Bacterial microbiota of the contact lens surface and associated care behaviours

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ABSTRACT

Introduction: Contact lens (CL) wear has been reported to cause changes to the microbiome of the ocular surface. More insight into the alteration of this microenvironment can help to understand the pathogenesis of CL-related eye infections. Knowledge of the relationship between the CL wearer's behaviours and pathogens would help health care providers focus on each step of proper CL care. This study aims to determine the behaviours that might be associated with the community of bacteria on CL.

Methods: A cross-sectional design was performed using anonymous questionnaires to obtain demographic data and assess hygiene practices among volunteering wearers. The CLs used were collected to evaluate the prevalence of pathogenic bacteria associated with ocular infections by PCR and microbiota analysis.

Results: The bacterial microbiota study revealed a total of 19 genera and 26 isolated strains from 20 eligible CLs. Enterobacter, Staphylococcus, and Achromobacter were the main genus in this subject population. Staphylococcus pasteuri and Achromobacter agilis were the most common pathogens at 65% and 35%, respectively. Enterobacter mori, a nonpathogenic organism, was found to be the most predominant strain, accounting for 27.51% of the total bacterial constituents. The risk behaviour of CL wear that was significantly associated with A. agilis contamination was cleaning the CL case with tap water (P value = 0.04).

Conclusions: This is the first study focusing on the association between the culture selected microbial community on the CL surface and comprehensive behavioural characteristics. Environmental contamination was the main source of microbes found on CL surfaces. An emphasis in patient education should be placed on careful handling during the CL care routine and managing the hygiene of the surroundings.

1. Introduction

The contact lens (CL) is a preferred choice for a large number of people for correcting refractive errors due to its ability to correct a wide range of refractive errors, ease of adaptation, and its practicality for an active lifestyle. Even in the absence of refractive errors, CLs are still a popular choice for cosmetics. More than 140 million people worldwide use CLs on a regular basis [1]; however, this may also come with complications, some of which are sight-threatening. Wearing a CL compromises the ocular surface in many ways, both from the lens itself and from unfavourable behaviours accompanied by CL wear. CL is a foreign body in the eye that can potentially foster pathogenic microorganisms.

Recently, over one million visits for keratitis and CL-related complications occurred each year in the USA [2], and one of the major important risk factors for microbial keratitis is the use of CLs [3]. Bacteria are the most common pathogen of CL-related eye infections [4, 5]. Both gram-negative and gram-positive bacteria, including Staphylococcus aureus, S. epidermidis, Klebsiella sp. Acinetobacter sp. and Pseudomonas aeruginosa, are recognized as the main bacterial pathogens of keratitis [6]. These organisms possessed the ability to attach and adhere to the CL surface [7, 8]. In particular, the biofilm formation of the bacteria promoted an interplay between specific properties of the CL surface and the organism [9].

Each type of CL material's unique chemical and physical properties, such as hydrophobicity, ionicity, and surface roughness, all contribute to the risk of infection. Interaction between the CL and the eye can lead to an altered state of the ocular surface [10]. The front and back surfaces demand more tears for covering, leading to dryness. The movement of
the CL on the lubricant-deprived ocular surface puts the cornea at an even greater vulnerability to microabrasions [3]. Corneal oxygenation is reduced as the lens acts as a physical barrier, blocking normal tear-gas exchange. Prolonged wearing only worsens dryness and cellular hypoxia, making the cornea more susceptible to pathogens [11]. Environmental exposure to dust and water brings pathogenic bacteria into the already compromised corneal surface. Apart from the inevitable risk from the lens acting on the corneal surface, another modifiable but hazardous complication associated with CL wear is mainly due to human behaviour [12]. Mishandling of CLs can both compromise the ocular surface and bring microbial contamination into the CL care system and the eye. The majority of CL users fail to adhere to good CL care behaviours, an important risk factor for CL-related eye infection, putting 40.9 million CL wearers in the United States at risk for serious eye infections [7]. Poor CL hygiene accounts for 12–66% of CL-related eye infection cases [6]. Poor hygiene is an important problem that is perhaps underestimated. A surprising 50% of CL wearers are not compliant with simple hygiene, such as hand washing [13].

Regarding the many steps involved in cleaning, disinfecting, and storing reusable CLs, mistakes in different steps may affect bacterial contamination differently. The ability to identify the causative risk behaviour may greatly help eye care professionals and CL wearers to focus on the steps and important points to avoid CL-related eye infection. Normal conjunctival bacterial normal flora has traditionally been considered predominantly gram-positive, reflecting those found on the skin [14]. The scientific advancements of the second decade of this century has allowed researchers to overcome the limitations of traditional culture. Microbiome analysis allows a deeper understanding of the unique microbial community in each niche. The conjunctival core microbiota is composed of the genera Corynebacterium, Pseudomonas, Staphylococcus, Acinetobacter, Streptococcus, Millisita, Anaerococcus, Finegoldia, Simonsiella, and Veillonella [15]. Human microbiomes help to regulate the homeostasis of human health and disease. The imbalance of the normal gut microbiota causes many noninfectious diseases, such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), and obesity [16,17,18]. From one study that investigated the ocular surface microbiota, a relationship between Streptococcus infection of the lens storage container and allergy symptoms related to CL wear was found [19].

