Next-Generation Sequencing of the Ocular Surface Microbiome

DOI:
10.1097/ICL.0000000000000697

Document Version
Accepted author manuscript

Eye & Contact Lens

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

--Manuscript Draft--

Manuscript Number:	ECL-19-266R2
Full Title:	Next Generation Sequencing of the Ocular Surface Microbiome: in Health, Contact Lens Wear, Diabetes, Trachoma and Dry Eye
Article Type:	Review Article
Keywords:	Microbiome; microbiota; eye; high-throughput nucleotide sequencing; Contact Lenses
Corresponding Author:	Arthur Okonkwo, MBBS MRes PgCert Manchester Royal Eye Hospital Manchester, UNITED KINGDOM
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Manchester Royal Eye Hospital
Corresponding Author's Secondary Institution:	
First Author:	Arthur Okonkwo, MBBS MRes PgCert
First Author Secondary Information:	
Order of Authors:	Arthur Okonkwo, MBBS MRes PgCert
	Victoria Rimmer, BSc
	Andrew Walkden, MBChB, FRCOphth
	Arun Brahma, MBChB, MD, FRCOphth
	Fiona Carley, MBChB, FRCOphth
	Andrew J McBain, PhD
	Hema Radhakrishnan, PhD
Order of Authors Secondary Information:	
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 Pseudomonas and Neisseria species. Bacterial DNA that make up the microbiome in other parts of the body such as Acinetobacter , Actinomyces, Aquabacterium, Bradyrhizobium, Corynebacterium , Sphigomonas, Staphylococcus and Streptococcus were found. The putative ocular microbiome is consistent between right and left eye and is affected by contact lens use (higher Pseudomonas levels) and blepharitis (higher Staphylococcus 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 contaminates (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.

Arthur Okonkwo
Manchester Royal Eye Hospital
Arthur.okonkwo@doctors.org.uk

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.	The search/inclusion/exclusion criteria used has been better explained. Lines 153-164 The reference below uses a mouse model to investigate the gut microbiome in sjogrens syndrome which is beyond the scope of the review. de Paiva CS, Jones DB, Stern ME, et al. Altered Mucosal Microbiome Diversity and Disease Severity in Sjögren Syndrome. Sci Rep. 2016;6:23561.
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

Arthur Okonkwo MBBS MRES PgCert[1], Victoria Rimmer BSc[2], Andrew Walkden MBChB FRCOphth[1], Arun Brahma MBChB MD FRCOphth[1], Fiona Carley MBChB FRCOphth[1], Andrew J McBain PhD[2], Hema Radhakrishnan PhD[2]

1. Manchester Royal Eye Hospital, Manchester University NHS Foundation Trust
2. Division of Pharmacy & Optometry, School of Health Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, United Kingdom

Corresponding author: Arthur Okonkwo, Manchester Royal Eye Hospital, Manchester, M13 9WL; Arthur.okonkwo@doctors.org.uk; 0161 276 5533

Five figures.

Word count – 4247

Date submitted – 14/10/2019

Dates revised – 1/1/2020 and 21/1/2020

No funding was received for this study and none of the authors have conflicts of interest to declare.

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 1st 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 *Pseudomonas* and *Neisseria* species. Bacterial DNA that make up the microbiome in other parts of the body such as *Acinetobacter*, *Actinomyces, Aquabacterium, Bradyrhizobium, Corynebacterium*, *Sphigomonas, Staphylococcus* and *Streptococcus* were found. The putative ocular microbiome is consistent between right and left eye and is affected by contact lens use (higher *Pseudomonas* levels) and blepharitis (higher *Staphylococcus* levels).

Conclusions

There is significant likelihood that there is at least a transitory 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 contaminates (*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

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 host[1]. These commensal microbes play a role in preventing infection and in return we provide them with an environment to live[1]. 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 syndrome[3-6].

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 inflammation[2].

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 term[1]. 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 heath 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 infection[7]. 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 billion[8]. With the

advent of next generation sequencing, this is possible to complete in one day at a cost of under £1,000[9].

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 £10[10].

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 threshold[11]. 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 growth[11]. 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 RNA[11].

In 1907 Axenfeld et al. first reported the culture of ocular surface microorganisms in healthy individuals[12]. 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 canal[13]. 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 life[13].

