Evaluating Contact Lens Microbial Contamination related to Hand Hygiene Compliance

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1. Introduction

The use of contact lenses (CLs) has become popular due to the convenience that these medical devices provide to wearers, especially to those who have active lifestyles. CLs nowadays come in different prescription ranges, wearing modalities, or materials that wearers can choose to suit their needs and lifestyles. CLs are primarily used for vision correction, therapeutic, and cosmetic purposes with a significant evolving of the contact lens materials over time to lessen negative side effects, support a normal corneal metabolism, and maintain the stability of the tear film (Moreddu *et al.*, 2019). Unlike conventional glasses which have frames that might block the peripheral field of vision, CLs are placed directly on top of the eyes and provide their wearers with a full field of vision (Kirkliauskienė *et al.*, 2024). CLs thus come into direct contact with the living cells and tissues of the eyes and require more care compared to glasses.

Hand hygiene compliance is important in contact lens wear and care as it affects the levels of contact lens contamination. Noncompliance with hand hygiene is the main source of lens contamination and the level of lens contamination is significantly affected by techniques of hand hygiene (Barlow *et al.*, 1994; Ly *et al.*, 1997). Poor hand hygiene prior to lens handling is a risk factor for the development of microbial keratitis and inflammatory events of the corneal (Fonn & Jones, 2019). Thus, it is hypothesized in this study that compliance with good hand hygiene would statistically significantly reduce the levels of microbial contamination found on handled CLs prior to lens insertion, further reducing risk of contact lens-related microbial keratitis development.

Although there is not yet a known cause and effect relationship between a common contact lens-related inflammation such as microbial keratitis and hand hygiene compliance, few studies have been done on examining the efficacy of various hand hygiene protocols on the changing levels of microbial contamination of CLs. This scientific study looks into how various

hand hygiene protocols affect the levels of contact lens microbial contamination associated with the handling procedure of the lens insertion, with a focus on risk-related microbial keratitis development.

2. Materials and Methods

2.1. Hand hygiene methods

Prior to handling the sterile contact lens, the experimenter performed each hand hygiene protocol amongst a total of 4 hand hygiene methods include no handwashing; hand washing with tap water and dry with paper towel; handwashing with soap (Softsoap brand), rinsing with tap water, and dry with paper towel; and hand hygiene using an alcohol-based hand sanitizing gel (Lifebuoy brand) (Ly *et al*., 1997; Morgan *et al.*, 2011). Adapting from a study by Barlow *et al.* (1994), each hand hygiene method was performed on different occasions of the experimental days given a gap of at least 3 hours between each conducted hand hygiene method in order to mitigate the mix-up effects of these hand hygiene methods; and a total of 4 replicates were included for each hand hygiene procedure.

2.2. Handling of sterile contact lenses

All contact lenses used for this experiment were MyDay brand daily disposable, soft contact lenses with -0.5D prescription. Each pre-unpacked lens was handled under a ducted fume hood to minimize the potential contamination of the surrounding environment. All typical lens manipulations prior to lens insertion were conducted in each replicate including eversion of the lens, taco test, and placement of lens on the fingertips for at least 15 seconds (Barlow *et al.*, 1994).

2.3. Culturing of the contaminated CLs

These experimental methods are adapted from the studies by Ly *et al*. (1997) and Mowrey-McKee *et al*. (1992) with modifications. Each handled contact lens was placed in a test tube containing approximately 3.3 mL sterile 0.1% peptone water and then agitated at high speed using a vortex mixer for at least 15 seconds. An approximate 750µL extract of the total

3.3mL the peptone water was inoculated onto tryptic soy agar (TSA), BD BBL™ MacConkey agar, Mannitol salt agar (MSA), and Sabouraud dextrose agar (SDA) using a spread plate method. The TSA, MacConkey agar, and MSA were incubated at 37^0C for 48 hours before manual colony counting was performed and morphologies of colonies were recorded. The SDA was incubated at 25^0C and checked daily for the visible growth of colonies before manual colony counting and morphological recording of colonies were performed.

2.4. Gram stain and biochemical tests on the isolated colonies

Using a streak plate method, each colony from the inoculated TSA was further isolated into a new TSA based on their characterized morphologies to ensure that isolates were pure colonies. The inoculated TSA with presumptive isolates were incubated for 48 hours and then stored in plastic bags in the refrigerator at 5^0C for a maximum of 2 weeks to keep the isolates from dehydration and died off.

