



Libraries and Learning Services

University of Auckland Research Repository, ResearchSpace

Version

This is the Accepted Manuscript version. This version is defined in the NISO recommended practice RP-8-2008 <http://www.niso.org/publications/rp/>

Suggested Reference

Watters, G. A., Turnbull, P. R., Swift, S., Petty, A., & Craig, J. P. (2017). Ocular surface microbiome in meibomian gland dysfunction. *Clinical and Experimental Ophthalmology*, 45(2), 105-111. doi: [10.1111/ceo.12810](https://doi.org/10.1111/ceo.12810)

Copyright

Items in ResearchSpace are protected by copyright, with all rights reserved, unless otherwise indicated. Previously published items are made available in accordance with the copyright policy of the publisher.

This is the peer reviewed version of the article above which has been published in final form at [10.1111/ceo.12810](https://doi.org/10.1111/ceo.12810)

This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for self-archiving.

For more information, see [General copyright](#), [Publisher copyright](#), [SHERPA/RoMEO](#).

Ocular surface microbiome in meibomian gland dysfunction in Auckland, New Zealand

Grant A Watters MscOptom,^{1,3} Philip R Turnbull PhD BOptom,³ Simon Swift PhD,²
Alex Petty BOptom^{1,3} and Jennifer P Craig PhD MCOptom¹

¹ Department of Ophthalmology, New Zealand National Eye Centre, The University of Auckland, Auckland, New Zealand,

² Department of Molecular Medicine and Pathology, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand,

³ School of Optometry and Vision Science, New Zealand National Eye Centre, The University of Auckland, Auckland, New Zealand

Correspondence: A/P Jennifer P Craig, Department of Ophthalmology, The University of Auckland, Auckland, 1142, New Zealand

Email: jp.craig@auckland.ac.nz

Short running title: Microbiome in MGD in Auckland, NZ

Received 14 March 2016; accepted 20 July 2016

Conflict of interest: None

Funding sources: None

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ceo.12810

ABSTRACT

Background: To investigate the ocular microbiome in meibomian gland dysfunction (MGD) relative to the normal eye in Auckland, New Zealand (NZ).

Design: Prospective, cross-sectional, observational study in a University setting.

Participants: A total of 157 participants, resident in NZ for ≥ 2 years were classified as normal (n=66), mild MGD (n=41), and moderate-to-severe MGD (n=50). Contact lens (CL) wear and anterior blepharitis status were recorded, as well as symptoms and clinical features.

Methods: Bacteria collected from lid margin swabs, before and after gland expression, were isolated and identified by conventional microbiological culture techniques. Aerobic isolates were identified in all 157 participants, and both aerobic and anaerobic bacteria, isolated in a subset of 87 subjects.

Main Outcome Measures: Bacterial incidence according to MGD status

Results: Symptoms, bulbar hyperaemia, conjunctival staining, lipid layer grade and tear film stability, but not corneal staining, showed moderate association with MGD severity. Participants with and without MGD showed a similar microbiome, unaffected by gland expression. Anterior blepharitis often co-existed with MGD, but was not an independent predictor of the microbiome. Sterile cultures were more common in CL wearers than non-wearers. The incidence of *Staphylococcus aureus* was higher than anticipated across all severity groups while that of coagulase-negative *Staphylococcus*, *Corynebacterium* and streptococci, were lower.

Conclusions: Modest differences in relative proportions of bacteria compared with other studies supports climatic variations in the ocular surface microbiome. Similarity in microbiome profile, irrespective of MGD severity, anterior blepharitis presence, or CL wear, suggests potential for commonality in treatment.

Key words: microbiome, dry eye, meibomian gland dysfunction, blepharitis, bacteria

INTRODUCTION

Meibomian gland dysfunction (MGD) is the most common form of posterior blepharitis and is recognised to be the main cause of evaporative dry eye.¹ Insufficient or poor quality meibum released by the glands in MGD, creates an inadequate tear film lipid layer that permits excessive tear evaporation.^{2,3} Anterior blepharitis, observed clinically as crusting and debris of the lashes and eyelid margin, creates a toxic environment which is disruptive to the tear film and promotes ocular surface inflammation.⁴ The local microbiome is believed to play a role in both forms of blepharitis.^{5,6} and topical antibiotics can be utilised to reduce the bacterial load and provide symptomatic relief.^{6,7}

An improved understanding of the ocular surface microbiome is essential for developing novel strategies for maintaining and promoting eyelid health, however characterisation of the microbiome in a number of studies⁸⁻¹⁰ has demonstrated geographic variability in the range of bacteria isolated. The ocular surface microbiome has not previously been described in MGD for any New Zealand (NZ) population.