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 microbiome[14]. 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 contamination[16]. 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 eye[17]. Limbal and fornix

conjunctival tissue samples were found to have significantly higher levels of *Pseudomonas* species when compared to ocular surface swab samples[17].

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 "Conjuntiva" 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* species[17]. 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 use[18]. 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 that previously thought[18].
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 patients[19]. 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 physiology[19].
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 use[20]. These results were
179 compared to traditional culture methods[20]. 21.5% of swabs were culture negative, despite
180 next generation sequencing revealing a diverse community of bacteria on the ocular
181 surface[20]. *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 insignificant[20].
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 sequencing[21]. 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 microbiome[21]. They were unable to reveal a
189 microbiome common to all 43 individuals, however, they identified that *Corynebacterium*,

190 *Sphingomonas* and *Streptococcus* were the most prevelant bacteria with next generation
191 sequencing (Figure 3)[21].

192 ## Ocular Surface Microbiome and Age

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

203 Wen et al. investigated the ocular surface microbiome using next generation sequencing of
204 90 healthy individuals classifying them as young (23-44 years of age) or old (47-84 years of
205 age)[23]. They again showed that there was no difference in the ocular surface microbiome
206 between an individual's right and left eye[23]. Although relative abundances are not stated,
207 Wen et al. showed younger volunteers had significantly higher levels of *Propionibacterium*
208 and *Mycoplasma*, whilst older volunteers had significantly higher levels of *Escherichia* and
209 *Micrococcus*. Wen et al. also demonstrated that the bacterial diversity differed from patient
210 to patient[23]. Furthermore, the study showed significantly higher levels of *Propionibacterium*
211 and *Staphylococcus* in men and higher levels of *Escherichia* in women[23]. Although bacteria
212 made up 98.2% of microorganisms detected by next generation sequencing, fungi and viral
213 species were reported to make up 0.9% each[23].

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 wear[24].

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 1[25]. 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 not[25]. 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 wearers[25]. *Haemophilus*, *Streptococcus*, *Staphylococcus*, and *Corynebacterium* were more prevalent in non-lens wearers[25]. Furthermore, they showed that the use of topical anaesthetic significantly reduced the quantity of bacteria available for analysis[25].

Zhang et al. went further in comparing 14 non-contact lens wearers with 13 soft contact lens wearers and 12 orthokeratology lens wearers, Table 1[26]. 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 swabs[26].

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 wearers[26]. 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 gender[26]. 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 stabilise[26].

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 1[27]. *Pseudomonas*, *Ralstonia*, *Enterococcus*, *Streptococcus*, *Halomonas*, *Corynebacterium*, *Staphylococcus*, *Acinetobacter*, *Shewanella*, *Rhodococcus*, and *Cobetia* were found to be present, however relative abundances were not mentioned[27]. 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 microbiome[28]. 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

proliferative diabetic retinopathy[29]. Conjunctival swabs were taken without local anaesthetic[29]. *Acinetobacter* was significantly more prevalent in diabetics, whereas *Bradyrhizobium* and *Streptophyta* were more prevalent in healthy subjects (Figure 4)[29].

Trachoma

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

Dry Eye/Blepharitis

Graham et al. compared the microbiome 57 normal subjects and 34 patients with dry eye both with culture-based methods and next generation sequencing[31]. Again, showing that next generation sequencing was capable of identifying bacteria that culture was not[31]. Samples were obtained with a sterile swab after instillation of topical anaesthetic. The author reported no significant difference in the microbiome between the two groups[31]. *Staphylococcus* species were commonly sequenced[31].

Lee et al. used next generation sequencing to investigate how the microbiome differed in 7 individuals with blepharitis and 4 healthy controls[32]. Blepharitis is a very common cause of dry eye disease in which there is a deficiency of the lipid layer of the tear film, secondary to inflammation of the lid margins where this aspect of the tear film is produced. Lee et al. found that *Propionibacterium*, *Staphylococcus*, *Streptophyta*, *Corynebacterium* and *Enhydrobacter* 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 controls[32]. 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 tubes[32]. 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 results[32].