Considering the available information, the ocular surface microbiome most likely plays a key role in maintaining ocular surface homeostasis, and its alteration could be linked to the development and progression of eye diseases. The purpose of this study was to clarify how CL care behaviours affect bacterial contamination by using microbiota analysis, which may help to reveal the causal relationship between microorganisms and behaviour. It would also allow health personnel to emphasize the importance of certain steps in CL care and would aid future studies in developing strategies to avoid such pitfalls. Furthermore, microbiome data reflect the state of the microenvironment, revealing changes in ocular microbiotas in persons wearing CLs that are a plausible cause for allergic symptoms [19]. A cross-sectional study was conducted to examine the relationship between risk behaviours and the main pathogens causing eye infections. The findings of this study provide more insights into the behaviour of Thai CL wearers that have been rarely studied.

2. Materials and methods

2.1. Study design and sample collection

A cross-sectional study was performed from November 2020 to March 2021. The study protocol was reviewed and approved by the Human Research Ethics Committee of Walailak University (WUEC-20-321-01) before the first volunteer was enrolled, in accordance with the tenets of the Declaration of Helsinki and with international restrictions on this study. A CL wearer was defined as a person who wore the CLs at least 5 days per week during the past month. The study population consisted of participants aged between 17 and 58 years who attended the ophthalmology clinic at Walailak University Hospital, Walailak University, and academic colleges in Nakhon Si Thammarat, Thailand. The subjects were recruited by a research assistant. Participants who were administered any topical medications of anti-allergic agents and/or antimicrobial agents within 2 months prior to initiation of the present study were excluded. The eligible lens wearers were advised to bring their CL with them for collection on the last study day. Written informed consent was obtained from all subjects after the explanation of the study. Participants completed a validated, anonymous, self-administered questionnaire regarding personal demographic information, the use of CL behaviours, and CL hygiene practice. Furthermore, the CLs used were collected to study the characteristics of bacterial accumulation and community, which might be related to the personal hygiene of those individuals. The optometrist placed the CLs in the sterile CL storage case containing normal saline solution, along with the questionnaire, put these in the sample envelope and returned them to the laboratory on the same day.

2.2. Questionnaire

Each participant completed a 47-item, anonymous, standardized paper questionnaire, which provided demographic data and behaviour of CL wear. In total, 20 soft CLs were obtained from 20 CL wearers. The questionnaire was divided into 3 parts: personal information (5 items), CL-related behaviours (20 items), and assessment of hygienic practices (22 items). Demographic information was collected, including sex, age, educational level, underlying disease, and history of antimicrobial agent administration as exclusion criteria.

2.3. Laboratory sampling, bacterial isolation and DNA extraction

The CLs from the participants were obtained within 4 h of CL wear on the same day, received at the laboratory and aseptically transferred to a culture tube. The CLs were grown on brain heart infusion broth (BHI, HiMedia, India) at 37 °C for 48 h for recovery of bacterial cells. The culture was centrifuged at 10,000 × g for 5 min at 4 °C, washed once with Tris-ethylenediaminetetraacetic acid (TE) buffer (10 mM Tris-HCl (pH 8.0), 1 mM ethylenediaminetetraacetic acid), and resuspended in 0.5 mL of TE buffer. An aliquot of 1 mL of all samples was centrifuged for 1 min at 15,000 × g, and then the supernatant was discarded from the tubes for DNA extraction. Genomic DNA (gDNA) was extracted from the bacterial pellet in accordance with the manufacturer’s protocol of the Presto Mini gDNA Bacteria Kit (Geneaid Biotech, Ltd., New Taipei City, Taiwan). The pellet was resuspended in 200 μL of lysozyme and incubated at 37 °C for 30 min. The supernatant was removed, 20 μL of proteinase K was added to the tube, and the tube was incubated at 60 °C for 10 min. DNA was lysed and bound to the GD column. The gDNA was washed and eluted in a collection tube. The purified gDNA was collected into one micro-centrifuge tube and centrifuged at 15,000 × g for 30 s. The concentration of gDNA was determined spectrophotometrically in a Nanodrop instrument and kept at -20 °C until library construction.

