Microbial contamination of contact lens storage cases during daily wear use

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Optometry and Vision Science

Microbial Contamination of Contact Lens Storage Cases During Daily Wear Use

--Manuscript Draft--

Manuscript Number:	OVS15496R2
Full Title:	Microbial Contamination of Contact Lens Storage Cases During Daily Wear Use
Article Type:	Original Article
Keywords:	contact lens, storage case, solution, contamination, biofilm, bacteria
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Abstract:	<p>Purpose: To evaluate contact lens (CL) storage case contamination when used with four different CL care solutions, during daily wear of three different CL materials.</p>
	<p>Methods: A parallel, prospective, bilateral, randomized clinical trial (n=38) was conducted. Subjects were randomly assigned to use one of three CL materials (etafilcon A, senofilcon A or galyfilcon A) on a daily wear basis. Subsequently, each subject randomly used one of four different CL care solutions (Biotrue, OPTI-FREE PureMoist, RevitaLens OcuTec and CLEAR CARE) for two weeks, along with their respective storage cases. After every two-week period, their storage cases were collected and the right and left wells of each storage case were randomized for two procedures: (i) microbial enumeration by swabbing the storage case surface and (ii) evaluation of biofilm formation (multipurpose solution cases only) using a crystal violet staining assay.</p>
	<p>Results: More than 80% of storage cases were contaminated when used in conjunction with the four CL care solutions, irrespective of the CL material worn. Storage cases maintained with CLEAR CARE (mean Log colony forming units (CFU)/well $\pm$ SD, 2.0 ± 1.0) revealed significantly ($p < 0.001$) greater levels of contamination, compared to those maintained with Biotrue (1.3 ± 0.8) and RevitaLens OcuTec (1.2 ± 0.8). Predominantly, storage cases were contaminated with Gram-positive bacteria ($\geq 80\%$). There were significant differences ($p = 0.013$) for the levels of Gram-negative bacteria</p>

recovered from the storage cases maintained with different CL care solutions. Storage cases maintained with OPTI-FREE PureMoist (0.526 ± 0.629) showed significantly higher biofilm formation ($p=0.028$) compared to those maintained with Biotrue (0.263 ± 0.197).

Conclusions: Levels of contamination ranged from 0 to 6.4 Log CFU/storage case well, which varied significantly ($p<0.001$) between different CL care solutions and storage case contamination was not modulated by CL materials.

Synopsis for “Microbial contamination of contact lens storage cases during daily wear use”

This study evaluated the frequency and levels of microbial contamination in contact lens (CL) storage cases, during the daily wear of three different CL materials that were used in combination with three contemporary CL care solutions with dual disinfectants and a hydrogen-peroxide based solution. Overall, at least 80% of storage cases were contaminated. The levels of storage case contamination varied significantly between different CL care solutions and was not modulated by CL materials. It is worthwhile, to evaluate the association between microbial contamination and the occurrence of adverse events during CL wear in a future study.

Short title for OVS announces: Microbial contamination of contact lens storage cases

Ref.: Ms. No. OVS15496R1- “Microbial Contamination of Contact Lens Storage Cases During Daily Wear Use”

Dear Dr Twa,

Many thanks for providing comments on our revised manuscript. Kindly, please find our responses (text in red) to the editors’ comments below.

Best regards,

Jaya

Jaya Dantam PhD, B.Optom
Laboratory Scientist

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This email and any attachments are confidential. If you are not an intended recipient or an authorised representative of an intended recipient, please notify the sender immediately by return email and delete all copies of this email and any attachments.

Editor in Chief's comments:

Jaya, the revisions have improved this manuscript. Thank you.

Thank you.

There are a few outstanding point to address:

While reasonable, the journal generally does not add or subtract authors form those listed originally without ample justification and clear consent of others involved.

This is an author not a journal responsibility to address. If you wish to change the author list, you must provide written consent from all other authors before we will even consider such a request.

This must include a statement that they specifically agree to adding this specific individual, that the proposed new author meets the ICMJE author criteria, and that the position in the list is acceptable to all:

(<http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html>).