METHODS

Study design and enrolment

Individuals, resident in NZ for a minimum of two years, were invited to participate in this cross-sectional, observational study. Participants comprised individuals responding to advertising around University campuses, or patients attending for routine eye examination, responding to adverts in optometry clinics. Those reporting use of topical therapy (other than lubricant use for dry eye), use of oral medications known to affect the tear film or ocular surface, a history of ocular trauma or surgery, or showing signs of active ocular disease, other than blepharitis, were excluded from the study, to minimise the risk of influencing the microbiome. Contact lens wearers were required to discontinue lens wear for at least 48 hours prior to testing. All participants were recruited in the New Zealand winter months, between May and September.

Symptomatic evaluation with the McMonnies dry eye questionnaire¹¹ preceded clinical ocular surface examination. In alignment with the MGD severity staging proposed by the Meibomian Gland Dysfunction Workshop (MGDW) in 2011, clinical lid margin signs of MGD were classified on a three point severity scale as 0 (normal; MGDW L0 or L1), 1 (mild; MGDW L2 or L3) or 2 (moderate-to-severe; MGDW L4 or L5).¹² Evidence of MGD signs on slitlamp biomicroscopy included diffuse abnormalities of the meibomian glands including terminal duct obstruction, lid margin thickening, hyperaemia, irregularity or telangiectasia, and/or altered meibomian gland secretions. Meibum expression, was graded as 0 (clear, smooth), 1 (cloudy/slightly viscous) or 2 (opaque/toothpaste-like). Lower lid MG drop-out observed by infra-red transillumination was also graded as 0 ($\leq 25\%$ loss), 1 (26 – 50% loss) or 2 ($\geq 51\%$ loss). Slit-lamp signs of anterior blepharitis including lid crusting, debris, collarettes, and telangiectasia were graded by visual analogue scale. Corneal and conjunctival staining with sodium fluorescein and lissamine green were graded using the Oxford Grading Scheme.¹³ The more severely affected eye was selected for analysis unless signs were indistinguishable between the two eyes, and then the right eye was selected.

The study adhered to the tenets of the Declaration of Helsinki, and received local ethical approval (UAHPEC 6423). Written informed consent was obtained from all participants.

Microbiology

Bacterial swabs for aerobic analysis were collected from all participants (n=157), immediately prior to gland expression and within 1 minute of performing gland expression. To enable culture of both aerobic and anaerobic bacteria, a subset of 87 participants provided a second set of swabs, before and after MG expression. Swabs were administered by wiping a saline-moistened sterile cotton bud with rotation for several seconds across the lower lid margin. After expressing the lower meibomian glands between two sterile cotton-tipped applicators, the swabbing process was repeated. The microbiology process employed to identify the aerobic bacteria isolated from the pre- and post-expression eyelid swabs is summarised in Figure 1. All agars, catalase and coagulase test reagents, and bacitracin and optochin disks

were sourced from Fort Richard Laboratories, and Microbact™ GNB12A strips were sourced from Thermo Fisher, Auckland, NZ. Agars and other reagents were used according to the manufacturer's instructions. Swabs for anaerobic culture were placed into Amies transport media (Fort Richard Laboratories, Auckland, NZ) for isolation and identification of *P. acnes* and other *Propionibacterium* spp. by a commercial medical laboratory (LabPlus, Auckland, NZ).

Statistical Analysis

Equality of the distribution of age and contact lens wear was compared between severity groups with one-way ANOVA, with Tukey post-hoc. Paired pre- and post-MGD expression incidence of bacterial types was compared with McNemar chi-square test, by severity group. Differences between the incidence of bacterial types between severity groups, and trends over severity groups were assessed with polytomous universal modelling (PLUM), with age, anterior blepharitis and contact lens wear as covariates. Correlations between continuous and ordinal variables are reported as Spearman's rho, with Bonferroni correction for multiple comparisons. Odds ratios (OR) were calculated from the PLUM independent variable ($OR = e^{-\beta}$). Results were considered significant at $p < 0.05$.

RESULTS

A total of 157 eyes from 157 individuals (aged 36 ± 21 years, 55% female) were included in the study, including 61 (39%) contact lens wearers. The participants of mixed ethnicity (45% Caucasian, 39% Asian, 8% Maori or Pacific Islander, 7% Indian and 2% Middle Eastern) were assigned to one of the three defined severity groups: non-MGD (normal, $n=66$), mild MGD ($n=41$), or moderate-to-severe MGD ($n=50$, Table 1). A significant difference in mean age was found to exist between groups (ANOVA, $F_{(2, 154)}=7.369$, $p=0.001$), with the moderate-to-severe group being older than the normal group (44 ± 22 vs 30 ± 18 , $p=0.001$), but not the mild MGD group (38 ± 23 , $p=0.294$), therefore age was included as a covariate in all analyses. The proportion of CL wearers was not significantly different between the groups

($p=0.696$), and there was no difference in the gender ratio ($p=0.134$) or ethnic distribution ($p=0.961$) between groups.