These methods are adapted from studies by Ly *et al*. (1997) and Kirkliauskienė *et al.* (2024) with modifications.Gram staining was performed on these isolated colonies as well as SDA colonies. Bacterial isolates were further characterized using biochemical tests where catalase test, bacitracin susceptibility test (0.04U), mannitol salt, and coagulase test were included for identification of the unknown Gram-positive bacteria while the 5% sheep blood agar was performed to characterize the unknown Gram-negative bacteria.

2.5. Statistical analysis of data

Data are presented as mean ± standard deviation. The F-test for variance was conducted to determine if the variance of each hand hygiene method's replicate was equal or unequal to the variance of the corresponding replicate of the no hand washing method. The two independent sample t-test with either unequal variance or equal variance was then performed to examine for statistically significant difference between the average number of colony growth in each replicate of each hand hygiene method and no hand washing method. Statistical significance was determined at $p < 0.05$ in all tests.

3. Results

3.1. Microbial colony growth on the inoculated TSA media

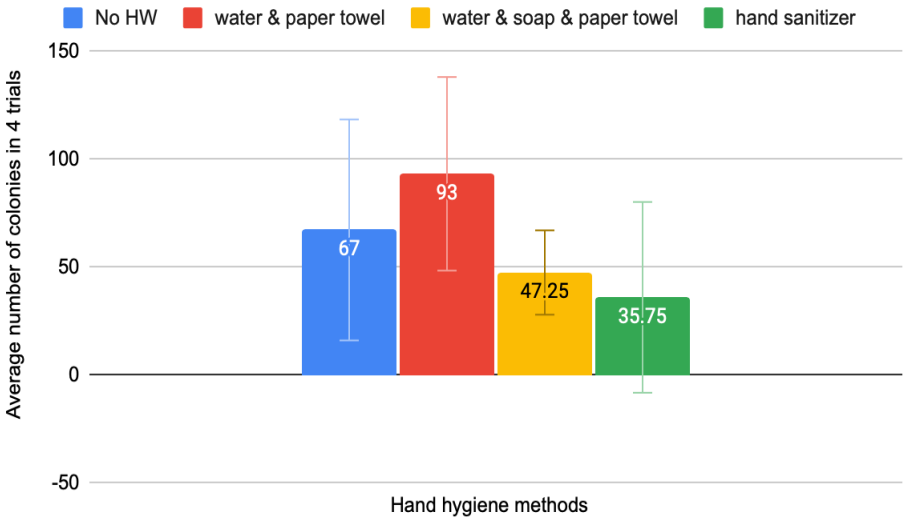
Table 1

Summary of manually counted number of colony growth on the inoculated TSA

Trial	1	2	3	4
No HW	26	27	82	133
Water & towel	>300 (TNTC)	113	128	38
Soap & water & towel	19	53	53	64
Sanitizing gel	5	13	24	101

Figure 1

Average number of microbial colony growth on the inoculated TSA



Note: Number of colony growth in the trial 1 of water + towel use is not included as it was TNTC

The manually counted numbers of microbial growth on the inoculated TSA in all replicates were summarized in **Table 1** and the morphologies of 4 identifiable microbial isolates across experimental replicates were described in **Table 2**. The average number of microbial growth was highest (93 ± 48.22 colonies) in the hand hygiene method where the experimenter's hands were washed with tap water and dried with a paper towel (**Figure 1**). The method of no handwashing was recorded having the second highest average number of microbial growth (67

± 51.19 colonies) in all replicates (**Figure 1**). The method of handwashing using soap, water, and dry with a paper towel had the second lowest mean number of microbial growth (47.25 ± 19.53 colonies) while the use of alcohol-based sanitizing gel had the lowest mean number of microbial isolates (35.75 ± 44.19 colonies) throughout all replicates (**Figure 1**). The differences in the average number of microbial colony growth between each method of hand hygiene versus no handwashing was found to be not statistically significant using two independent sample t-tests with equal variances ($p > 0.05$).

Table 2

Characterized morphologies of microbial isolates on the inoculated TSA

Unknown	Recorded morphologies of isolated colony
1	White, circular, entire edge, raised elevation, opaque, shiny
2	Grey, circular, entire edge, raised, opaque, dull
3	Grey-yellowish, circular, entire edge, umbonate, opaque, dull
4	White, rhizoid, raised, opaque