Yes, as per your suggestion, we have obtained the written consent from all the co-authors (enclosed), for your consideration, to add Dominik as a co-author.

Please follow the Managing Editor's instructions below regarding your OVS Announces synopsis image and image licensing agreement form.

Yes, sure.

Topical Editor's comments:

The manuscript has been appropriately revised based on the reviewers comments.

Some additional comments below:

line 121 "The storage cases maintained with OPTI-FREE PureMoist (0.3 ± 0.9) showed" what are the numbers? cfu? or? please clarify. similarly at line 125 "OcuTec (0.1 ± 0.3) were not significantly different" what are these numbers?

Apologies for not clarifying the units earlier. These numbers refer to mean Log CFU/ storage case well $\pm$ SD. As per your suggestion, we have now added these units beside the numbers (please refer to lines 121, 122 and 125).

Again - lines 160-160 - what are the numbers? Log cfu?

As above, these numbers refer to mean Log CFU/ storage case well $\pm$ SD. We have now added the units in lines 160 and 161.

Abstract - add key words at the end of the abstract text file.

We have now added the key words at the end of the abstract.

Acknowledgments - I don't understand why the houston, Manchester sites are acknowledged -

the data did not come from these sites - I would suggest removing all of the "site" acknowledgments"

We agree that the data was not collected or analyzed at the Manchester and Houston sites. However, as this study was part of the main multi-site study (assessing the clinical performance of contemporary care systems), we have included these sites in the acknowledgement section.

On this topic - was there a significant contribution from; listed authors other than those at the Canada site and sponsor to warrant an authorship? Even the stats seems to have been performed in Canada with help from Canadian (German) person.

Please note that the contributions from each author listed in this manuscript have been identified as per ICMJE criteria and we have enclosed these declaration forms for your reference.

Table 3 - column 2 - I presume this is log₁₀ CFU/ml? Needs to have this added above (mean +/- SD).

Yes, as per your suggestion we have now added the units in Table 3.

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Yes we have uploaded a synopsis image.

Declaration for the addition of a co-author

I, Jaya Dantam (print first, middle, last name) agree to the addition of Dr Dominik Papinski as a co-author in our manuscript entitled "*Microbial contamination of contact lens storage cases during daily wear use*" (manuscript number: OVS15496R1) in recognition of his significant contributions to the statistical analysis (as per the stated authors list below).

I also agree that he meets the ICMJE (<http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html>) author criteria.

Jaya Dantam* PhD, BOptom; David J McCanna* PhD; Lakshman N Subbaraman* PhD, BSOptom; **Dominik Papinski*** PhD; Carol Lakkis[†] PhD, PGCertOcTher, FAAO; Aftab Mirza[#] MSc, MCOptom; David A Berntsen[§], PhD, OD, FAAO; Philip Morgan[#] PhD, MCOptom, FAAO; Jason J Nichols[‡] PhD, M.P.H, FAAO; Lyndon Jones* PhD, FCOptom, FAAO; for the Performance of Contact Lens Solutions Study Group

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Signature D. Jaya Date Jan 15, 2016

Declaration for the addition of a co-author

I, David Joseph McCanna (print first, middle, last name) agree to the addition of Dr Dominik Papinski as a co-author in our manuscript entitled "*Microbial contamination of contact lens storage cases during daily wear use*" (manuscript number: OVS15496R1) in recognition of his significant contributions to the statistical analysis (as per the stated authors list below).

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Declaration for the addition of a co-author

I, LAKSHMAN N. SUBBARAMAN (print first, middle, last name) agree to the addition of Dr Dominik Papinski as a co-author in our manuscript entitled "*Microbial contamination of contact lens storage cases during daily wear use*" (manuscript number: OVS15496R1) in recognition of his significant contributions to the statistical analysis (as per the stated authors list below).

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Date

Jan 22, 2016

Declaration for the addition of a co-author

I, Carol Lakkis (print first, middle, last name) agree to the addition of Dr Dominik Papinski as a co-author in our manuscript entitled "*Microbial contamination of contact lens storage cases during daily wear use*" (manuscript number: OVS15496R1) in recognition of his significant contributions to the statistical analysis (as per the stated authors list below).