There was strong agreement between MG clinical lid margin signs and lipid layer grade ($n=157$ $\rho=-.322$, $p<0.001$), amount of bulbar hyperaemia ($\rho=.475$, $p<0.001$), crusting ($\rho=.466$, $p<0.001$), conjunctival staining ($\rho=.423$, $p<0.001$), tear breakup time ($\rho=-.435$, $p<0.001$) and McMonnies symptom scores ($\rho=.560$, $p<0.001$). Fluorescein corneal staining was not significantly correlated with lid margin score ($\rho=.207$, $p=.009$), falling just short of the required Bonferroni corrected significance of $p=0.0083$.

Microbiological findings

Pre and post MG expression

No significant difference in the bacterial profile was observed before and after gland expression, in any group (all $p>0.1$), therefore only pre-MGD expression results are reported. Coagulase-negative staphylococcus (C-NS) was the most prevalent organism isolated, identified in almost two-thirds of individuals, but the frequency of isolation was not related to MGD severity ($p=0.129$). Alpha and beta-haemolytic streptococci were present in very low numbers ($<5\%$) and a small number ($<10\%$) of other gram positive bacteria was noted in each severity group.

Contact lens wearers exhibited no significant differences in bacterial profile ($p>0.1$) between MGD severity grades, with the exception of *P. acnes*, which was positively associated with increasing MGD severity (OR:3.7, $p=0.004$). *Enterobacteriaceae* were present in only 3 non-CL wearing individuals, while *Pseudomonas* spp. was present only in two contact lens wearers and one non-wearer.

Anterior blepharitis

Anterior blepharitis was seen in 75.82% of subjects with MGD, and 22.7% of those without MGD (Table 1). The severity of anterior blepharitis signs correlated with increasing MG severity grade ($n=157$, $\rho=0.631$, $p<0.001$), however the microbiome was not significantly different between those with and those without signs of anterior blepharitis ($p=0.452$).

Contact lens wear

Overall, contact lens (CL) wearers had a similar bacterial profile to non-CL wearers, with the exception that just over one in four (27.9%) contact lens wearers demonstrated no aerobic cultures, which was significantly more common than in non-contact lens wearers (13.50%, $p=0.022$, Figure 2). As the absence of culturable bacteria could adversely skew the distribution within each cohort, contact lens wearers were excluded from the MGD severity group comparison.

MGD classification

In the non-CL wearing group, there was a negative correlation between the presence of *S. aureus* and MGD severity (Normal:49%, Mild:21%, Moderate-severe:18%, OR: 0.455, $p=0.006$, Table 2).

DISCUSSION

Eyelid signs of MGD were noted to be highly correlated with dry eye symptoms as well as with a number of clinical features such as meibomian gland function, denoted by lipid layer grade, and tear film stability, as described by the tear break up time (both $p<0.001$). While this might have been anticipated,¹ such a relation is not consistently noted, and often there is a reported mismatch between signs and symptoms.^{14,15} Interestingly, in the sample included in the current study, individuals with MGD demonstrated a predisposition to exhibiting signs of anterior blepharitis as evidenced by crusting of the lashes. Lid margin signs of MGD were also found to correlate with the degree of bulbar hyperaemia, supporting the concept that destabilisation of the tear film secondary to lid disease, promotes downstream inflammation of the ocular surface.^{4,16} MGD severity was associated with increased fluorescein conjunctival staining, but its correlation with corneal staining failed to reach statistical significance, supporting the recent evidence which reports that corneal staining performs better as a marker for aqueous deficiency than evaporative dry eye.¹⁷

Beyond clinical assessment, evaluation of the microbiome is acknowledged to be useful for understanding pathophysiological mechanisms, as well as directing novel therapies for blepharitis.¹⁸ *Staphylococcus aureus* has a historical association with

dry eyes and blepharitis,^{4,5} and many studies also report significantly higher coagulase-negative staphylococci (C-NS) levels than *S. aureus* levels in subjects diagnosed with dry eyes (Table 3). Our NZ-based results reflect this trend although the relative difference in prevalence of C-NS and *S. aureus* was less marked (2:1 vs 5:1). Within the non-lens wearing subjects, an inverse relationship between severity of MGD and *S. aureus* was identified, such that the incidence was highest in the normal group and lowest in those with moderate to severe MGD. The reason for this somewhat counterintuitive finding is not understood, although similar mixed *S.aureus* results have been observed in other overseas studies¹⁹⁻²² with one study also reporting a similar inverse relationship.²³ Notably, a relatively high incidence of *S. aureus* and a relatively low incidence of C-NS was observed in both normal and MGD eyes (Table 4) in the current study relative to reports from other countries.¹⁹⁻²⁸ Climatic and environmental conditions are recognised to have the potential to affect the relationship between populations of *S. aureus* and C-NS.^{19,28,29} Further, differences in pollens, dust and soil particles in different environments have also been postulated to influence bacterial populations.^{5,9,19,29} Our data, collected over the relatively cool and wet winter months (maximum 14.5 °C; average monthly precipitation = 130mm) may simply reflect this seasonal effect, although analysis of seasonal and geographical variation of the microbiome within our sample is precluded by the cross-sectional nature of our single-site study.