3.2. Bacterial growth on the inoculated MSA media

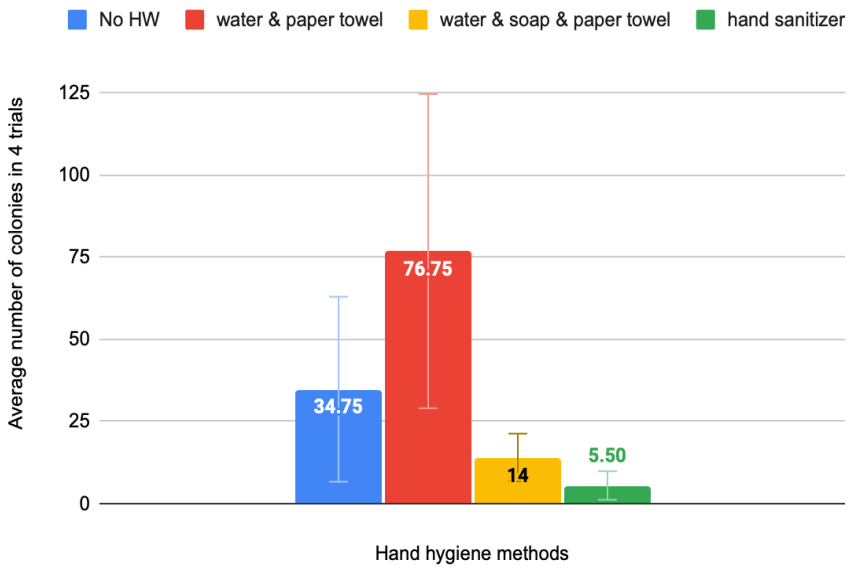
Table 3

Summary of manually counted number of colony growth on the inoculated MSA

Trial	1	2	3	4
No HW	29	19	15	76
Water & towel	117	50	117	23
Soap & water & towel	6	16	11	23
Sanitizing gel	3	4	3	12

Figure 2

Average number of microbial colony growth on the inoculated MSA



The manually counted numbers of bacterial isolates on the inoculated MSA in all replicates of experimental hand hygiene methods were summarized in **Table 3** and a total of 3 different growth patterns of bacterial colonies on the inoculated MSA were recorded in **Table 4**. There was the highest average number of bacterial growth in all replicated trials of hand hygiene method using tap water and drying with a paper towel (76.75 ± 47.77 colonies), followed by the method of no handwashing having the second highest average number of bacterial growth (34.75 ± 28.12 colonies) (**Figure 2**). Hand sanitizing using an alcohol-based gel showed the lowest mean number of bacterial isolates (5.5 ± 4.36 colonies), followed by the handwashing using soap, water, and dry with a paper towel having the second lowest mean number of bacterial growth (14 ± 7.26 colonies) in all replicates (**Figure 2**).

The two independent sample t-test with equal variances were performed for the group of no hand washing versus hand washing using water and dry with a paper towel while two independent sample t-tests with unequal variances were performed for the group of no hand washing versus hand washing using soap, water and dry with a paper towel and hand sanitizing

gel. The differences in the average number of bacterial colony growth between every method of hand hygiene versus no handwashing was found to be not statistically significant ($p > 0.05$).

Table 4

Growth patterns of bacterial colonies on the inoculated MSA

Unknown	Pattern of colony growth on the inoculated MSA media
1	Colony growth that turned agar into white/yellowish
2	Colony growth that turned agar into pink/reddish
3	Colony growth that did not change the agar's color

3.3. Fungal growth in the inoculated SDA media

The manually counted numbers of fungal isolates on the inoculated SDA in all experimental replicates were summarized in **Table 5**. A single type of fungal isolates are identified on the inoculated SDA across the replicates which were morphologically described as white, circular, entire edge, umbonate, opaque, and non-shiny. This type of fungal isolates had a slow growth where it took an average 3-4 days to be visibly observable on the inoculated SDA.

Table 5

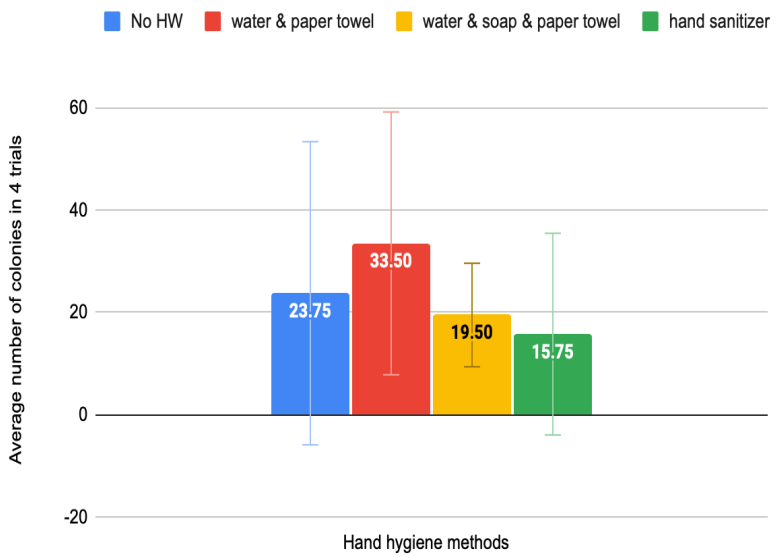
Summary of manually counted number of colony growth on the inoculated SDA

