Moraxella* (8%) were all found in both eyes²². Older children had a similar number of bacteria on the ocular surface but a greater diversity, with the significant addition of *Oceanospirillaceae*, *Psychomonadaceae* and *Leuconostocaceae*²². These results may indicate that the ocular surface microbiome may change depending on age, however, these patients had a diverse past ophthalmic history with over half previously having undergone eye surgery²².

Wen et al. investigated the ocular surface microbiome using next generation sequencing of 90 healthy individuals classifying them as young (23-44 years of age) or old (47-84 years of age)²³. They again showed that there was no difference in the ocular surface microbiome between an individual's right and left eye²³. Although relative abundances are not stated, Wen et al. showed younger volunteers had significantly higher levels of *Propionibacterium* and *Mycoplasma*, whilst older volunteers had significantly higher levels of *Escherichia* and *Micrococcus*. Wen et al. also demonstrated that the bacterial diversity differed from patient to patient²³. Furthermore, the study showed significantly higher levels of *Propionibacterium* and *Staphylococcus* in men and higher levels of *Escherichia* in women²³. Although bacteria made up 98.2% of microorganisms detected by next generation sequencing, fungi and viral species were reported to make up 0.9% each²³.

Ocular Surface Microbiome and Contact Lens Wear

The ocular surface microbiome has been theorised to competitively inhibit proliferation of pathogenic bacteria that have the potential of causing ocular surface infection. As a result, any bacteriostatic/bactericidal substance could potentially leave the ocular surface vulnerable to infection or inflammation. Contact lens wear is a risk factor for microbial keratitis and conjunctivitis. This is largely theorised to be due to a breach in epithelial integrity or an alteration in the tear film that may occur during lens wear²⁴.

Shin et al. compared next generation sequencing of eye swabs from 9 lens wearers with 11 non-lens wearers over a period of 6 weeks, Table 1²⁵. Simultaneously, they compared the ocular surface microbiome to the periorbital skin microbiome by both tear and skin swabs. Firstly, Shin et al. showed that the ocular surface microbiome of contact lens wearers was similar to their periorbital skin, whereas in non-contact lens wearers it was not²⁵. Again, they showed that gender did not influence the ocular surface microbiome. Although abundances were not reported; *Pseudomonas*, *Acinetobacter*, *Methylobacterium*, and *Lactobacillus* were more prevalent in contact lens wearers²⁵. *Haemophilus*, *Streptococcus*, *Staphylococcus*, and *Corynebacterium* were more prevalent in non-lens wearers²⁵. Furthermore, they showed that the use of topical anaesthetic significantly reduced the quantity of bacteria available for analysis²⁵.

Zhang et al. went further in comparing 14 non-contact lens wearers with 13 soft contact lens wearers and 12 orthokeratology lens wearers, Table 1²⁶. Orthokeratology lenses are relatively new rigid contact lenses that are typically worn overnight to temporarily change the curvature of the surface of the cornea. This provides the wearer with good visual acuity without lenses during the day. Again, cotton swabs were used, although the authors did attempt to control for confounding factors by removing any resident genetic material found on blank swabs²⁶.

Orthokeratology lens wearers had significantly less *Bacillus*, *Delftia*, and *Lactobacillus* species compared to non-lens wearers, and soft contact lens wearers had significantly less *Delftia* and significantly more *Elizabethkingia* than non-lens wearers²⁶. Contrary to Shin et al., the microbiome of non-lens wearers significantly differed by gender (although the specifics of how the microbiome differed by gender were not reported). Interestingly, soft contact lens wearers and orthokeratology lens wearers did not differ with gender²⁶. Additionally, duration of soft contact lens or orthokeratology lens wear did not affect the microbiome, suggesting that any changes that occur may occur early in use and stabilise²⁶.