Anaerobic *P.acnes* has previously been linked to anterior blepharitis³¹ and to acne rosacea,³² a condition strongly associated with MGD.^{33,34} In the current study *P.acnes* was the second most frequently isolated microorganism in participants with MGD, and the third most prevalent in the group without MGD, consistent with Australian¹⁹ and other studies (Tables 3 & 4). While one study, with limited sample size, has reported a lower incidence of *P.acnes* in blepharitis,⁵ the present study showed a rising incidence of *P. acnes* with increasing MGD severity, in agreement with the majority of the literature. This trend observed in the non-CL wearers (p=0.140; Table 2), reached significance within the CL-wearing group (p=0.004). The ocular microbiota is affected by CL wear,³⁵⁻³⁷ for a number of reasons, but including possible disruption of the synergistic relationship between *Corynebacterium* spp. and C-NS to favour C-NS. CL wear serves to decrease the aerobic conjunctival

microbiome population,³⁸ which might explain the higher proportion of sterile (“no aerobic”) counts noted in the CL wearers (Figure 2). CL wear is also reported to encourage *Pseudomonas* spp colonisation, although the low incidence of *Pseudomonas* in this study did not reflect this association.^{35,39} Our results do support the findings of Fleiszig and Efron, however, which confirm the non-transient effect of CL wear on the microbiome, as samples were collected a minimum of 48 hours after lens removal.³⁵

Corynebacterium and streptococci were notably low in incidence in the current subject population but have featured in reports of a number of studies globally.²¹⁻²³ Studies in Texas and Florida, USA²¹⁻²³ (Table 4) found a higher incidence of *Corynebacterium* spp. than those in Australia or Ireland.^{19,20} Similarly, the low incidence of streptococci in the present study, supports the argument that commensal ocular microbial populations are likely to be climate-specific, and collectively influence the microbiome through symbiotic interactions.^{6,8,18}

Interestingly anaerobic *P.acnes* incidence in both normal and MGD groups in the current study is consistent with international reports¹⁹⁻²³ (Tables 3 & 4), perhaps suggesting a lesser environmental influence on these anaerobic bacteria. The involvement of *P. acnes* in the pathogenesis of acne is worth consideration here as although *P. acnes* is a dominant member of the microbiota on healthy human skin⁴⁰ genetic analysis has revealed that disease is associated with a specific subpopulation.⁴¹ It is possible that there will be a high incidence of *P. acnes* in non-MGD and MGD samples, with MGD only associated with the presence of a pathogenic subpopulation. In a major NZ study on keratitis, C-NS was the most common bacterium isolated in corneal scrapes, followed by *P.acnes*.⁴² Although differences between resident microbiota and opportunistic infection are acknowledged, these similarly culture-based findings appear to correspond to the ocular surface findings in the present study, and emphasise the contributing relationship of the commensal microbiome to ocular infection.^{29,32,36}

This report on the ocular surface microbiome in MGD, in Auckland, NZ, provides useful information despite the limitations of having sampled from one location at a single time point, and having analysed the data according to traditional microbial/biochemical methods. Modern PCR-based evaluations have the potential to

provide more in depth information about the microbiome via DNA sequencing, although technological limitations exist with regard to distinguishing viable, culturable bacteria from those that have already yielded to the antimicrobial defence of the normal tear film, and also in establishing permanent communities rather than transient, non-viable bacterial populations.¹⁸

This study represents a snapshot of the microbiome in MGD at a single site in Auckland, New Zealand. Bacterial species present in subjects with MGD in the current study were found to closely match those of the normal population, and are largely consistent with those observed in previous reports from studies conducted within similar climates. Higher proportions of *S. aureus* relative to coagulase-negative *Staphylococcus* sp., and a lower incidence of *Corynebacterium* and streptococci, were observed compared with other reports from across the globe. No differences in the cultured bacteria were noted between individuals with and without co-existing anterior blepharitis and only minimal differences were noted in contact lens wear suggesting that therapies addressing symptoms attributed to bacterial over-colonisation could be effective in all three groups. To further explain the findings of this study, investigations of other factors that might influence the microbiome, for example climate, are warranted.

Acknowledgements

The authors are grateful to Sam Sharples, Logan Cooke, Richard Coakley, Meldody She, Defini Tau'alupe Tai, Nafisa Slaimankhel and Juntao Tang for assistance in data collection.