Trial	1	2	3	4
No HW	13	8	6	68
Water & towel	33	70	16	15
Soap & water & towel	11	25	11	31
Sanitizing gel	2	9	7	45

The mean number of fungal growth was highest in the hand hygiene method using tap water and drying with a paper towel (33.50 ± 25.70 colonies) and the method of no handwashing showed the second highest mean number of of fungal growth (23.75 ± 29.65 colonies) (**Figure 3**). Hand sanitizing using an alcohol-based gel showed the lowest mean number of fungal isolates (15.75 ± 19.72 colonies), followed by the handwashing using soap, water, and dry with a paper towel having the second lowest mean number of fungal growth (19.50 ± 10.12 colonies) in all replicates (**Figure 3**). The differences in the mean number of fungal colony growth between each method of hand hygiene versus no handwashing was found to be not statistically significant using two independent sample t-tests with equal variances ($p > 0.05$).

Figure 3

Average number of fungal colony growth on the inoculated SDA media



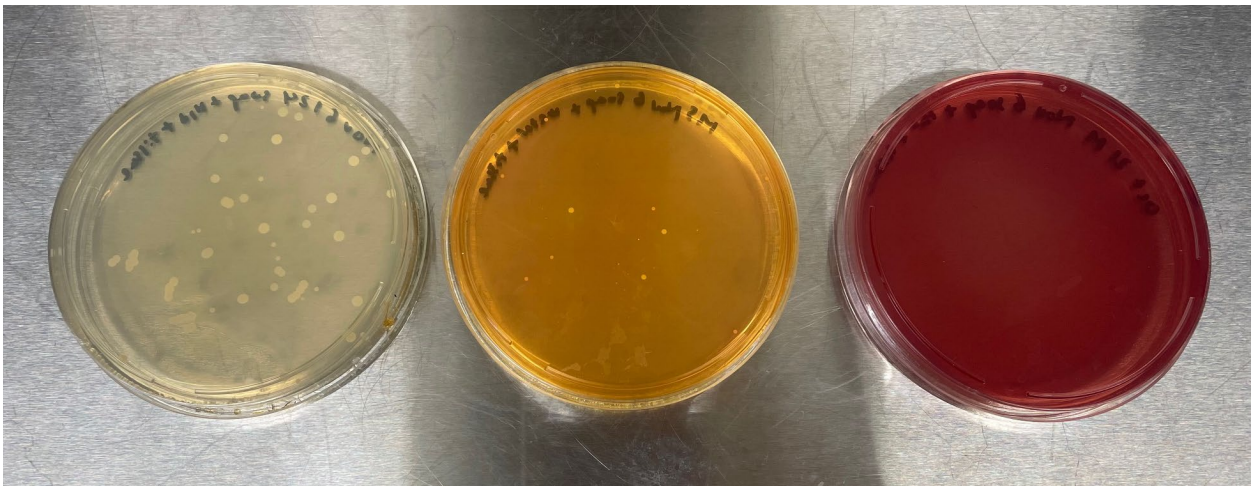
3.4. No bacterial growth on the inoculated MacConkey agar

There was no bacterial growth observed on the inoculated MacConkey media in all replicates of 4 experimental hand hygiene methods and this can be explained by the use of BBL™ MacConkey agar. In the Difco & BBL manual : manual of microbiological culture media

(2nd ed.) by Zimbro and Power (2009), BBL™ MacConkey agar does not support a wide range of non-fastidious Gram negative bacilli.