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Date 18 January 2016

Declaration for the addition of a co-author

I, AFTAB MIRZA (print first, middle, last name) agree to the addition of Dr Dominik Papinski as a co-author in our manuscript entitled “*Microbial contamination of contact lens storage cases during daily wear use*” (manuscript number: OVS15496R1) in recognition of his significant contributions to the statistical analysis (as per the stated authors list below).

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Declaration for the addition of a co-author

I, David A. Berntsen, OD PhD (print first, middle, last name) agree to the addition of Dr Dominik Papinski as a co-author in our manuscript entitled "*Microbial contamination of contact lens storage cases during daily wear use*" (manuscript number: OVS15496R1) in recognition of his significant contributions to the statistical analysis (as per the stated authors list below).

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Signature Date 1-19-16

Declaration for the addition of a co-author

I, PHILIP B. MORGAN (print first, middle, last name) agree to the addition of Dr Dominik Papinski as a co-author in our manuscript entitled "*Microbial contamination of contact lens storage cases during daily wear use*" (manuscript number: OVSI5496R1) in recognition of his significant contributions to the statistical analysis (as per the stated authors list below).

I also agree that he meets the ICMJE (<http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html>) author criteria.

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Signature

Date January 17, 2016

Declaration for the addition of a co-author

I, Jaya Dantam (print first, middle, last name) agree to the addition of Dr Dominik Papinski as a co-author in our manuscript entitled "*Microbial contamination of contact lens storage cases during daily wear use*" (manuscript number: OVS15496R1) in recognition of his significant contributions to the statistical analysis (as per the stated authors list below).

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Signature Date 1/25/16

Declaration for the addition of a co-author

I, Lyndon Jones (print first, middle, last name) agree to the addition of Dr Dominik Papinski as a co-author in our manuscript entitled "*Microbial contamination of contact lens storage cases during daily wear use*" (manuscript number: OVS15496R1) in recognition of his significant contributions to the statistical analysis (as per the stated authors list below).

I also agree that he meets the ICMJE (<http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html>) author criteria.

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Date 15 JAN 2016

Declaration of contribution and authorship

To qualify for authorship, for the manuscript # **OVS15496R1** entitled "*Microbial contamination of contact lens storage cases during daily wear use*", by checking the appropriate boxes below, I declare that I meet all the four ICMJE (<http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html>) criteria, stated as follows:

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- ☒ Drafting the work or revising it critically for important intellectual content; AND
- ☒ Final approval of the version to be published; AND
- ☒ Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Print name (first, middle, last) JAYA DANTAM

Signature D. Jaya

Date Jan 15, 2016

Declaration of contribution and authorship

To qualify for authorship, for the manuscript # **OVS15496R1** entitled "*Microbial contamination of contact lens storage cases during daily wear use*", by checking the appropriate boxes below, I declare that I meet all the four ICMJE (<http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html>) criteria, stated as follows:

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- ☒ Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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David Joseph McCanna

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Daniel McCanna

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January 15, 2016

Declaration of contribution and authorship

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Print name (first, middle, last) LAKSHMAN SUBBARAMAN

Signature N.S. Subbaraman

Date Jan 22, 2016

Declaration of contribution and authorship

To qualify for authorship, for the manuscript # **OVS15496R1** entitled "*Microbial contamination of contact lens storage cases during daily wear use*", by checking the appropriate boxes below, I declare that I meet all the four ICMJE (<http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html>) criteria, stated as follows:

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- ☒ Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Date 18/01/2016

Declaration of contribution and authorship

To qualify for authorship, for the manuscript # **OVS15496R1** entitled "*Microbial contamination of contact lens storage cases during daily wear use*", by checking the appropriate boxes below, I declare that I meet all the four ICMJE (<http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html>) criteria, stated as follows:

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Print name (first, middle, last) Carol Lakkis

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Declaration of contribution and authorship

To qualify for authorship, for the manuscript # **OVS15496R1** entitled “*Microbial contamination of contact lens storage cases during daily wear use*”, by checking the appropriate boxes below, I declare that I meet all the four ICMJE (<http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html>) criteria, stated as follows:

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Declaration of contribution and authorship

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- ☒ Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Date 1-19-16

Declaration of contribution and authorship

To qualify for authorship, for the manuscript # **OVS15496R1** entitled "*Microbial contamination of contact lens storage cases during daily wear use*", by checking the appropriate boxes below, I declare that I meet all the four ICMJE (<http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html>) criteria, stated as follows:

- ☒ Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
- ☒ Drafting the work or revising it critically for important intellectual content; AND
- ☒ Final approval of the version to be published; AND
- ☒ Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Date January 17, 2016

Declaration of contribution and authorship

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MICROBIAL CONTAMINATION OF CONTACT LENS STORAGE CASES DURING DAILY WEAR USE

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Purpose: To evaluate contact lens (CL) storage case contamination when used with four different CL care solutions, during daily wear of three different CL materials.

Methods: A parallel, prospective, bilateral, randomized clinical trial (n=38) was conducted. Subjects were randomly assigned to use one of three CL materials (etafilcon A, senofilcon A or galyfilcon A) on a daily wear basis. Subsequently, each subject randomly used one of four different CL care solutions (Biotrue, OPTI-FREE PureMoist, RevitaLens OcuTec and CLEAR CARE) for two weeks, along with their respective storage cases. After every two-week period, their storage cases were collected and the right and left wells of each storage case were randomized for two procedures: (i) microbial enumeration by swabbing the storage case surface and (ii) evaluation of biofilm formation (multipurpose solution cases only) using a crystal violet staining assay.

Results: More than 80% of storage cases were contaminated when used in conjunction with the four CL care solutions, irrespective of the CL material worn. Storage cases maintained with CLEAR CARE (mean Log colony forming units (CFU)/well $\pm$ SD, 2.0 ± 1.0) revealed significantly ($p < 0.001$) greater levels of contamination, compared to those maintained with Biotrue (1.3 ± 0.8) and RevitaLens OcuTec (1.2 ± 0.8). Predominantly, storage cases were contaminated with Gram-positive bacteria ($\geq 80\%$). There were significant differences ($p = 0.013$) for the levels of Gram-negative bacteria recovered from the storage cases maintained with different CL care solutions. Storage cases maintained with OPTI-FREE PureMoist (0.526 ± 0.629) showed significantly higher biofilm formation ($p = 0.028$) compared to those maintained with Biotrue (0.263 ± 0.197).

Conclusions: Levels of contamination ranged from 0 to 6.4 Log CFU/storage case well, which varied significantly ($p < 0.001$) between different CL care solutions and storage case contamination was not modulated by CL materials.

Key words: contact lens, storage case, solution, contamination, biofilm, bacteria

INTRODUCTION

Approximately 140 million people worldwide and 40.939.2 million people in the United States use contact lenses (CLs) as a vision correction aid.¹ Contact lens wear has been associated with a variety of ocular complications, including sight threatening complications such as microbial keratitis (MK).² When used on a daily wear basis, the incidence of MK varies between 2.2 and 6.9 per 10,000 wearers.^{3, 4} The majority of CL-related MK are caused by Gram-negative bacteria,^{5, 6} with *Pseudomonas aeruginosa* frequently being recovered from culture-positive corneal scrapings.^{2, 6-11} Given the widespread use of CLs,¹² even a low incidence of MK incurs substantial public health concerns. During CL wear, microbial contamination of CLs and their associated care solutions and storage cases is a common finding.¹³⁻¹⁷ Of particular note is contamination of the storage cases,^{14, 18} which occurs far more frequently than contamination of either the CL^{14, 18} or disinfection solutions.¹⁴ Additionally, studies have indicated that the leading causative microorganism '*P. aeruginosa*' that was recovered from corneal scrapings of patients with MK has been traced to storage cases.^{19, 20} Furthermore, certain retrospective studies^{21, 22} have suggested that culturing either the CL^{21, 22} or storage case,²³ in the event of negative or unavailable corneal cultures, may facilitate the identification of the microorganisms causing MK. Recently, certain emerging Gram-negative bacterial genera such as *Achromobacter*, *Stenotrophomonas* and *Delftia* have been recovered from CL storage cases²⁴ and associated with cases of MK in CL wearers.²⁵ Taken together, these findings indicate the notable role that storage cases contaminated with Gram-negative bacteria may play in the pathogenesis of MK.