2.4. Sequencing of 16S rRNA gene and microbiota analysis

For each sample, 10 ng of precipitate was used to amplify the V3 and V4 region of the 16S rRNA gene following the procedure developed by Illumina MiSeq System. (Primer:16S Amplicon PCR Forward Primer = 5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCTACGGGNGGCWCGAGACAG™ GCAG, 16S Amplicon PCR Reverse Primer = 5'TCTCGTGGGCTGCTGGTAATTCCGGACTACHVGGGTATCTAATCC and adaptor sequences: Forward overhang: 5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG™ GCAGACAG-locus specific sequence) Reverse overhang: 5'TCTCGTGGCAGAGCGGTATCTAATCC and locus specific sequence); this method also used molecular barcodes to enable multiplex sequencing as previously described [20]. Paired-end sequencing (2 × 150 base pairs [bp]) of these amplicons was performed on a desktop sequencer (MiSeq; Illumina, Inc., San Diego, CA, USA). 16S rRNA gene pipeline data acquisition incorporated phylogenetic and alignment-based approaches to
maximize data resolution. Read pairs were demultiplexed based on the unique molecular barcodes, and reads were merged using FlASH v1.2.11 [21] with at least a 50-bp overlap and no more than 1-bp mismatch. Merged sequences were clustered into operational taxonomic units (OTUs) at a similarity cut-off value of 97% using the CD-HIT-OTU program. An expected error rate of 0.5 was applied for quality filtering. We mapped OTUs to the rDnaTools database to determine taxonomies [22]. QIIME was used to cluster the operational taxonomic units (OTUs), and constructed an OTU table from the output files generated in the previous two steps for downstream analyses of alpha diversity (observed OTUs, Chao 1 estimator, and Shannon diversity index; confirm species diversity), beta diversity (unweighted and weighted UniFrac; visualize the community diversity), and taxonomic trends (at the phylum and genus level).

2.5. Scanning electron microscopy (SEM)

Bacterial accumulation on the used CLs was examined by SEM on the representative CL surface as the contamination subjects. The lenses were cut into two pieces and fixed in 2.5% glutaraldehyde/0.1 M cacodylate buffer pH 7.4, at 4°C overnight. The sample was resuspended in 0.1 M cacodylate buffer pH 7.4 at 4°C and secondarily fixed with 1% osmium tetroxide (OsO₄) (OsO₄; Electron Microscopy Sciences, Hatfield, PA) in cacodylate buffer for 1 h at room temperature. All samples were dehydrated in a graded ethanol series of 20%, 40%, 60%, 80%, 90%, and finally two changes of absolute ethanol [23]. The lenses were subjected to electron microscopy at the Center for Scientific and Technological Equipment (CSE), Walailak University, according to the following protocol. Samples were dried immediately, mounted on aluminium stubs, and sputter-coated with gold in the vacuum chamber of a Cressington 108 Auto Sputter Coater (Cressington Scientific Instruments, UK). Visualization was performed under a scanning electron microscope (Merlin Compact, Zeiss, Germany).

2.6. Statistical analysis

All statistical analyses were performed using SPSS software (version 23.0; SPSS Inc., Chicago, IL). The data were analyzed for both descriptive and inferential statistics. Continuous variables were described using the mean and standard deviation. Independent categorical variables were
Table 2 CL wearers hygiene behaviors.

| CL care behaviors                                      | N = 20 (%) |
|-------------------------------------------------------|------------|
| Always check expiration date and integrity of packaging before use |            |
| Yes                                                   | 17 (85%)   |
| No                                                    | 3 (15%)    |
| Check for the correct side (inside-outside) before use |            |
| Yes                                                   | 18 (90%)   |
| No                                                    | 2 (10%)    |
| Start inserting and removing the lens from the same eye |            |
| Yes                                                   | 17 (85%)   |
| No                                                    | 3 (15%)    |
| Continued using the lens that had been dropped        |            |
| Yes                                                   | 10 (50%)   |
| No                                                    | 10 (50%)   |
| Hand wash before putting in the CLs                   |            |
| With water only                                       | 4 (20%)    |
| With soap                                             | 10 (50%)   |
| Not done                                              | 6 (30%)    |
| Routine before putting in the CLs                     |            |
| Rub the lenses                                        | 1 (5%)     |
| Rinse the lenses                                      | 7 (35%)    |
| Rub and rinse the lenses                              | 8 (40%)    |
| No management                                         | 4 (20%)    |
| Hand wash before CLs removal                          |            |
| With water only                                       | 4 (20%)    |
| With soap                                             | 10 (50%)   |
| Not done                                              | 6 (30%)    |
| Routine after CLs removal                             |            |
| Rub the lenses                                        | 1 (5%)     |
| Rinse the lenses                                      | 3 (15%)    |
| Rub and rinse the lenses                              | 9 (45%)    |
| None                                                  | 7 (35%)    |
| Products used to clean the CL                         |            |
| CL cleaning solution                                  | 13 (65%)   |
| NSS                                                   | 5 (25%)    |
| Tap water                                             | 2 (10%)    |
| Soaking CLs in the cleaning solution for ≥6 h before reuse |        |
| Yes                                                   | 19 (95%)   |
| No                                                    | 1 (5%)     |
| Fill CL case with fresh CL solution every day         |            |
| Yes                                                   | 20 (100%)  |
| No                                                    | 0          |
| Topping off the old cleaning solution                 |            |
| Yes                                                   | 7 (35%)    |
| No                                                    | 13 (65%)   |
| Close the cap of the cleaning solution tightly after use |          |
| Yes                                                   | 15 (75%)   |
| No                                                    | 5 (25%)    |
| Keep using the same bottle of cleaning solution for more than 3 months |       |
| Yes                                                   | 1 (5%)     |
| No                                                    | 19 (95%)   |
| Close the CL case tightly after use                   |            |
| Yes                                                   | 19 (95%)   |
| No                                                    | 1 (5%)     |
| Keep using the same case for more than 3 months       |            |
| Yes                                                   | 4 (20%)    |
| No                                                    | 16 (80%)   |
| Clean the CL case with                                 |            |
| Water only                                            | 9 (45%)    |
| Water and soap                                        | 5 (25%)    |