Figure 4

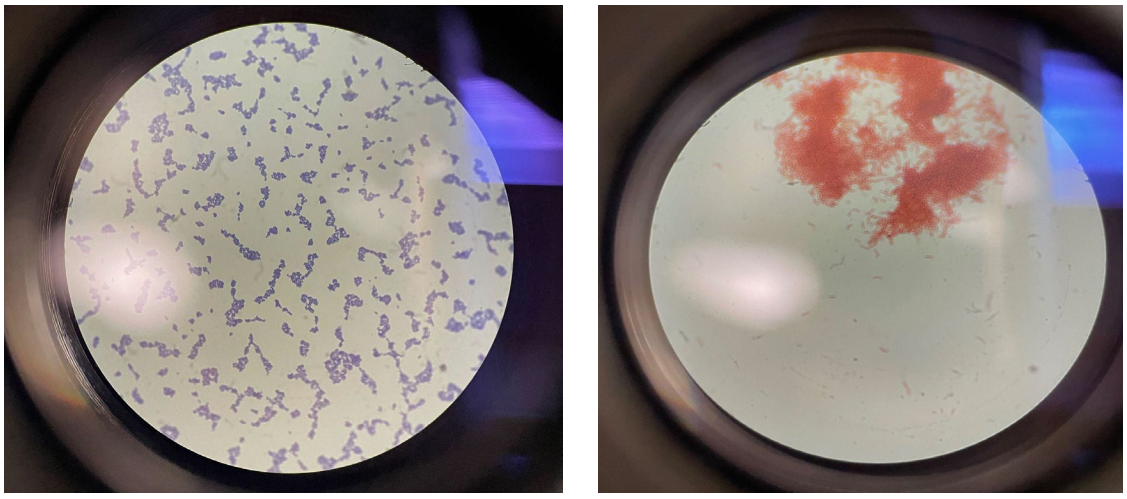
No bacterial growth observed on an inoculated BBL™ MacConkey agar



3.5. Gram stain and biochemical tests for the identification of unknowns

Figure 5

Gram stain results of unknown microbial colonies isolated from the inoculated TSA

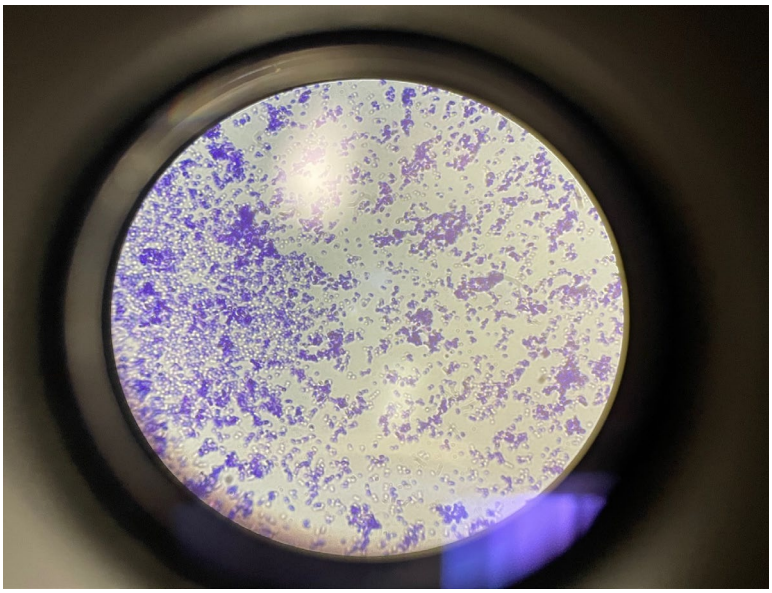


The isolated colonies that are listed as unknown 1, 2, and 3 in the **Table 2** appeared to be Gram-positive cocci under the Gram staining test while the unknown 4 listed in the **Table 2** was characterized as Gram-negative bacilli under Gram-staining (**Figure 5**). The unknown SDA

isolates - characterized by the Gram-staining test, appeared to be larger than cocci-shaped bacteria with many of them being in pairs as they were probably budding or reproducing, and they were also not colored with resistance to take up a safranin counterstain (**Figure 6**).

Figure 6

Gram stain result of the unknown fungal isolates on the inoculated SDA



The unknown Gram-positive cocci number 1, 2, and 3 listed in the **Table 2** all showed positive results in the catalase tests. In the bacitracin susceptibility tests (0.04U), these unknowns showed to be susceptible (or sensitive) to bacitracin and no hemolysis of the blood agar (**Figure 7**). This result seems to be contradictory with the observations in the inoculated MSA in the **Table 4** where some colony growth turned the agar into pink/reddish or white/yellowish, suggesting the presence of *Staphylococcus* spp. It was speculated that the results of these bacitracin tests were false-positive due to the non-confluent growth of bacterial isolates on the tested blood agar. The unknown Gram-negative bacilli number 4 listed in the **Table 2** showed resistance to bacitracin and no hemolysis of the blood agar.

The isolated Gram-positive, catalase-positive cocci unknowns were streaked onto the mannitol salt agar media where their growth turned the agar either into pink/reddish or

white/yellowish (**Figure 8**). The subsequent coagulase tests of these unknowns did not show visibly clear results with minor coagulations observed in some replicates of the tests.