REFERENCES

1. Nelson JD, Shimazaki J, Benitez-del-Castillo JM, et al. The international workshop on meibomian gland dysfunction: report of the definition and classification subcommittee. *Investigative ophthalmology & visual science* 2011;52:1930-7.
2. Lemp MA. Report of the National eye institute/ industry workshop on clinical trials in dry eyes. *The CLAO journal : official publication of the Contact Lens Association of Ophthalmologists, Inc* 1995;21:221-32.
3. Smith RE, Flowers CW, Jr. Chronic blepharitis: a review. *The CLAO journal : official publication of the Contact Lens Association of Ophthalmologists, Inc* 1995;21:200-7.
4. Bernardes TF, Bonfioli AA. Blepharitis. *Semin Ophthalmol* 2010;25:79-83.
5. Lee SH, Oh DH, Jung JY, Kim JC, Jeon CO. Comparative ocular microbial communities in humans with and without blepharitis. *Investigative ophthalmology & visual science* 2012;53:5585-93.
6. Miller D, Iovieno A. The role of microbial flora on the ocular surface. *Curr Opin Allergy Clin Immunol* 2009;9:466-70.
7. Lindsley K, Matsumura S, Hatef E, Akpek EK. Interventions for chronic blepharitis. *The Cochrane database of systematic reviews* 2012;5:CD005556.
8. Dong Q, Brulc JM, Iovieno A, et al. Diversity of bacteria at healthy human conjunctiva. *Investigative ophthalmology & visual science* 2011;52:5408-13.
9. Karimian F, Zarei-Ghanavati S, A BR, Jadidi K, Lotfi-Kian A. Microbiological evaluation of chronic blepharitis among Iranian veterans exposed to mustard gas: a case-controlled study. *Cornea* 2011;30:620-3.
10. Yactayo-Miranda Y, Ta CN, He L, et al. A prospective study determining the efficacy of topical 0.5% levofloxacin on bacterial flora of patients with chronic blepharoconjunctivitis. *Graefe's archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie* 2009;247:993-8.
11. McMonnies CW, Ho A. Patient history in screening for dry eye conditions. *Journal of the American Optometric Association* 1987;58:296-301.

12. Tomlinson A, Bron AJ, Korb DR, et al. The international workshop on meibomian gland dysfunction: report of the diagnosis subcommittee. *Investigative ophthalmology & visual science* 2011;52:2006-49.
13. Bron AJ, Evans VE, Smith JA. Grading of corneal and conjunctival staining in the context of other dry eye tests. *Cornea* 2003;22:640-50.
14. Shimazaki J, Sakata M, Tsubota K. Ocular surface changes and discomfort in patients with meibomian gland dysfunction. *Archives of ophthalmology* 1995;113:1266-70.
15. Nichols KK, Foulks GN, Bron AJ, et al. The international workshop on meibomian gland dysfunction: executive summary. *Investigative ophthalmology & visual science* 2011;52:1922-9.
16. Luo L, Li DQ, Doshi A, Farley W, Corrales RM, Pflugfelder SC. Experimental dry eye stimulates production of inflammatory cytokines and MMP-9 and activates MAPK signaling pathways on the ocular surface. *Investigative ophthalmology & visual science* 2004;45:4293-301.
17. Ichihashi YI, T; Kaido, M ; Ishida, R ; Hatou, S ; Tsubota, K. Short break-up time type dry eye has potential ocular surface abnormalities. *Taiwan J Ophthalmol* 2015;5:68-71.
18. Zegans ME, Van Gelder RN. Considerations in understanding the ocular surface microbiome. *American journal of ophthalmology* 2014;158:420-2.
19. Albietz JM, Lenton LM. Effect of antibacterial honey on the ocular flora in tear deficiency and meibomian gland disease. *Cornea* 2006;25:1012-9.
20. Graham JE, Moore JE, Jiru X, et al. Ocular pathogen or commensal: a PCR-based study of surface bacterial flora in normal and dry eyes. *Investigative ophthalmology & visual science* 2007;48:5616-23.
21. Bowman RW, Dougherty JM, McCulley JP. Chronic blepharitis and dry eyes. *International ophthalmology clinics* 1987;27:27-35.
22. Dougherty JM, McCulley JP. Comparative bacteriology of chronic blepharitis. *The British journal of ophthalmology* 1984;68:524-8.
23. Groden LR, Murphy B, Rodnite J, Genvert GI. Lid flora in blepharitis. *Cornea* 1991;10:50-3.