Table 2 (continued)

| CL care behaviors                                      | N = 20 (%) |
|-------------------------------------------------------|------------|
| With CL solution                                      | 6 (30%)    |
| Not done                                              | 0          |
| Clean the CL case daily                               |            |
| Yes                                                   | 9 (45%)    |
| No                                                    | 11 (55%)   |

described using frequencies and expressed as percentages. For the continuous variables, a t-test (two groups) was used to compare the groups. The chi-square test was used to examine bivariate associations between independent variables and pathogen-related eye infections. A P value <0.05 was considered statistically significant for the group comparison.

3. Results

3.1. Demographic data and behaviour of the CL wearers

A total of 20 CL wearers were enrolled and completed the questionnaires in this study. A summary of the participants’ demographic data is shown in Table 1. All participants were female with a mean age of 35.2 years, ranging from 17-58 years. Forty-five percent of the participants had a postgraduate degree education. All subjects wore soft reusable CLs, with the majority wearing monthly disposable lenses and 80% of the participants wearing CLs every day. Although it is generally recommended not to wear CLs more than 8 h per day, this survey found that 90% of subjects wore CLs longer than the recommendation. All CLs were purchased from non-health care professionals. Most of the participants (95%) also bought CL care solutions from non-health care professionals. Moreover, data revealed that 35% had a history of eye infections, including keratitis, conjunctivitis, and blepharitis. The undesirable activities found in this study were skipping annual eye check-ups, wearing CLs in water (such as swimming, diving, and shower), exceeding the recommended planned replacement of CLs and storage cases, and applying eye makeup.

The CL wearers’ hygiene behaviour is demonstrated in Table 2. Most of the subjects had good practices in CL care, such as checking the expiration of the CL product and solutions, checking the side of the lens, and washing hands with soap before putting in and taking off the CL. Impressively, most performed the correct routine by the drop-rub-rinse regimen after wearing the CLs and even before. Moreover, they mostly followed the correct routine regarding the use of the CL care solution and its storage case, such as always renewing the cleaning solution, keeping the bottle clean, and not using the same CL case for more than 3 months. However, half of the participants continued to use lenses that had been dropped, which might have been contaminated, and had an improper CL case care regimen by cleaning them with tap water only.

3.2. Culture selected bacterial community on CLs

The 20 CL samples were coded as CL-1 to CL-20. The bacteria that were cultured from the CLs were detected and identified by microbiota analysis at the phylum to species level by 16S rRNA amplicon sequencing data. A bacterial microbiota study revealed that a total of 19 genus and 26 isolated trains were obtained from all CLs. Among the genus, Enterobacter, Staphylococcus, and Achromobacter were the most abundant representing 27.51%, 26.18% and 17.41% of total population, respectively. The isolated strains and CL care behaviours of each subject are shown in Table 3. The bacterial constituents of each sample are illustrated in Figure 1. The overall abundance of bacteria showed that Enterobacter mori, Staphylococcus pasteuri, and Achromobacter agilis were the 3 most predominant species, representing 27.51%, 26.17%, and
Table 3. Sample collection and culture-selected microbial community.