Figure 7

Results of the bacitracin susceptibility test (0.04U)

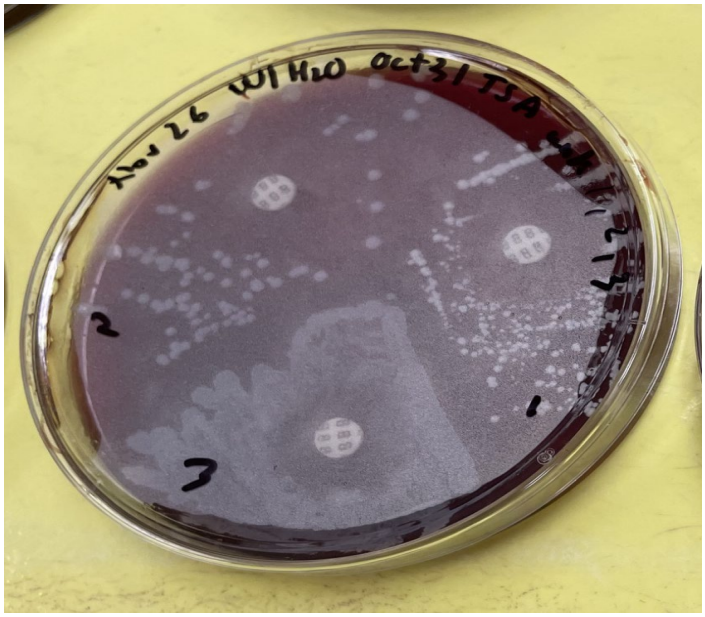
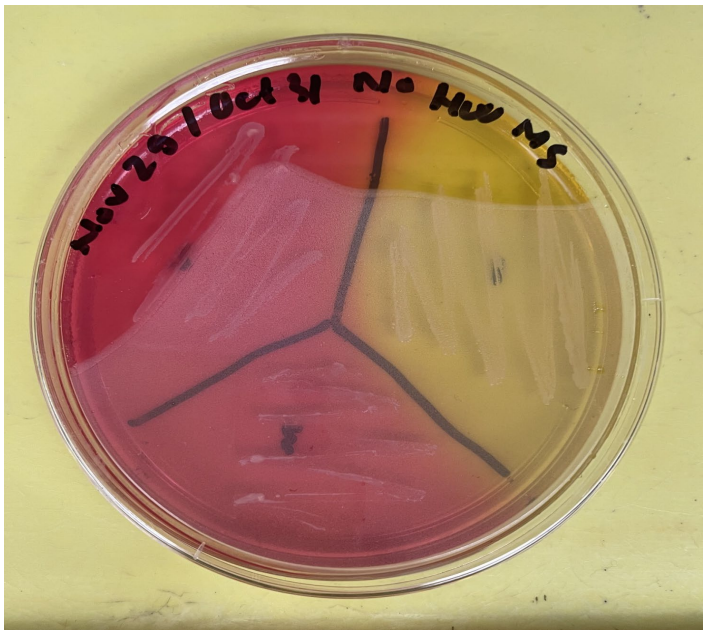


Figure 8

The growth of Gram-positive, catalase-positive unknowns in the MSA



4. Discussion

The main result of this research showed that there was a higher level of microbial, bacterial, and fungal contamination on handled soft CLs when there was poor hand hygiene practiced prior to lens wear. This result somewhat aligns with the previous study's result by Barlow *et al.* (1994) where it was found that the practice of hand washing with antibacterial liquid soap prior to lens insertion was more effective than other hand hygiene methods including the use of non-antibacterial soap bar, hand washing with water only, and no hand washing in controlling the transferred amount of bacterial contamination from hands to the handled hydrogel CLs. Fonn and Jones (2019) emphasized that hand hygiene is recommended by eye care practitioners as it helps to minimize the level of microbial contamination transferred from hands to contact lenses and contact lens wearers' poor hand hygiene is specifically indicated by inadequacy or absence of hand washing.

The absence of or inadequate hand hygiene in contact lens wear and care has been statistically examined in several studies where a significant proportion of contact lens wearers reported to have poor hand hygiene. An online survey conducted in the U.S. showed that 41% of the total of 950 surveyed individuals who self-described themselves as daily disposable contact lens wearers did not do hand washing with soap prior to inserting lenses and 15% of this surveyed population hardly or did not wash their hand ever before lens insertion (Osborn *et al.*, 2017). Another internet-based survey conducted in the U.S. showed that just slightly more than half of the participants who are non-daily soft contact lens wearers wash their hands with soap prior to lens handling in the morning and in the evening (Hickson-Curran *et al.*, 2011). These statistics on hand washing behaviors of contact lens wearers emphasize the use of soap in the hand washing procedure, so inadequate hand hygiene might include the lacking use of soap in the hand washing procedure of contact lens wearers. A study on behaviors and attitudes of contact lens wearers towards lens hygiene and care suggested that poor or

inadequate hand washing protocols should be worded with the no use of soap (Wu *et al.*, 2010).