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 conistently culture in past studies, these bacteria can be detected using next generation sequencing[18]. 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 host[1]. *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 Kingdom[33]. 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 resistance[34]. Other bacteria, such as *Propionibacterium* are also a cause
315 of microbial keratitis[18]. *Neisseria* species are common commensal species elsewhere on the
316 body, however, species such as *Neisseria gonorrhoea* and *Neisseria meningitidis* cause severe
317 corneal ulcers[35]. 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 injection[36]. Bacillus and *Propionibacterium* have both been
323 reported as a cause of postoperative endophthalmitis, potentially correlating with their
324 presence on the ocular surface[36]. 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 kits[37-38].
330 Furthermore, as previously mentioned *Bacillus* and *Pseudomonas* have both been picked up
331 by next generation sequencing of sterile cotton swabs[9]. 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

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

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

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

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

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

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

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

References

1. Boost M, Cho P, Wang Z. Disturbing the balance: effect of contact lens use on the ocular proteome and microbiome. Clinical & Experimental Optometry. 2017;100(5):459-72.
2. Pleyer U, Baatz H: Antibacterial Protection of the Ocular Surface. Ophthalmologica 1997;211(suppl 1):2-8. doi: 10.1159/000310878
3. Horai R, Caspi RR. Microbiome and Autoimmune Uveitis. Front Immunol. 2019;10:232.
4. Zaheer M, Wang C, Bian F, et al. Protective role of commensal bacteria in Sjögren Syndrome. *J Autoimmun*. 2018;93:45–56.
5. Wang C, Schaefer L, Bian F, et al. Dysbiosis Modulates Ocular Surface Inflammatory Response to Liposaccharide. Invest Ophthalmol Vis Sci. 2019;60(13):4224–4233.
6. de Paiva CS, Jones DB, Stern ME, et al. Altered Mucosal Microbiome Diversity and Disease Severity in Sjögren Syndrome. Sci Rep. 2016;6:23561
7. Di Bella, S.; Ascenzi, P.; Siarakas, S.; Petrosillo, N.; Di Masi, A. *Clostridium difficile* Toxins A and B: Insights into Pathogenic Properties and Extraintestinal Effects. *Toxins* 2016, *8*, 134.
8. Harris A. Lewin, Gene E. Robinson, W. et al. Earth BioGenome Project: Sequencing life for the future of life Proceedings of the National Academy of Sciences Apr 2018, 115 (17) 4325-4333
9. Touzani R, Perrier L, Rouleau E et al. Cost of cancer diagnosis using next-generation sequencing targeted gene panels in routine practice: a nationwide French study. Eur J Hum Genet. 2018 Mar;26(3):314-323
10. NHS Fife (2010) Wound Formulary and Wound Management Guidelines
11. Willcox MD. Characterization of the normal microbiota of the ocular surface.Exp Eye Res 2013;117:99e105.Exp Eye Res 2013;117:99e105
12. Burns BP. Indigenous flora of the lids and conjunctiva. In: Tasman W, Jaeger E, eds. Duan's Ophthalmology [CD-ROM]. Philadelphia: Lippincott, 1998.
13. Osato MS. Normal ocular flora. In: JS Pepose, GN Holland, KR Wilhelmus, eds. Ocular Infection and Immunity, 1st edn. St Louise: Mosby, 1996. pp 191– 19
14. Salter SJ, Cox MJ, Turek EM, et al. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biol. 2014 Nov 12;12:87