Irrespective of the care solutions used, microbial contamination of storage cases ranges from 19% to 92%.^{13, 14, 16, 17, 26-33} Often, the contaminated storage cases illustrate polymicrobial colonization, with varying frequencies of bacteria, fungi and protozoa.^{17, 28, 34} Willcox et al. reported that in addition to the levels of contamination, the spectrum of microbes recovered from the contaminated storage cases varied with the different formulations of disinfecting solutions.¹⁷ Recently, manufacturers have introduced multipurpose disinfecting solutions (MPDS) with newly formulated dual disinfectants, in an attempt to increase the efficacy of the CL care solutions. However, controlling storage case contamination remains a challenge, even with disinfecting solutions that contain dual disinfectants.¹⁷

Practitioners recommend a wide variety of care solutions and CL material combinations, which may vary in their ability to resist microbial contamination of their respective storage cases. The purpose of this study was to determine the frequency and levels of microbial contamination in storage cases used with different CL material and care solution combinations, during a two-week exposure period. Further, this study aimed to classify the type of microorganisms recovered from storage cases and to quantify the biofilm formation on the interior surface of storage cases used with a variety of MPDS.

MATERIALS AND METHODS

Contact Lens Care Solutions, Storage Cases and Contact Lenses

The four care solutions used in this study are listed in Table 1. All care solutions were supplied with their respective storage cases by the manufacturers. A hydrogel material (etafilcon A; ACUVUE 2; Johnson & Johnson Vision Care, Inc.) and two silicone hydrogel materials (ACUVUE OASYS; senofilcon A; and ACUVUE Advance Plus; galyfilcon A; Johnson & Johnson Vision Care, Inc.) were used in this study.

Clinical Study Design

As part of an international multi-site clinical study consisting of 236 subjects (sites were Eurolens Research, University of Manchester, UK, the Ocular Surface Institute, College of Optometry, University of Houston, USA and the Centre for Contact Lens Research, University of Waterloo, Canada) assessing the clinical performance of contemporary care systems, a sub-set of subjects at the University of Waterloo (n=38) were included in a parallel study to specifically address the issue of case contamination. This was a cross-over, bilateral, and randomized clinical study with nine scheduled visits. All study procedures were conducted in compliance with the International Conference on Harmonization Good Clinical Practice E6 (ICH-GCP) guidelines, tenets of Declaration of Helsinki for Experimentation in Humans and applicable regulatory requirements.

Subjects between 18 and 69 years of age with previous CL wear experience and who had no systemic or ocular contraindications for CL wear were enrolled in the study, after obtaining informed consent. After the initial random assignment to one of the three CL materials (which were worn on a daily wear basis), subjects were assigned to use one of the four care solutions in a random order for two-weeks, along with their

respective storage cases. In addition to verbal instructions, subjects were given an instruction guide with regards to the use of their CLs and storage cases, specific to a care solution. After the initial two-week period, subjects were randomly assigned the remaining three care solutions, each for two weeks of use. The storage cases were collected after every two-week period. There was a washout period of at least four days between the care solutions, during which the subjects wore daily disposable CL (1-DAY ACUVUE MOIST; etafilcon A; Johnson & Johnson Vision Care, Inc) and/or spectacles. All subjects were masked to the CL material assigned and the care solutions were de-identified as much as possible. This was achieved by removing the identifying manufacturer labels from the CL care solution bottles. Complete masking of solution identification was not possible for either OPTI-FREE PureMoist (that has the manufacturer name embossed on the upper portion of the bottle) or CLEAR CARE (which uses a barrel-shaped storage case with platinum disk). The investigators were masked to the CL material only. Subjects were also masked as to the reason for their cases being collected and their subsequent culturing, to ensure that they did not modify their habitual behaviors during the study period, by instructing them to return all the study supplies at the end of each wearing period.