| Subject ID | Age, sex | Past-history of eye infection or symptoms | Behavior                                                                 | Isolated species | % found on CL |
|------------|----------|------------------------------------------|--------------------------------------------------------------------------|------------------|---------------|
| CL-1       | 38, Female | Abscess                                  | - Sleeping or napping in CLs                                              | S. periodonticum  | 98.91         |
|            |          |                                          | - Exceed the recommended period of CL                                    |                  |               |
|            |          |                                          | - Wearing in water                                                       |                  |               |
|            |          |                                          | - No hand-washing before taking out - Clean the CL case with tap water   |                  |               |
|            |          |                                          | - Use CL case more than 3 months                                         | S. adiacens      | 1.09          |
| CL-2       | 18, Female | Never                                    | - Sleeping or napping in CLs                                              | S. pasteurii     | 87.24         |
|            |          |                                          | - Exceed the recommended period of CL                                    |                  |               |
|            |          |                                          | - Wearing in water                                                       | S. periodonticum | 9.03          |
|            |          |                                          | - Without soap                                                           | A. junii         | 3.67          |
|            |          |                                          | - Soaking CL with normal saline solution                                 | S. epidermidis   | 0.04          |
|            |          |                                          | - Reuse old CL solution                                                  | B. albigilva     | 0.01          |
| CL-3       | 17, Female | Never                                    | - Exceed the recommended period of CL and solution                        | S. pasteurii     | 98.21         |
|            |          |                                          | - Wearing in water                                                       |                  |               |
|            |          |                                          | - Without soap                                                           | P. aeruginosa    | 1.19          |
|            |          |                                          | - Soaking CL with normal saline solution                                 | P. stutzeri      | 0.53          |
|            |          |                                          | - Reuse old CL solution                                                  | A. agilis        | 0.07          |
| CL-4       | 17, Female | Never                                    | - Sleeping or napping in CLs                                              | S. pasteurii     | 99.99         |
|            |          |                                          | - Exceed the recommended period of CL and solution                        |                  |               |
|            |          |                                          | - Wearing in water                                                       |                  |               |
|            |          |                                          | - Without soap                                                           |                  |               |
|            |          |                                          | - Reuse old CL solution                                                  |                  |               |
| CL-5       | 46, Female | Keratitis                                 | - Wearing in water                                                       | S. pasteurii     | 50.20         |
|            |          |                                          | - Clean the CL case with tap water                                       |                  |               |
|            |          |                                          | - No clean CL case                                                       | B. albigilva     | 49.80         |
| CL-6       | 37, Female | Never                                    | - Exceed the recommended period of CL                                    | S. pasteurii     | 99.99         |
|            |          |                                          | - Wearing in water                                                       |                  |               |
|            |          |                                          | - Shower while wearing CL                                                |                  |               |
|            |          |                                          | - Soaking CL with normal saline solution                                 |                  |               |
| CL-7       | 33, Female | Never                                    | - Exceed the recommended period of solution                              | S. pasteurii     | 100.00        |
|            |          |                                          | - Wearing in water                                                       |                  |               |
|            |          |                                          | - Soaking CL with normal saline solution                                 |                  |               |
| CL-8       | 27, Female | Conjunctivitis                            | - Exceed the recommended period of solution                              | S. pasteurii     | 99.99         |
|            |          |                                          | - Wearing in water                                                       |                  |               |
|            |          |                                          | - Soaking CL with normal saline solution                                 | S. geniculata    | 0.10          |
| CL-9       | 31, Female | Never                                    | - Exceed the recommended period of CL and solution                        | S. pasteurii     | 100.00        |
|            |          |                                          | - Wearing in water                                                       |                  |               |
|            |          |                                          | - Without soap                                                           |                  |               |
|            |          |                                          | - No rub and rinse CL before soaking                                      |                  |               |
| CL-10      | 49, Female | Never                                    | - Exceed the recommended period of CL                                    | S. pasteurii     | 72.89         |
|            |          |                                          | - Wearing in water                                                       |                  |               |
|            |          |                                          | - Clean the CL case with tap water                                       | P. geniculata    | 24.63         |
|            |          |                                          | - Soaking CL with normal saline solution                                 | S. maltophilia   | 2.45          |
|            |          |                                          | - No rub and rinse CL before soaking                                      | E. mori          | 0.02          |
|            |          |                                          | - Reuse old CL solution                                                  | S. pasteurii     | 0.01          |
| CL-11      | 48, Female | Conjunctivitis                            | - Exceed the recommended period of CL                                    | S. pasteurii     | 75.03         |
|            |          |                                          | - Wearing in water                                                       |                  |               |
|            |          |                                          | - No hand washing before putting on and taking out - Reuse old CL solution|                  |               |
|            |          |                                          | - Clean the CL case with tap water                                       | E. mori          | 8.71          |
| CL-12      | 33, Female | Keratitis                                 | - Sleeping or napping in CLs                                              | S. pasteurii     | 89.93         |
|            |          |                                          | - Exceed the recommended period of CL                                    |                  |               |
|            |          |                                          | - Wearing in water                                                       | E. mori          | 8.71          |
|            |          |                                          | - No hand washing before putting on and taking out - Reuse old CL solution| S. pasteurii     | 1.24          |
| CL-13      | 33, Female | Abscess                                  | - Wearing in water                                                       | S. pasteurii     | 54.07         |
|            |          |                                          | - No rub and rinse CL before putting on - Soaking CL with tap water       |                  |               |
|            |          |                                          | - Reuse old CL solution                                                  | A. lactucae      | 32.62         |
|            |          |                                          | - Clean the CL case with tap water                                       | S. pasteurii     | 8.84          |
| CL-14      | 58, Female | Never                                    | - Sleeping or napping in CLs                                              | S. pasteurii     | 50.20         |
|            |          |                                          | - Exceed the recommended period of CL                                    |                  |               |
|            |          |                                          | - Wearing in water                                                       | E. mori          | 49.80         |
|            |          |                                          | - No rub and rinse CL                                                     |                  |               |
|            |          |                                          | - Use CL case more than 3 months                                         |                  |               |