15. Kaewjiaranai T, Srisatjaluk RL, Sakdajeyont W, Pairuchvej V, Wongsirichat N. The efficiency of topical anesthetics as antimicrobial agents: A review of use in dentistry. J Dent Anesth Pain Med. 2018;18(4):223–233.
16. Posa A, Bräuer L, Schicht M, Garreis F, Beileke S, Paulsen F. Schirmer strip vs. capillary tube method: non-invasive methods of obtaining proteins from tear fluid. Ann Anat. 2013 Mar;195(2):137-42.
17. Ozkan J, Coroneo M, Willcox M, Wemheuer B, Thomas T; Identification and Visualization of a Distinct Microbiome in Ocular Surface Conjunctival Tissue. Invest. Ophthalmol. Vis. Sci. 2018;59(10):4268-4276.
18. Dong Q, Brulc JM, Iovieno A, Bates B, Garoutte A, Miller D et al. Diversity of bacteria at healthy human conjunctiva. Investigative Ophthalmology and Visual Science. 2011 Jul 1;52(8):5408-5413. https://doi.org/10.1167/iovs.10-6939
19. Huang Y, Yang B, Li W. Defining the normal core microbiome of conjunctival microbial communities. Clinical Microbiology and Infection. 2016;22(7):643.e7-.e12.
20. Doan T, Akileswaran L, Andersen D, Johnson B, Ko N, Shrestha A, et al. Paucibacterial microbiome and resident DNA virome of the healthy conjunctiva. Investigative Ophthalmology and Visual Science. 2016;57(13):5116-26.
21. Ozkan J, Nielsen S, Diez-Vives C, Coroneo M, Thomas T, Willcox M. Temporal Stability and Composition of the Ocular Surface Microbiome. Scientific Reports. 2017;7(1):9880.
22. Cavuoto KM, Banerjee S, Miller D, Galor A. Composition and comparison of the ocular surface microbiome in infants and older children. Translational Vision Science and Technology. 2018;7(6).
23. Wen X, Miao L, Deng Y, Bible PW, Hu X, Zou Y, et al. The Influence of Age and Sex on Ocular Surface Microbiota in Healthy Adults. Invest Ophthalmol Vis Sci. 2017;58(14):6030-7.
24. Fleiszig SM. The Glenn A. Fry award lecture 2005. The pathogenesis of contact lens-related keratitis. Optom Vis Sci. 2006 Dec;83(12):866-73
25. 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.
26. Zhang H, Zhao F, Hutchinson DS, Sun W, Ajami NJ, Lai S, et al. Conjunctival microbiome changes associated with soft contact lens and orthokeratology lens wearing. Investigative Ophthalmology and Visual Science. 2017;58(1):128-36.

27. Retuerto M, Szczotka-Flynn L, Mukherjee P, Stoll G, Chandra J, Debanne S, et al. Microbiome diversity on worn contact lenses and bacterial communities associated with care solution use and lid bioburden. Investigative Ophthalmology and Visual Science. 2014;55 (13):5458.
28. Skarbez K, Priestley Y, Hoepf M, Koevary SB. Comprehensive Review of the Effects of Diabetes on Ocular Health. Expert Rev Ophthalmol. 2010;5(4):557–577. doi:10.1586/eop.10.44
29. 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.
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(11):99.
31. Graham JE, Moore JE, Jiru X, Moore JE, Goodall EA, Dooley JSG, Hayes VEA, Dartt DA, Downes S, Moore TCB; Ocular Pathogen or Commensal: A PCR-Based Study of Surface Bacterial Flora in Normal and Dry Eyes. Invest. Ophthalmol. Vis. Sci. 2007;48(12):5616-5623.
32. Lee SH, Oh DH, Jung JY, Kim JC, Jeon CO. Comparative ocular microbial communities in humans with and without blepharitis. Invest Ophthalmol Vis Sci. 2012 Aug 15;53(9):5585-93.
33. Carnt N, Hoffman JJ, Verma S, Hau S, Radford CF, Minassian DC, Dart JKG. Acanthamoeba keratitis: confirmation of the UK outbreak and a prospective case-control study identifying contributing risk factors. Br J Ophthalmol. 2018 Dec;102(12):1621-1628.
34. Fleiszig SM, Evans DJ. Pathogenesis of contact lens-associated microbial keratitis. Optom Vis Sci. 2010;87(4):225–232.
35. Liu G, Tang CM, Exley RM. Non-pathogenic Neisseria: members of an abundant, multi-habitat, diverse genus. Microbiology. 2015 Jul;161(7):1297-1312
36. Miller JJ, Scott IU, Flynn HW Jr, Smiddy WE, Murray TG, Berrocal A, Miller D. Endophthalmitis Caused by Bacillus Species. Am J Ophthalmol. 2008 May;145(5):883-8
37. Salter, S; Cox, M; Turek, E; Calus, S; Cookson, W; Moffatt, M; Turner, P; Parkhill, J; Loman, N; Walker, A. Reagent contamination can critically impact sequence-based microbiome analyses. BMC Biology. 2014 Dec;12(1):87
38. Lee, M.R., Huang, Y.T., Liao, C.H. et al. Bacteremia caused by Brevundimonas species at a tertiary care hospital in Taiwan, 2000–2010. Eur J Clin Microbiol Infect Dis. 2011;30: 1185.