Microbial Analysis of Contact Lens Storage Cases

Storage cases were collected from subjects by the lab investigators and processed within two hours. All cases were handled aseptically while performing the microbial procedures. Prior to analysis, any residual care solution was discarded; however, no additional rinsing or neutralization steps were performed. The interior surface of a randomly selected well of the flat storage case and the inner surface of the lid were swabbed using a calcium alginate swab, pre-moistened with sterile phosphate buffered saline (PBS). For the barrel (stand-up) storage cases (used with the hydrogen-peroxide solution), one CL storage basket was randomly selected and the CL holder, interior and exterior surfaces of the CL basket, top hinge and the interior surface of the lid were swabbed. The swab was placed in a sterile plastic vial containing 2 mL PBS containing 1% sodium hexametaphosphate, which was vortexed at high speed for 30 seconds. From this sample, aliquots were removed and plated (up to 3 dilutions) on two sets of chocolate blood agar plates (without antibiotics or supplements) and a set of Sabouraud Dextrose agar (SAB) with Chloramphenicol plates for the recovery of bacterial and fungal isolates respectively. The chocolate agar plates were

incubated at 37°C, one set under aerobic (O₂) conditions for 7 days and the other under anaerobic conditions (ANO₂) for 4 days. The SAB with chloramphenicol plates were incubated at 25°C for 7 days. The colony forming units (CFUs) were enumerated after the designated incubation period. For bacterial isolates recovered from aerobic and anaerobic cultures, Gram-staining was performed to classify their group, either as Gram-positive or Gram-negative, from the representative colonies of similar morphology and these data were combined for analysis. Fungal isolates were identified based on their morphological characteristics.

Additionally, a biofilm assay^{30, 35} was performed with flat-bottomed cases only. Following the aforementioned microbial procedures on one of the wells, each storage case was air dried in an upside down position, allowing the evaporation of any residual moisture. The second (unswabbed) well was stained with 5% crystal violet stain for 45 minutes at room temperature, followed by a gentle wash with running tap water. The biofilms were then extracted using 95% ethanol. Finally, the solubilized biofilm extracts were transferred into 24-well microtiter plates and the absorbance of the samples was measured at 595 nm, using a spectrophotometer (Thermo Scientific Multiskan Spectrum, USA).

Statistical Analysis:

A chi-square test was used to evaluate the differences in the frequency of microbial contamination of storage cases maintained with different care solutions. The levels of contamination (CFUs) were log transformed for the data analysis. For the exploration of biofilm data, absorbance values were used. Both the level of contamination and biofilm formation in storage cases were analyzed using a linear mixed model to compare the differences between care solutions. The model included CL material, care solution and the interaction between CL and solution along with sequence of CL care solution and visit (as appropriate) as fixed effect factors. All these factors were tested simultaneously. Age and gender were not included in the model. A Bonferroni correction was used for multiple pairwise comparisons. Only those factors that were significant in the model were reported in the results section. All statistical tests were two-sided and conducted at the 5% level of significance. Statistical software SPSS (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY) was used for the data analysis.

RESULTS

Of the 38 subjects included in this study, 76% were female and 24% were male. The majority of subjects were university students. The mean age ($\pm$ SD) of the subjects was 30 ± 11 years. A total of 152 storage cases (as each subject used four different CL care solutions) were sampled and analyzed.

At least 80% of the storage cases cultured were contaminated (Figure 1), and the frequency of contamination did not vary significantly between different solutions ($p=0.134$), regardless the CL material combination, Table 2. The highest percentage of contamination was found in storage cases maintained with OPTI-FREE PureMoist (98%); those maintained with RevitaLens OcuTec exhibited the lowest contamination (80%). Figure 2 details the levels of contamination, which varied significantly between the four CL care solutions ($p<0.001$).