Other studies, however, showed contradictory results regarding how good hand hygiene would reduce the microbial contamination of CLs. Ly *et al.* (1997) showed that the use of alcohol wipes was associated with the least number of bacterial contaminants followed by no hand washing where they proposed that various techniques of hand washing possibly dislodge microbes from the hands' finger nails and palms which later attached to the handled lenses. Mowrey-McKee *et al.* (1992) found that hydrogel CLs which were handled by hand washing with soap and water and dried with a paper towel had a higher level of microbial contamination compared to lenses that were worn and later aseptically removed from the eyes. These contradictory results potentially suggest the importance of hand drying in the hand washing process to minimize the re-contamination of hands with pathogenic microbes. In this research, hand drying was done using a paper towel obtained from the biological laboratory where there were possible contaminations, highlighting a possibility for confounding effects of this factor on the actual levels of microbial contamination transferred from the experimenter's hands to lenses. Hands are re-contaminated as microbes in the air blown by air blowing driers deposit on the skin of wet hands, so air blowing driers are recommended for hand drying as an alternative if single use paper towels are not available (McMonnies, 2012). However, the use of a possibly contaminated paper towel in this research might reflect the fact that reusable textile towels that are used to dry the lens wearers' hands prior to lens wear are contaminated with daily use. Kato *et al.* (2023) found that there was a statistically significant higher microbial load and area of microbial film formation in textile towels used for a 6-month period compared to those used for a 2-month period.

The use of alcohol-based hand sanitizer in this research had the lowest levels of microbial contamination transferred from hands to soft CLs which is supported by the study by Ly *et al.* (1997) where hands sanitized using an alcohol wipe had the lowest level of bacterial contamination transferred to hydrogel CLs. This result suggests that a certain amount of

microbial contamination was not transferred from a contaminated towel to hands and later from hands to lens. In fact, the use of hand sanitizer is considered as an alternative to regular handwashing with soap in studies. In an international online survey where roughly 40% of surveyed individuals reported to practice proper handwashing, contact lens wearers' hand hygiene compliance includes hand washing with soap, or an alternative use of hand sanitizer or wet wipe (Morgan *et al.*, 2011). McMonnies (2012) also indicated that when hand washing is not possible, alcohol-based solutions, gels or wipes can be alternatively used with a caution to not leave any remaining alcohol on the hands before lens handling to prevent lens damage and eye irritation.

It is important to reduce the levels of microbial contamination transferred from wearers' hands to CLs because handled CLs are directly placed in the wearers' eyes. Contact lens handling is a source of contact lens-related microbial contamination where lens insertion procedure involves the transfer of contaminants from the hands to lens and then directly from lens to the eyes (Fonn & Jones, 2019; Szczotka-Flynn *et al.*, 2010; Lievens *et al.*, 2017). Specifically, contact lens handling prior to lens insertion includes several manipulation steps such as lens eversion, taco test, and the placement of lens on the fingertips to ensure lens' rightness and free of defects, preventing discomfort and distorted vision resulting from the lens wear (Barlow *et al.*, 1994; Lievens *et al.*, 2017). However, as contact lens wearers have different habits in lens wear, they might not practice all these manipulation steps prior to lens insertion and handled CLs are thus less or more contaminated with the transient flora of wearers' hands. Pathogens mainly attach to contact lens material and form a biofilm which weakens the defense mechanism related to immunity in promoting the development of microbial keratitis (MK) (Maier *et al.*, 2022).

In 2019, about 140 million people use CLs to correct various types of refractive impairments globally and MK is one of the most frequent adverse consequences induced by contact lens use (Moreddu *et al.*, 2019). MK is a sight-threatening corneal inflammation resulting

from scarring and perforation of patients' cornea (Cheng *et al.*, 1999). Causative organisms of MK are commonly found in the environment including bacteria, fungi, and acanthamoebae where bacteria are the most common causative pathogens and usually associated with more severe consequences (Lievens *et al.*, 2017; Cheng *et al.*, 1999; Maier *et al.*, 2022). At least two circumstances should occur in the pathogenesis of contact lens-related MK include a defective epithelial layer of the cornea and the presence of a sufficient amount of pathogens (Maier *et al.*, 2022). Contact lens wear interferes with defense mechanisms evolved by the corneal epithelium in protecting the ocular surface against the invading of microbes such as *P.aeruginosa* (Robertson & Cavanagh, 2008). Although differences in the levels of microbial contamination was found to be not statistically significant in this research, a higher number of microbial contamination transferred from the experimenter's hands with poor hand hygiene to the lens implies a possibility that the amount of microbes carried by the hands with poor hand hygiene are sufficient and pathogenic to cause MK in cases with a defective corneal epithelia caused by contact lens wear.