(continued on next page)
16.16% of the total population, respectively. Moreover, the bacterial isolates were justified as pathogens related to eye infections according to their contamination sources and background of causing the disease, as shown in Table 4. The main pathogens that were found in the present population were represented by 13 strains: *Serratia surfactanfaciens* (10%), *Staphylococcus epidermidis* (5%), *S. pasteuri* (65%), *Stenotrophomonas maltophilia* (5%), *Pseudomonas aeruginosa* (10%), *Delftia tsuruhatensis* (5%), *Acinetobacter calcoaceticus* (5%), *Granulicatella adiacens* (5%), *Raoultella planticola* (10%), *R. ornithinolytica* (5%), *A. agilis* (35%), *Pseudomonas stutzeri* (5%), and *Acinetobacter junii* (5%). Sources of
contamination are normally residential water, rivers, soil, mud, and some plants [19, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41], which might be related to the CL care routines and nonhygienic environments. For contamination by nonpathogenic species, 13 strains were found in the rhizosphere and sometimes cause plant diseases, the bacterial genera consisting of Aquabacterium, Sphingomonas, Streptococcus, Streptophyta, and Methylobacterium [42, 43, 44]. The members of this putative core are the permanent residents of the ocular surface, despite occasional changes and the introduction of other bacteria. Recently, alteration of the ocular microbiome associated with CLs has been reported [45]. The microbial community in the presence of CL wear was found to be more variable than the community from the normal ocular surface, reflecting more of the skin flora, with higher abundances of Methyllobacterium, Lactobacillus, Acinetobacter, and Pseudomonas, while Haemophilus, Streptococcus, Staphylococcus, and Corynebacterium showed lower abundances. Despite the close contact of the CL and the ocular surface, the microbiota of the CL surface was markedly different from those of the conjunctiva. The major microbes isolated from CL were previously reported, including coagulase-negative staphylococci (CNS), Propionibacterium, and Haemophilus, which was found in 5% of the studied population. This was shown to be statistically significant in this study, with P values of 0.04 and 0.01, respectively.

3.4. Visualization of CLs

SEM was used to visualize the ultrastructure of bacterial accumulation on the CL surface. Figure 2A shows the surface of a CL that was taken from a control following the correct CL routine, such as hand washing with soap before applying and taking out the CL, not exceeding the recommended wearing period, and dropping-rubbing-rinsing the surface with CL care solution. This control was utilized as a reference for comparison with the CL from a subject with unfavourable behaviours (Figure 2B, C). The control CL shows a clear surface without bacterial attachment, whereas Figure 2B shows large amounts of bacterial adhesion. Additionally, the SEM photograph revealed typical biofilm morphologies and dense networks of cells arranged in multiple layers, forming microcolonies with the visible granular extracellular matrix of both gram-positive and gram-negative bacteria (Figure 2C).

4. Discussion

The ocular surface is a newly described niche with the unique characteristics of microbiota. Its consortium comprises a ‘putative core’ of 12 bacterial genera consisting of Pseudomonas, Propionibacterium, Bradyrhizobium, Corynebacterium, Acinetobacter, Brevundimonas, Staphylococci, Aquabacterium, Sphingomonas, Streptococcus, Streptophyta, and Methylobacterium [42, 43, 44]. The members of this putative core are the permanent residents of the ocular surface, despite occasional changes and the introduction of other bacteria. Recently, alteration of the ocular microbiome associated with CLs has been reported [45]. The microbial community in the presence of CL wear was found to be more variable than the community from the normal ocular surface, reflecting more of the skin flora, with higher abundances of Methyllobacterium, Lactobacillus, Acinetobacter, and Pseudomonas, while Haemophilus, Streptococcus, Staphylococcus, and Corynebacterium showed lower abundances. Despite the close contact of the CL and the ocular surface, the microbiota of the CL surface was markedly different from those of the conjunctiva. The major microbes isolated from CL were previously reported, including coagulase-negative staphylococci (CNS), Propionibacterium sp., and Corynebacterium sp [46]. Another study of alterations in soft CL wearers also detected the constituents of Streptococcus, Methyllobacterium, and Acinetobacter in the bacterial microbiome data [45].

This present study provided more insight into the nature of the bacterial microbiota on CLs. The 20 collected CLs contained 19 genera and 26 strains of bacteria. Additionally, 13 of these strains have been recognized as the causative pathogens of ocular infections or CL-related infections. The species with the highest predominance was E. mori. This bacteria is not a major pathogen causing disease in humans but rather in plants. However, it was mentioned in a case report as causing otitis externa in a 59-year patient in Austria [47]. Since bacteria are usually found in the rhizosphere and sometimes cause plant diseases, the detection of this pathogen at high levels indicates the contamination of soil and water [47].