24. Hsu HY, Lind JT, Tseng L, Miller D. Ocular flora and their antibiotic resistance patterns in the midwest: a prospective study of patients undergoing cataract surgery. *American journal of ophthalmology* 2013;155:36-44 e2.
25. Capriotti JA, Pelletier JS, Shah M, Caivano DM, Ritterband DC. Normal ocular flora in healthy eyes from a rural population in Sierra Leone. *International ophthalmology* 2009;29:81-4.
26. Karthika. N NA, Ramchandran S. A study of normal bacterial flora of the conjunctiva in patients undergoing cataract surgery in a rural teaching hospital in R. R. district. *Journal of Scientific and Innovative Research* 2014;3:164-7.
27. Mino de Kaspar H, Koss MJ, He L, Blumenkranz MS, Ta CN. Antibiotic susceptibility of preoperative normal conjunctival bacteria. *American journal of ophthalmology* 2005;139:730-3.
28. Rubio EF. Climatic influence on conjunctival bacteria of patients undergoing cataract surgery. *Eye* 2004;18:778-84.
29. Fernandez-Rubio ME. Geographic location and endophthalmitis. *Ophthalmology* 2012;119:2655-6 e1-5; author reply 6-7.
30. Weiland SK, Husing A, Strachan DP, Rzehak P, Pearce N, Group IPOS. Climate and the prevalence of symptoms of asthma, allergic rhinitis, and atopic eczema in children. *Occup Environ Med* 2004;61:609-15.
31. Dougherty JM, McCulley JP. Bacterial lipases and chronic blepharitis. *Investigative ophthalmology & visual science* 1986;27:486-91.
32. Oltz M, Check J. Rosacea and its ocular manifestations. *Optometry* 2011;82:92-103.
33. Driver PJ, Lemp MA. Meibomian gland dysfunction. *Surv Ophthalmol* 1996;40:343-67.
34. Arita R, Itoh K, Maeda S, et al. Proposed diagnostic criteria for obstructive meibomian gland dysfunction. *Ophthalmology* 2009;116:2058-63 e1.
35. Fleiszig SM, Efron N. Microbial flora in eyes of current and former contact lens wearers. *J Clin Microbiol* 1992;30:1156-61.
36. Cabrera JVR, J.B. Ocular bacterial flora in contact lens wearers. *Contact lens & anterior eye : the journal of the British Contact Lens Association* 1996;23:149-51.

37. Stapleton F, Willcox MD, Fleming CM, Hickson S, Sweeney DF, Holden BA. Changes to the ocular biota with time in extended- and daily-wear disposable contact lens use. *Infection and immunity* 1995;63:4501-5.
38. Sankaridurg PR, Markoulli M, de la Jara PL, et al. Lid and conjunctival micro biota during contact lens wear in children. *Optom Vis Sci* 2009;86:312-7.
39. Jiang Y, Jacobs M, Bajaksouzian S, et al. Risk factors for microbial bioburden during daily wear of silicone hydrogel contact lenses. *Eye & contact lens* 2014;40:148-56.
40. Bek-Thomsen M, Lomholt HB, Kilian M. Acne is not associated with yet-uncultured bacteria. *J Clin Microbiol* 2008;46:3355-60.
41. Lomholt HB, Kilian M. Population genetic analysis of *Propionibacterium acnes* identifies a subpopulation and epidemic clones associated with acne. *PloS one* 2010;5:e12277.
42. Wong T, Ormonde S, Gamble G, McGhee CN. Severe infective keratitis leading to hospital admission in New Zealand. *The British journal of ophthalmology* 2003;87:1103-8.

Table 1: Participant demographics by severity classification. Asterisks indicate statistically significant difference from the normal group ($p \leq 0.001$).

Participant Demographics	Clinical MGD lid margin signs classification		
	Normal (Grade = 0)	Mild (Grade = 1)	Moderate-Severe (Grade =2)
Number (%)	66 (42%)	41 (26%)	50 (32%)
Age \pm SD (years)	30 \pm 18	38 \pm 23	44 \pm 22*
Male (%)	26 (39%)	24 (59%)	21 (42%)
Contact lens wear (%)	27 (41%)	17 (41%)	17 (34%)
Anterior Blepharitis (%)	15 (23%)	26 (63%)*	43 (86%)*
<i>Ethnicity</i>			
Caucasian (%)	27 (41%)	14 (34%)	27 (54%)
Asian (%)	27 (41%)	20 (49%)	16 (32%)
Maori / PI (%)	4 (6.1%)	2 (4.9%)	3 (6%)
Indian SC (%)	5 (7.6%)	5 (12%)	2 (4%)
Middle Eastern (%)	1 (1.5%)	0	1 (2%)

Table 2: Distribution of bacteria isolated from *non-CL wearers according to MGD severity category. Asterisks denote significant differences between groups.*

Bacteria isolated	Normal group n: (%)	Mild n: (%)	Mod-Severe n: (%)	<i>p</i>
Coagulase-negative <i>Staphylococcus</i>	25: (64.1%)	14: (58.3%)	21: (63.6%)	0.129
<i>Staphylococcus aureus</i>	19: (48.7%) *	5: (20.8%)	6: (18.2%)	0.006 *
<i>Propionibacterium acnes</i> (anaerobic)	10: (25.6%)	7: (29.2%)	15: (45.5%)	0.142
<i>Pseudomonas</i> sp.	1: (2.6%)	0: (0%)	0: (0%)	-
Notable <i>Streptococcus</i> sp.	0: (0%)	1: (4.2%)	1: (3.0%)	0.401
<i>Enterobacteriaceae</i>	1: (2.6%)	1: (4.2%)	1: (3.0%)	0.757
Other G +ve	2: (5.1%)	2: (8.3%)	3: (9.1%)	0.508
No aerobic bacteria	3: (7.7%)	5: (20.8%)	5: (15.2%)	0.497