Table of Figures

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	• Contact lens wearers had significantly higher levels of *Pseudomonas*, *Acinetobacter*, *Methylobacterium*, *Lactobacillus*. • Non contact lens wearers had significantly higher levels of *Haemophilus*, *Streptococcus*, *Staphylococcus*, *Corynebacterium* • 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	• *Bacillus, Rothia, Massilia, Betaproteobacteria, Actinomyces, Arcobacter, Shewanella, Acinetobacter, Rhodocyclaceae, Comamonadacea,* and *Propionibacterium* were all identified • No significant difference in the ocular surface microbiome between groups was identified	• *Pseudomonas, Ralstonia*, *Enterococcus, Streptococcus*, *Halomonas*, *Corynebacterium*, *Staphylococcus*, *Acinetobacter*, *Shewanella*, *Rhodococcus*, and *Cobetia* were all identified • Contact lenses stored in peroxide had less bacterial abundance and diversity than contact lenses stored in multipurpose solution

Figure 1

Click here to access/download;Figure;Figure 1.jpg

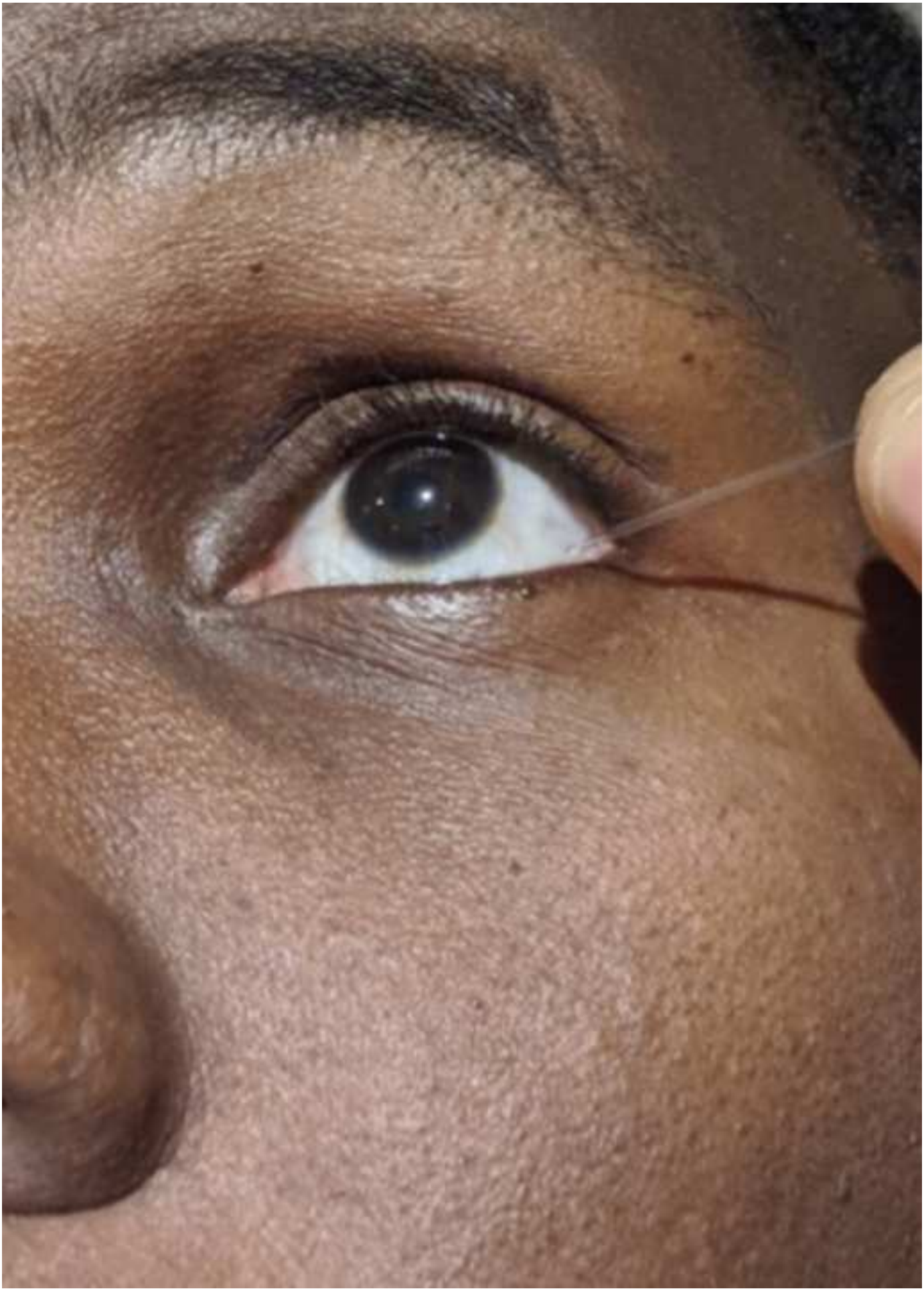

Figure 2

Click here to access/download;Figure;Figure 2.jpg

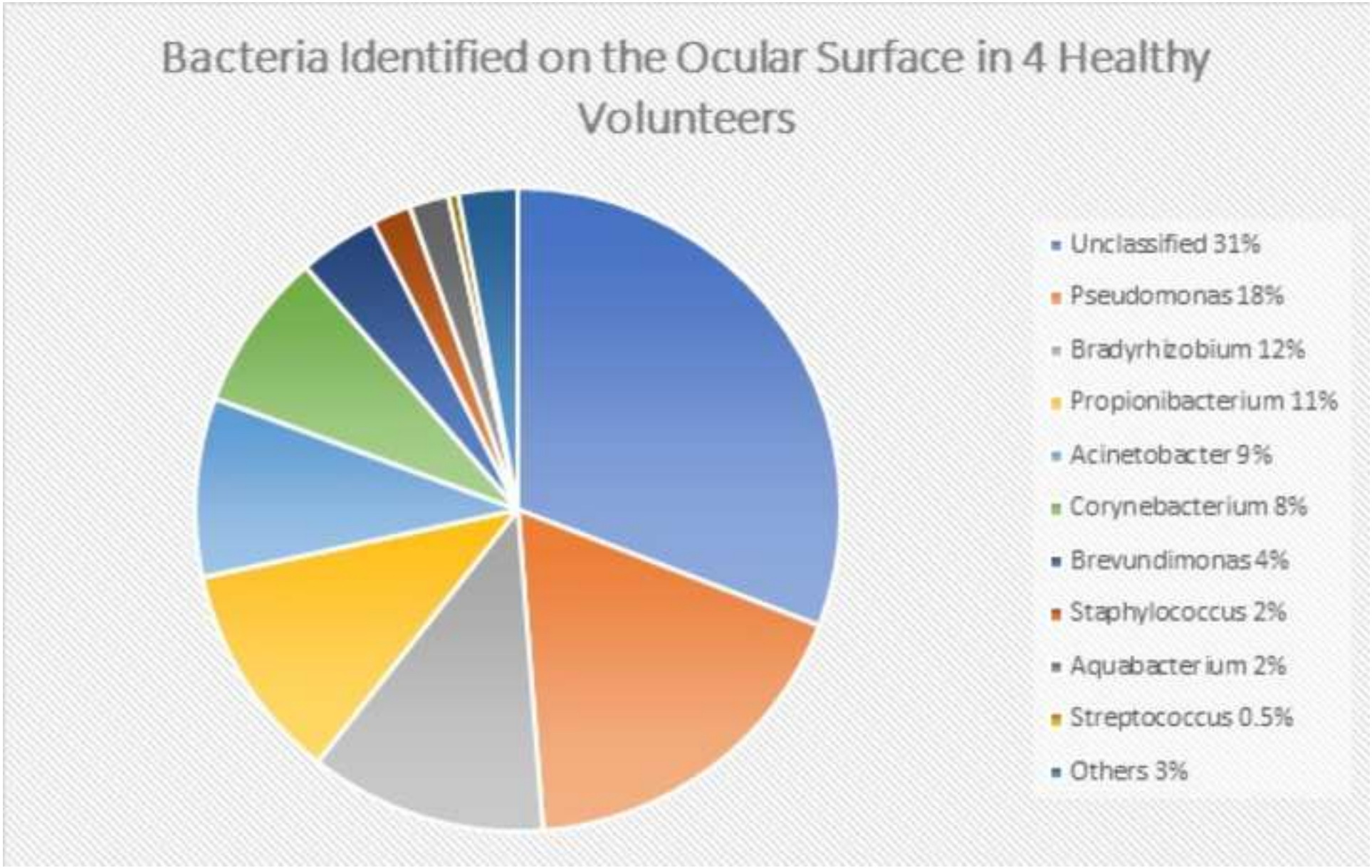

Figure 3

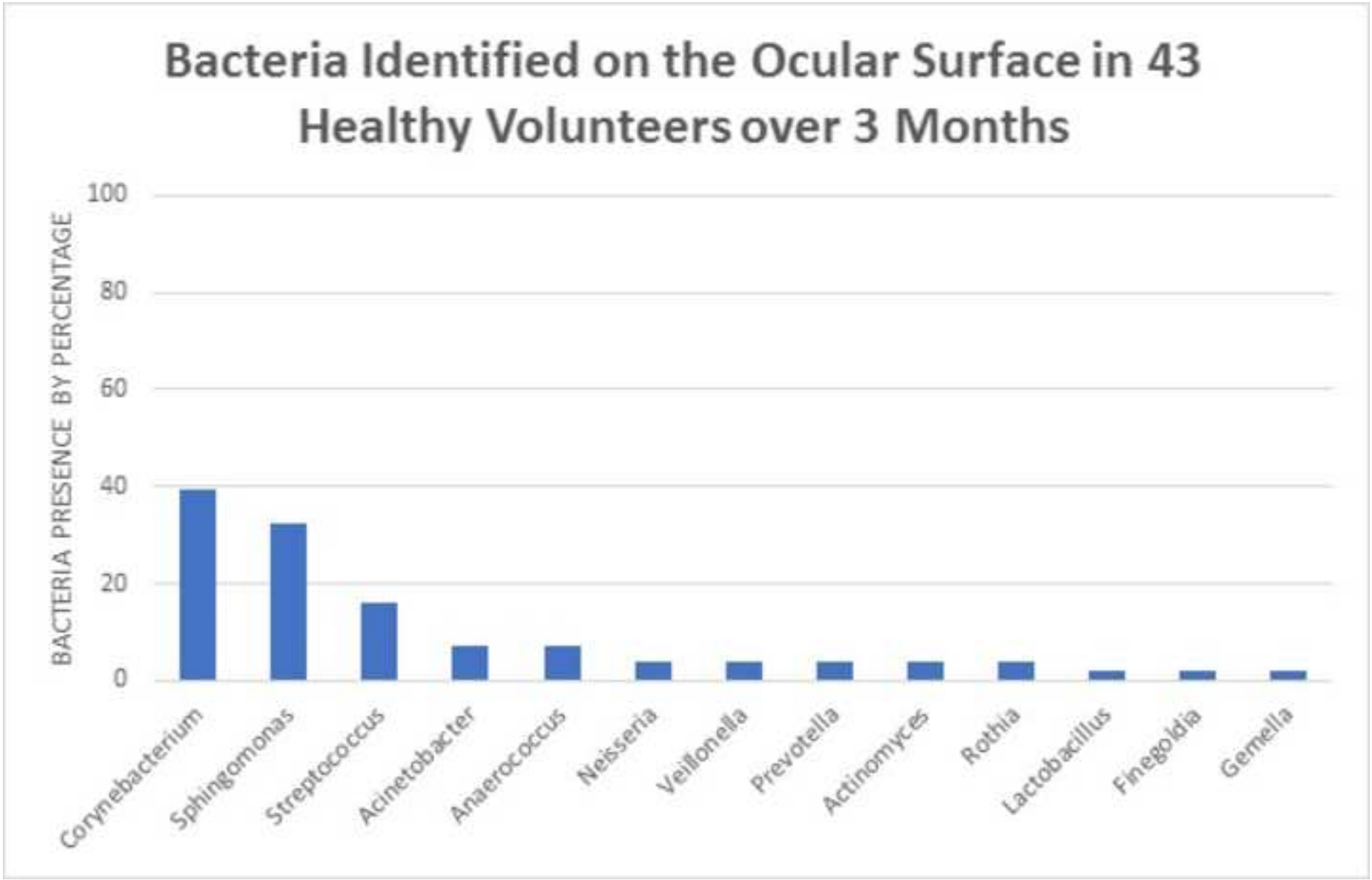

Figure 4

Click here to access/download;Figure;Figure 4.jpg

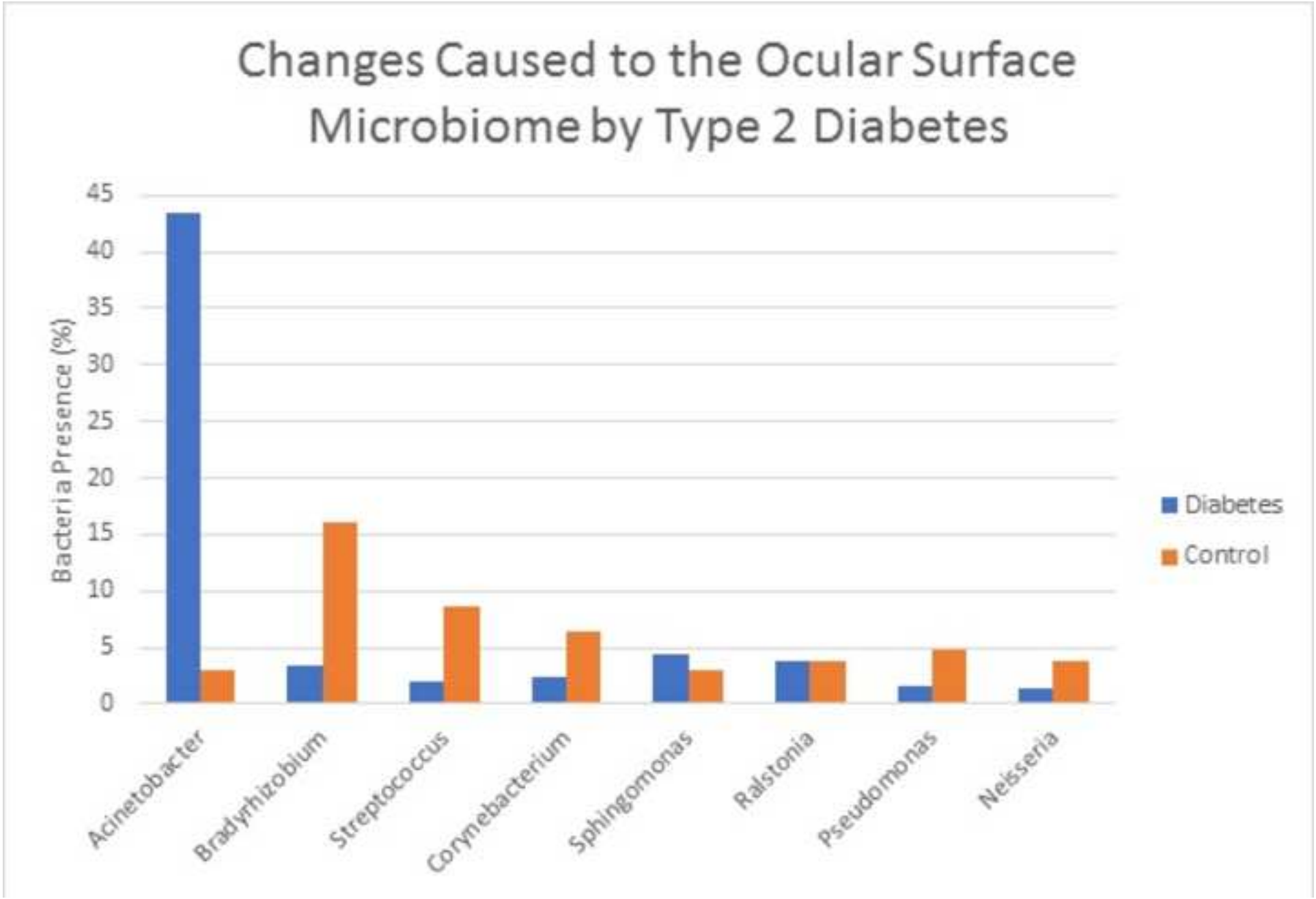

Figure 5

Click here to access/download;Figure;Figure 5.png