In a published abstract presented at the Association of Research in Vision and Ophthalmology in 2014 Retuerto et al. investigated the microbiome diversity on 84 worn contact lenses from 42 healthy volunteers, Table 1²⁷. *Pseudomonas*, *Ralstonia*, *Enterococcus*, *Streptococcus*, *Halomonas*, *Corynebacterium*, *Staphylococcus*, *Acinetobacter*, *Shewanella*, *Rhodococcus*, and *Cobetia* were found to be present, however relative abundances were not mentioned²⁷. The investigators went on to analyse if there was a difference in bacteria found on contact lenses cleaned with peroxide versus those by multipurpose solution. They concluded that multipurpose solution left lenses harboring a greater number of more diverse bacteria such as *Corynebacterium*, *Streptococcus*, *Aggregatibacter*, *Peptoniphilus* and *Haemophilus*²⁷.

Ocular Surface Microbiome in Sickness

Diabetes

Individuals with diabetes are relatively immunocompromised, and this is a risk factor for microbial keratitis and other ocular surface infections. Diabetes is associated with blepharitis, dry eye, reduced corneal sensation and delayed epithelial healing, all of which may contribute to changes in the ocular surface microbiome²⁸. Ham et al. compared the ocular surface microbiome of 19 healthy non-contact lens wearers with 30 type 2 diabetics that were awaiting vitrectomy for non-resolving vitreous haemorrhage secondary to severe

263 proliferative diabetic retinopathy²⁹. Conjunctival swabs were taken without local
264 anaesthetic²⁹. *Acinetobacter* was significantly more prevalent in diabetics, whereas
265 *Bradyrhizobium* and *Streptophyta* were more prevalent in healthy subjects (Figure 4)²⁹.

266 *Trachoma*

267 Zhou et al. also used next generation sequencing to analyse the ocular surface microbiome
268 comparing healthy eyes with eyes with clinical signs of trachoma (e.g. conjunctival scarring
269 trichiasis and subsequent corneal scarring) in the absence of detectable *Chlamydia*
270 *trachomatis* infection³⁰. The group analysed conjunctival swabs in Gambia from 105 healthy
271 individuals and 115 individuals with clinical signs of trachoma. The major constituents of the
272 healthy microbiome were *Corynebacterium*, *Streptococcus*, *Propionibacterium*, *Bacillus*,
273 *Staphylococcus* and *Ralsonia*³⁰. Following adjustment of confounders trachoma was not
274 found to significantly affect the microbiome in the study³⁰.

275 *Dry Eye/Blepharitis*

276 Graham et al. compared the microbiome 57 normal subjects and 34 patients with dry eye
277 both with culture-based methods and next generation sequencing³¹. Again, showing that next
278 generation sequencing was capable of identifying bacteria that culture was not³¹. Samples
279 were obtained with a sterile swab after instillation of topical anaesthetic. The author reported
280 no significant difference in the microbiome between the two groups³¹. *Staphylococcus*
281 species were commonly sequenced³¹.

282 Lee et al. used next generation sequencing to investigate how the microbiome differed in 7
283 individuals with blepharitis and 4 healthy controls³². Blepharitis is a very common cause of
284 dry eye disease in which there is a deficiency of the lipid layer of the tear film, secondary to
285 inflammation of the lid margins where this aspect of the tear film is produced. Lee et al. found
286 that *Propionibacterium*, *Staphylococcus*, *Streptophyta*, *Corynebacterium* and *Enhydrobacter*
287 made up a significant proportion of the ocular surface microbiome, although relative

abundances were not reported. Furthermore, in those with blepharitis *Staphylococcus* was more abundant (seen previously in skin flora of those with blepharitis) and *Propionibacterium* was less abundant than in healthy controls³². However, rather than sampling purely tears saline drops were instilled into eyes of volunteers who were then encouraged to blink before tear material was removed using capillary tubes³². As previously stated, next generation sequencing is capable of detecting small amounts of DNA, and therefore although the saline was sterile it may have affected results³².

Conclusion

Current research shows that the ocular surface microbiome may be more complex than previously thought (Figure 5). Although not present in high enough numbers to reliably or consistently culture in past studies, these bacteria can be detected using next generation sequencing¹⁸. Bacteria that were once thought to only be present during ocular pathology are being detected on the ocular surface under healthy physiological conditions. This may further indicate to us as practitioners the importance of contact lens hygiene. In addition, it is important to examine the corneal epithelium in contact lens wearers as this provides an important mechanical barrier to prevent some of these bacteria from penetrating into the stroma and potentially causing infection.