The most ubiquitous gram-positive bacterium found in this study was S. pasteuri, a pathogenic bacterium with a coagulase-negative reaction that normally colonizes human skin or acts as a contaminant in water, food products, and unsanitary environments [23]. Contamination can occur by inappropriate handling of the CL during the process of wearing and taking off. Another Staphylococcus sp. found in this study was S. epidermidis, which was found in 5% of the studied population. This was in contrast to results from a previous study, which suggested that Staphylococcus sp. established on ocular surfaces in healthy adults represents as much as 73% of the bacterial community, especially S. epidermidis [48]. Aside from the skin flora discussed above, the gram-negative bacteria A. agilis showed a high abundance in the bacterial microbiota constituents in this study, which indicated that the contamination might be from pollutants in the surroundings [36, 47]. The source of A. agilis was previously studied and the strain was obtained from soil as a rhizobacterial flora [37, 38]. The microbiota on the CLs reflected external

### Table 4. Pathogens related eye infections.

| Pathogens                | % found in population (n = 20) | Source                          | References             |
|-------------------------|-------------------------------|---------------------------------|------------------------|
| S. surfactanficiens     | 10 water and marine environments, contaminated soil, plants, animals, hospitalized patients | Grimmont (2006) [44]; Su et al. (2016) [25] |
| S. epidermidis          | 5 human skin, upper respiratory tract | Du et al. (2021) [26] |
| S. pasteurii            | 65 drinking water, common skin flora, food products, air | Santeoimma et al. (2020) [27] |
| S. malophilia           | 5 soil, sediment, wastewater, sputum | Ma et al. (2020) [28]; Al-Dhabi et al. (2021) [29] |
| P. aeruginosa           | 10 CLs, wet surfaces, chronic infection sites | Enzor et al. (2021) [30]; Riquelme et al. (2020) [31] |
| D. tsuruhatensis        | 5 soil, water, sludge, human microflora, CLs | Hotta et al. (2020) [19] |
| A. calcoaceticus        | 5 Soil, water | Roy et al. (2013) [32] |
| G. adiacens             | 5 human oral cavity, urogenital tract, gastrointestinal tract | Borroni (2002) [33] |
| R. planticola           | 10 vegetables, food, liquid soap | Vansalto et al. (2016) [34] |
| R. ornitholytica        | 5 CLs, water, urine, wounds | Eguici et al. (2017) [35]; |
| A. agilis               | 35 rivers, ponds, residential water sources, soil, mud, some plants | Price et al. (2020) [36]; Aghaji et al. (2020) [37]; Vansdamme et al. (2016) [38] |
| P. stutzeri             | 5 soil, water | Gildari (1972) [39]; Lalacat et al. (2006) [40] |
| A. junii                | 5 water, soil, animals | Broniek et al. (2014) [41] |
Excluding the recommended period of CL

Excluding the recommended period of CL solution

Wearing in water

Wearing in shower

Hand washing

With soap

Rub and rinse CL

Rub and rinse CL

Reuse old CL solution

Soaking CL with normal saline solution

Clean the CL case with tap water

History of eye infection

Table 5. Risk behaviors related to main pathogens causing eye infections.

| Risk factor                  | Achromobacter agilis | P-value | Entero bacteri mori | P-value |
|------------------------------|-----------------------|---------|---------------------|---------|
|                             | Yes N (%)            | No N (%)| Yes N (%)           | No N (%)|
| Sleeping or napping in CLs  | 5 (71.43)            | 6 (46.15)| 0.27               | 4 (44.44)| 0.39 |
| No                           | 11 (55)              | 7 (53.85)| 4 (57.14)          | 0.88    |
| Yes                          | 9 (45)               | 6 (46.15)| 3 (42.86)          |         |
| Exceed the recommended period of CL | 7 (28.57) | 7 (53.85) | 0.17               | 3 (27.27)| 0.75 |
| No                           | 5 (25)               | 2 (15.38)| 3 (42.86)          | 0.16    |
| Yes                          | 15 (75)              | 11 (84.62)| 4 (57.14)         |         |
| Exceed the recommended period of CL solution | 7 (100) | 8 (61.54) | 0.058              | 8 (72.73)| 0.77 |
| No                           | 13 (65)              | 8 (61.54)| 5 (71.43)          | 0.65    |
| Yes                          | 7 (35)               | 5 (38.46)| 2 (28.57)          |         |
| Wearing in water             | 2 (28.57)            | 4 (30.77) | 0.48               | 2 (18.18)| 0.82 |
| No                           | 4 (20)               | 1 (7.69) | 3 (42.86)          | 0.06    |
| Yes                          | 16 (80)              | 12 (92.31)| 5 (71.43)         |         |
| Wearing in shower            | 1 (14.29)            | 2 (15.38) | 0.94               | 3 (27.27)| 0.08 |
| No                           | 3 (15)               | 1 (7.69) | 2 (28.57)          | 0.21    |
| Yes                          | 17 (85)              | 12 (92.31)| 5 (71.43)         |         |
| Hand washing                 | 3 (22.08)            | 3 (23.08) | 0.35               | 2 (18.18)| 0.05 |
| No                           | 6 (30)               | 4 (30.77)| 2 (28.57)          | 0.91    |
| Yes                          | 14 (70)              | 9 (69.23)| 7 (51.43)          |         |
| With soap                    | 4 (30.77)            | 3 (23.08) | 0.35               | 2 (18.18)| 0.49 |
| No                           | 10 (50)              | 7 (53.85)| 3 (42.86)          | 0.63    |
| Yes                          | 10 (50)              | 6 (46.15)| 4 (57.14)          |         |
| Rub and rinse CL             | 5 (71.43)            | 5 (38.46) | 0.16               | 5 (45.45)| 0.65 |
| No                           | 7 (35)               | 6 (46.16)| 1 (14.29)          | 0.15    |
| Yes                          | 13 (65)              | 7 (53.84)| 6 (86.71)          |         |
| Reuse old CL solution        | 4 (30.77)            | 3 (23.08) | 0.58               | 2 (18.18)| 0.08 |
| No                           | 13 (65)              | 7 (53.85)| 6 (85.71)          | 0.15    |
| Yes                          | 7 (35)               | 6 (46.15)| 1 (14.29)          |         |
| Soaking CL with normal saline solution | 4 (30.77) | 3 (23.08) | 0.58               | 2 (18.18)| 0.88 |
| No                           | 15 (75)              | 10 (76.92)| 5 (71.43)         | 0.78    |
| Yes                          | 5 (25)               | 3 (23.08)| 2 (28.57)          |         |
| Clean the CL case with tap water | 4 (30.77) | 3 (23.08) | 0.17               | 8 (72.73)| 0.79 |
| No                           | 11 (55)              | 6 (46.15)| 5 (71.43)          | 0.27    |
| Yes                          | 9 (45)               | 7 (53.85)| 2 (28.57)          |         |
| History of eye infection     | 6 (85.71)            | 5 (38.46) | 0.04*              | 7 (63.64)| 0.39 |
| No                           | 13 (65)              | 10 (76.92)| 3 (42.86)         | 0.12    |
| Yes                          | 7 (35)               | 3 (23.08)| 4 (57.14)          |         |