Table 3: Global comparison of the microbiome of the MGD population. Variations in the diagnostic criteria exist. The NZ population shows a lower prevalence of C-NS, *Corynebacterium* sp. and gram-negative rods. † aerobic testing only; ‡ anaerobic testing only. Abbreviations used are N = number of participants in the study; C-NS = coagulase negative staphylococci; *S. aureus* = *Staphylococcus aureus*; *P. acnes* = *Propionibacterium acnes*; *Corynebact.* sp. = *Corynebacterium* sp.; Gm neg. rods inc. = Gram-negative rods including. A hyphen (-) indicates the number is not available – either not measured, or not reported.

Authors (year published) ^{reference}	Country	N	C-N S (%)	<i>S. aureus</i> (%)	<i>P. acnes</i> (%)	<i>Corynebact.</i> sp.(%)	<i>Streptococcus</i> sp.(%)	Gm-neg. rods inc. <i>Pseudomonas</i> (%)
Albietz & Lenton (2006)¹⁹	Australia	20	95.0	20.0	30.0	35.0	0	15.0
Graham et al (2007)²⁰	Ireland	12	83.3	0	16.7	16.7	16.7	16.7
Bowman et al (1987)²¹	Texas USA	21	100.0	35.0	58.0	62.0	19.0	8.0
Dougherty & McCulley (1984)²²	Texas USA	85	97.6	27.0	94.1	48.2	-	-
Groden et al (1991)²³	Florida USA	332 † 56‡	95.8	10.5	92.8	77.4	-	11.4
Watters et al (2016)	New Zealand	57† & 21‡	61.4	30.3	36.8	3.2	3.5	0

Table 4: Global variations in the ocular surface microbiome. † denotes normal subjects in studies comparing dry eye and normal subjects. ‡ denotes a consecutive and randomised healthy population. N = number of participants in the study; C-NS = coagulase negative staphylococci; *S. aureus* = *Staphylococcus aureus*; *P. acnes* = *Propionibacterium acnes*; *Corynebact. sp.* = *Corynebacterium sp.*; Gm neg. rods inc. = Gram-negative rods including. A hyphen (-) indicates the number is not available – either not measured, or not reported.

Author(s); (year published) ^{reference}	Country	N	C-N S (%)	<i>S. aureus</i> (%)	<i>P. acnes</i> (%)	<i>Corynebact. sp.</i> (%)	<i>Streptococcus p.</i> (%)	Gm neg. rods inc. <i>Pseudomonas</i> (%)
Albietz & Lenton (2006)¹⁹	Australia†	18	84.0	6.0	22.0	6.0	0	6.0
Graham et al (2007)²⁰	Ireland†	12	81.0	0	19.0	19.0	6.0	6.0
Bowman et al (1987)²¹	Texas USA†	21	100.0	13.0	31.0	69.0	6.0	9.0
Dougherty & McCulley (1984)²²	Texas USA†	47	95.7	8.5	87.2	63.8	-	-
Groden et al (1991)²³	Florida USA†	160	87.5	15.6	73.7	45.0	-	4.3
Watters et al (2016)	New Zealand†	39	64.1	48.7	25.6	1.3	0	5.1
Rubio (2004)²⁸	Spain ‡	4366	56.8	6.4	-	30.2	7.5	6.2
Hsu et al (2013)²⁴	Missouri USA ‡	183	74.8	4.9	-	7.6	0.9	9.4
Mino de Kaspar et al (2005)²⁷	California USA ‡	162	76.0	11.7	-	-	4.9	6.8
Capriotti et al (2009)²⁵	Sierra Leone (Rural) ‡	276	28.6	19.9	-	-	-	16.0
Karthika et al (2014)²⁶	India (Rural) ‡	100	32.0	10.0	-	11.0	2.0	-