Interestingly, *Bradyrhizobium* was found to be abundant in some studies. This bacterium is normally found in soil and interestingly is also an endosymbiont for *acanthamoeba*, living inside *acanthamoeba* and helping it survive whilst it finds a host¹. *Acanthamoeba* keratitis causes severe visual loss and is associated with poor contact lens hygiene in hard water areas and is currently on the rise in the United Kingdom³³. The potential presence of *Bradyrhizobium* on the ocular surface may be an interesting target in the development of contact lens solutions for prevention of *Acanthamoeba* infection for contact lens solution.

312 *Pseudomonas aeruginosa* is an opportunistic pathogen that can cause severe keratitis and is
313 typically associated with contact lens use. It can be difficult to treat as it is capable of
314 developing antibiotic resistance³⁴. Other bacteria, such as *Propionibacterium* are also a cause
315 of microbial keratitis¹⁸. *Neisseria* species are common commensal species elsewhere on the
316 body, however, species such as *Neisseria gonorrhoea* and *Neisseria meningitidis* cause severe
317 corneal ulcers³⁵. Their potential presence on the ocular surface poses the question as to
318 whether any reported microbiome plays a key role in suppressing pathogenic bacteria, leaving
319 the ocular surface more prone to infection when it is altered, or whether these pathogens are
320 indeed part of the healthy microbiome.

321 Endophthalmitis is an intraocular infection with poor visual prognosis that may rarely occur
322 after intraocular surgery or injection³⁶. *Bacillus* and *Propionibacterium* have both been
323 reported as a cause of postoperative endophthalmitis, potentially correlating with their
324 presence on the ocular surface³⁶. This is similar to the aetiology of endophthalmitis associated
325 with *Streptococcus* and *Staphylococcus*. This further reinforces the importance of sterilisation
326 the ocular surface with effective agents such as iodine preoperatively, as some of these
327 potential pathogens may be resident.

328 *Brevundimonas* is found in the environment and rarely isolated from clinical samples and
329 *Ralstonia* has been previously identified as a contaminate in DNA extraction kits³⁷⁻³⁸.
330 Furthermore, as previously mentioned *Bacillus* and *Pseudomonas* have both been picked up
331 by next generation sequencing of sterile cotton swabs⁹. Therefore, the importance of using
332 controls to adjust results is significant to assess true components of the ocular surface
333 microbiome.

334 It is likely that contact lens wear significantly changes any ocular surface microbiome.
335 However, these effects are likely to vary due to the variations in contact lenses, solution and

336 contact lens hygiene. Further investigation into the effect of contact lens cleaning solution
337 and contact lens wear should initially attempt to minimise these variables. It is apparent that
338 the microbiome does not vary from right eye to left eye in individuals; however, it is unclear
339 as to whether the microbiome varies by gender, age, ocular surface infection/inflammation
340 (e.g. dry eye) and systemic disease. These latter variables, therefore, warrant further
341 investigation.

342 There is significant evidence to suggest that bacteria capable of causing sight threatening
343 infections may reside on a healthy ocular surface. It is therefore important that we consider
344 this when discussing contact lens wear with patients. Proper lens insertion/removal
345 techniques, cleaning techniques, advice on length of wear and not
346 sleeping/showering/swimming in lenses should all be taught to prevent breaking of the
347 epithelial barrier. Furthermore, it is likely that ocular surface disease such as dry eye and
348 blepharitis can alter the delicate balance of the microbiome. In both contact lens wearers and
349 non-contact lens wearers treatment of dry eye and blepharitis, even whilst the patient is
350 asymptomatic can help reduce the risk of infection. If these conditions are deemed as severe
351 patients should not be offered contact lenses until they receive treatment to ensure the
352 epithelial barrier is likely to be maintained.