Note: *A P value < 0.05 was considered statistically significant from chi-square test.

contamination much more than that of the conjunctiva. The sources of the constituents on the lens surfaces were mostly from water, soil, the oral cavity, and the urogenital tract. This difference gave a clearer picture of the microbiological contamination of the CL surface. Furthermore, the association of bacterial contamination with CL wearer behaviour was determined. The results of this study emphasize the danger of using tap water to clean CL cases. CL wearers who used tap water to clean the CL case carried a significantly higher risk of A. agilis contamination (p = 0.04). The CL case was recently acknowledged as the bulk of microbial contamination. The accumulation of bacteria and biofilm formation on the case surface was a commonly susceptible part of the CL care system more than the CLs themselves [49, 50]. This study provided insight into factors that may be significant in maintaining lens case hygiene and explored some of the issues previously proven in the in vitro study that tap-water use was associated with the contamination rate of gram-negative bacteria, particularly the strains Pseudomonas sp., Stenotrophomonas maltophilia, and Achromobacter sp [50]. Moreover, the bacterial pathogens that were reported in this study are commonly found on human skin, the oral cavity, and the urogenital tract, reflecting the non-compliance of CL wearers with hand washing. Seventy percent of the subjects routinely washed their hands before putting in CLs, and only half of them did so before taking off their CLs. The fact that most of the CL care process and CL case drying occurred in restrooms may increase urogenital tract pathogen contamination into the CL care system. In agreement with previous research, this study again highlights the negative effect of improper CL behaviour. The five most common improper CL care practices in Thai CL wearers were wearing CL for longer than recommended, not changing the CL solution, swimming with CLs, rinsing CLs with tap water, and not washing hands before handling the CLs [51]. Although our result showed that cleaning the CL case with tap water is statistically significant associated with A. agilis contamination. The non-significance difference of other behavior might be due to the low number of participants. Further studies of greater sample size would be necessary to confirm these findings. The summation of this information suggests poor hygiene or overlooked pitfalls in CL handling. Most CL wearers received less-than-adequate to no education regarding CL handling at their time of purchase in conjunction with the surprisingly low proportion of CL wearers who seek their CLs and CL care solutions from a health care provider. Thus, no professional advice or patient evaluation for potential risks was ever provided. Appropriate behaviour remains a crucial point for eye care
professionals to emphasize with their patients for a safe CL-corrected vision.

Statement of Ethics

The study protocol was reviewed and approved by the Human Research Ethics Committee of Walailak University (WUEC-20-321-01) before the first volunteer was enrolled, in accordance with the tenets of the Declaration of Helsinki and with international restrictions on this study.

Declarations

Author contribution statement

Lunla Udomwech: Conceived and designed the experiments; Wrote the paper.
Kulwadee Karnjana and Juntamanee Jewboonchu: Performed the experiments; Analysed and interpreted the data.
Phisut Rattanathamma, Udomsak Narkkul and Jakkrit Juhong: Analysed and interpreted the data.
Auemphon Mordmuang: Performed the experiments; Analysed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article supplementary material/referenced in article.

Figure 2. Scanning electron micrographs of bacterial accumulation on the CL surface. (A) A clear surface of a control CL with the correct care routine, such as hand washing with soap before applying and taking out the CL, not exceeding the recommended wearing period, and dropping-rubbing-rinsing the surface with CL care solution. (B,C) Markedly contaminated CL surface of a subject with unfavorable behaviours, at scale bars of 10 μm and 1 μm, respectively.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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