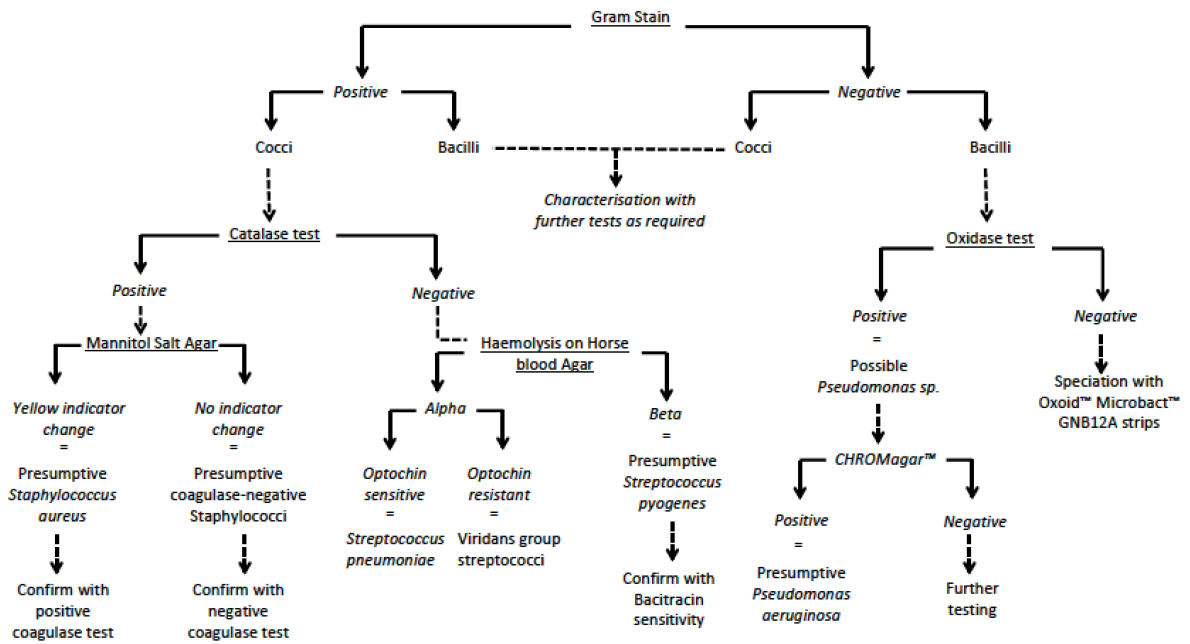


Figure 1: Summary microbiological speciation flowchart used to identify the aerobic bacteria isolated from lid margin swabs, which were taken both pre- and post-meibomian gland expression.

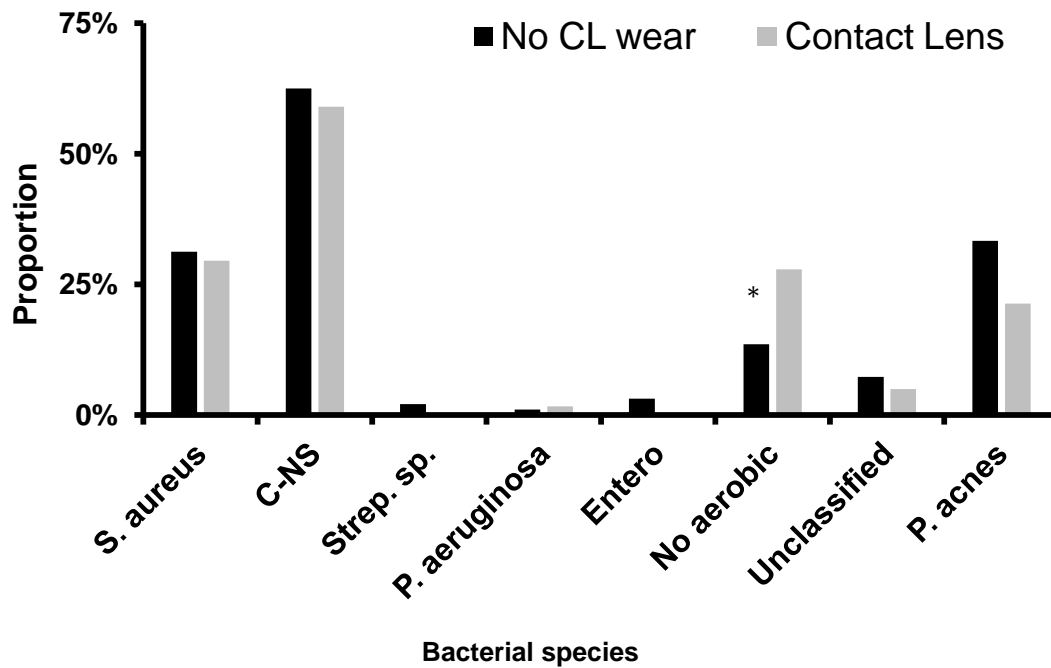


Figure 2: Bacterial frequency in non-CL (black bars) vs CL wearers (grey bars). No significant differences in bacterial distribution among *Staphylococcus aureus*, coagulase-negative staphylococci, haemolytic streptococcus, *Pseudomonas aeruginosa*, *Enterobacteriaceae*, *Propionibacterium* sp. or other, unclassified, bacteria were noted between non-contact lens (non-CL) and CL wearers in any severity group. Unclassified describes other gram-positive bacteria including *Corynebacterium* sp. However, no aerobic growth occurred more commonly in CL wearers. The asterisk denotes a statistically significant difference ($p= 0.022$).