353 The reported healthy ocular surface microbiome may differ in the literature due to large
354 methodological variations. It may also be possible that geographic variations due to the
355 environment may also exist. Furthermore, each paper analysed did show that there was some
356 variation from person to person in their samples. Further investigation with robust, well
357 controlled experiments is required to quantify the bacteria of the ocular surface microbiome
358 in physiological and pathophysiological states. Therefore, in seeking to minimise confounding

factors further research into the normal ocular surface microbiome should respect the following:

- Avoid the use of swabs without necessary negative controls for environmental contaminants
- Avoid use of local anaesthetic or sterile eye drops if sampling tears
- Unilateral tear sampling in healthy individuals
- Utilise negative controls of DNA extraction kits
- Assessment should occur at two different time points if investigating the stability of a microbiome

Investigation of the microbiome in any setting, let alone in the paucibacterial setting of the ocular surface is more difficult than the gut or skin as contamination is more likely to affect results. The bulk of the work is retrospective and in the early years as the field develops must be careful not to confuse correlation with causation when drawing conclusions. As an external mucosal surface temporal change may exist that need to be considered. However, much pathology has environmental factors that play a role in their pathogenesis.

With greater understanding, new therapeutic agents can be identified to treat and prevent ocular surface infection and inflammation. An increase in knowledge in this area can have a wide-ranging impact on contact lens practice. Finally, investigation of potential viral components of the ocular surface microbiome in sickness and in health should also be considered. The ocular surface microbiome provides both an interesting and complex topic to investigate, however, striving to minimise the risk of ocular surface infections (especially those associated with contact lenses) will likely require a better understanding of it and its role in ocular surface homeostasis.

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Table of Figures

Figure 1 – Collection of Tears using a Capillary Tube

Figure 2 – Bacteria Identified on the Ocular Surface Common to 4 volunteers (adapted from Dong et al.)¹⁸

Figure 3 – Ocular Surface Microbiome as Identified by Ozkan et al. (adapted from Ozkan et al.)²¹

Figure 4 – Changes Caused to the Ocular Surface Microbiome by Type 2 Diabetes (adapted from Ham et al.)²⁹

Figure 5 – Significant (>1%) Constituents of the Healthy Ocular Surface Microbiome Identified in >1 Paper (out of 10 studies)^{18-21, 23,25-26, 29-32}

Table 1 – Summary of Findings of Literature Describing how the Ocular Surface Microbiome is Affected by Contact Lenses²⁵⁻²⁷

Table 1

Reference	Shin et al. ²⁵	Zhang et al. ²⁶	Retuerto et al. (abstract) ²⁷
Study Population	Comparison of contact lens wearers with non-contact lens wearers	Comparison of soft contact lens wearers with Orthokeratology lens wearers and non-contact lens wearers	Worn contact lenses
Country	USA	China	USA
Number of patients (Number of eyes)	9 (11)	35 (35)	42 (84)
Next Generation Sequencing Technique	16s (Illumina)	16s (Illumina)	16s (Ion torrent)
Major Findings	<ul style="list-style-type: none"> Contact lens wearers had significantly higher levels of <i>Pseudomonas</i>, <i>Acinetobacter</i>, <i>Methylobacterium</i>, <i>Lactobacillus</i>. Non contact lens wearers had significantly higher levels of <i>Haemophilus</i>, <i>Streptococcus</i>, <i>Staphylococcus</i>, <i>Corynebacterium</i> Contact lens wearers had similar conjunctival microbiota to that of their periocular skin when compared to non-contact lens wearers. Local anaesthetic significantly alters the microbial community on the ocular surface 	<ul style="list-style-type: none"> <i>Bacillus</i>, <i>Rothia</i>, <i>Massilia</i>, <i>Betaproteobacteria</i>, <i>Actinomyces</i>, <i>Arcobacter</i>, <i>Shewanella</i>, <i>Acinetobacter</i>, <i>Rhodocyclaceae</i>, <i>Comamonadaceae</i>, and <i>Propionibacterium</i> were all identified No significant difference in the ocular surface microbiome between groups was identified 	<ul style="list-style-type: none"> <i>Pseudomonas</i>, <i>Ralstonia</i>, <i>Enterococcus</i>, <i>Streptococcus</i>, <i>Halomonas</i>, <i>Corynebacterium</i>, <i>Staphylococcus</i>, <i>Acinetobacter</i>, <i>Shewanella</i>, <i>Rhodococcus</i>, and <i>Cobetia</i> were all identified Contact lenses stored in peroxide had less bacterial abundance and diversity than contact lenses stored in multipurpose solution

Figure 1

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Bacteria Identified on the Ocular Surface in 4 Healthy Volunteers

Figure 5