In cases of contact lens-related bacterial keratitis, the 3 most frequently isolated bacterial species were *Pseudomonas aeruginosa*, *Staphylococcus* spp., and *Serratia marcescens* (Hatami *et al.*, 2021). This is aligned with the result of this research in which Gram staining and catalase tests detected the presence of unknown species of Gram-positive, catalase-positive cocci bacteria on handled CLs. Species of *Staphylococcus* are a part of human skin's microbiota and categorized as Gram-positive, catalase-positive bacteria (Moreddu *et al.*, 2019; Foster, 1996). Specifically, the results of Mannitol salt agar tests in this research indicated the presence of unknown species of Gram-positive, catalase-positive cocci with high salt-tolerance which either have the ability to ferment or non-ferment mannitol, suggesting the presence of presumptive *Staphylococcus aureus* and coagulase-negative species such as *Staphylococcus epidermidis* on handled CLs. Unlike other species of bacteria, Staphylococci can withstand the osmotic pressure of 7.5% NaCl contained in Mannitol salt agar for their

growth in which the yellow agar indicates the breakdown of mannitol by presumptive *Staphylococcus aureus* (Shields & Tsang, 2006). However, this interpretation should be supported with the results of other biochemical tests with a possibility of repeated coagulase tests.

Unfortunately, there was no growth of Gram-negative bacteria in all replicates of this research. Gram-negative bacteria are predominant pathogens in contact lens-related MK with the most common presence of *P. aeruginosa* (Stapleton & Carnt, 2012). This result can be mainly explained by the use of BBL™ MacConkey agar. According to the Difco & BBL manual : manual of microbiological culture media (2nd ed.) by Zimbro and Power (2009), BBL™ MacConkey agar does not support the growth of a wide range of Gram-negative bacteria including *P. aeruginosa*. Besides, the most common causative species of fungal keratitis are Fusarium, Aspergillus, and Candida (Castano *et al.*, 2024). While Fusarium and Aspergillus genera appear branching under microscopic views, the Candida species do not show to resist taking up stain in the Gram's staining (Wacira *et al.*, 2020; Aslam *et al.*, 2015). Therefore, staining and microscopic morphologies of the unknown fungal species in this research are not sufficient to relate it to common causative species of fungal keratitis and it can be presumptively categorized as an unknown yeast species of the human skin's microbiota.

This research also comes with several limitations. There was no inoculated media used as negative controls to test for the sterility of prepared media while the use of BD BBL™ MacConkey agar does not favor the growth of many Gram-negative bacteria, negatively affecting the measure of actual bacterial contamination levels on handled CLs. Several confounding factors such as the use of contaminated paper towels might also be controlled in the future research to examine for the actual microbial contamination that stemmed from different hand hygiene methods. It is also worth noting that the interpretation of this research might be constrained by a small number of replicates and time limit if there is growth of Gram-negative bacteria. Several experimental techniques such as the manual colony counting is

prone to error as some colonies grew small next to each other while biochemical tests for bacterial identification are prone to either false positive and false negative indicated in the results of this research's bacitracin susceptibility tests.

Although differences in the mean levels of contact lens microbial contamination resulted from poor and good hand hygiene techniques were found not to be statistically significant in this study, the reduced average levels of contact lens microbial contamination related to good hand hygiene methods including hand washing with soap and alternative using of alcohol-based gel suggest that compliance with good hand hygiene should be practiced in the contact lens wear to further reduce risk of developing contact lens-related ocular inflammations such as MK. A repeated experiment of this project can aim to have more replicates to test for any statistical significance of data. Contact lens-related MK can stem from different aspects of contact lens use with modifiable factors such as lens wearing time, lens materials, and patients' noncompliance with lens replacement schedule and hygiene guidelines (Stapleton & Carnt, 2012). Therefore, eye care professionals might also consider developing a detailed guideline for hand hygiene protocols to raise awareness and better educate their patients. There are also venues for future research to develop CLs with antimicrobial chemical properties. However, this technology is still mostly in its research and developmental state described in a review by Khan & Lee (2020).

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