Microorganisms recovered from the storage cases were classified by their group (either bacteria or fungi) and subsequently bacteria were classified by their type (Figure 3). Levels of Gram-positive bacteria recovered from the storage cases differed significantly ($p<0.001$) between different CL care solutions and the specific comparisons between different storage cases are shown in Table 3. The levels of Gram-negative bacteria recovered from different storage cases varied significantly ($p=0.013$), between different CL care solutions. The storage cases maintained with OPTI-FREE PureMoist (mean Log CFU/ storage case well $\pm$ SD, 0.3 ± 0.9) showed significantly higher levels of Gram-negative bacteria compared to those maintained with Biotrue and CLEAR CARE, which each showed no recovery of Gram-negative bacteria ($p=0.026$ and $p=0.028$ respectively). The levels of Gram-negative bacteria recovered from the storage cases maintained with RevitaLens OcuTec (mean Log CFU/ storage case well $\pm$ SD, 0.1 ± 0.3) were not significantly different compared to the storage cases maintained with any of the other three solutions (all $p>0.05$). There was statistically significant effect of visit on Gram-negative bacteria contamination ($p=0.009$), however post-hoc comparisons showed no significance between the different visits ($p>0.05$). For fungi, the number of observations was limited, preventing valid statistical comparisons.

The biofilm formation on the three flat cases varied significantly between products ($p=0.031$). Higher absorbance values refer to greater biofilm formation on the surface of storage cases. Storage cases maintained with OPTI-FREE PureMoist (mean absorbance $\pm$ SD = 0.526 ± 0.629) showed significantly greater biofilm formation compared to those maintained with Biotrue (0.263 ± 0.197 ; $p=0.028$). However,

the storage cases maintained with RevitaLens OcuTec (0.362 ± 0.300) did not vary significantly from the other two solutions ($p>0.05$).

DISCUSSION

A variety of care solutions are commercially available for the care and maintenance of reusable hydrogel and silicone hydrogel CL materials, leading to a wide-range of combinations of materials and care solutions being used in clinical practice. This study evaluated the microbial contamination of storage cases maintained with three contemporary dual disinfectant multipurpose solutions and a hydrogen peroxide based solution, when used with contemporary CL materials.

The results of the current study indicated that differences between CL materials did not impact microbial contamination of the storage cases, which has previously been reported by Willcox et al.¹⁷ At least 80% of the storage cases were contaminated (Figure 1), with no significant differences between the MPDS and the peroxide-based system.

These contamination rates are consistent with recent studies that have reported the frequency of contamination to be between 76% and 92%, when storage cases were maintained with MPDSs containing either polyhexanide-0.0001% or polyquarternium-1 0.001% + myristamindopropyl dimethylamine 0.0005% or a hydrogen peroxide based CL care solution.^{17, 30} In contrast, other studies have reported lower case contamination rates of 42-66%.^{16, 25, 36, 37} These variations could be attributable to the different study designs or locations from where the cases were sampled. For example, while processing flat-bottomed cases, certain studies swabbed the inner wells and the inner surface of the lids,^{17, 30} whereas others swabbed the inner base of the well and along the top rim,^{16, 37} which may underestimate the frequency of contamination. The sampling methods used in this study are consistent with those reported by Willcox et al., and Dantam et al.,^{17, 30} and the results are broadly in agreement. Given that storage cases were only used for two weeks, the contamination rates appear high. However, a previous study by Lakkis et al.,³⁸ also reported heavy contamination of storage cases within two-weeks of use, irrespective of the care solutions used.

The levels of contamination in the storage cases ranged from 0 to 6.4 Log CFU/storage case well (Figure 2). This is within the values previously reported, which vary from 0 to 7.0 log CFU/per sample.^{16, 17, 29, 34} The

microorganism load recovered from the storage cases maintained with CLEAR CARE (mean Log CFU/storage case well $\pm$ SD, 2.0 ± 1.0) were significantly greater than those maintained with Biotrue (1.3 ± 0.8) and RevitaLens OcuTec (1.2 ± 0.8) solutions; however, the magnitude of the difference was less than 1.0 log, which may not be clinically relevant.³⁹ The greater level of contamination with CLEAR CARE was driven by the degree of Gram-positive contamination. This observation is consistent with earlier studies,^{28, 40, 41} which reported heavier contamination with peroxide-based care systems when compared to chemical disinfection systems. More recently, Jiang et al. identified that peroxide-based systems were associated with frequent storage case contamination.²³ However, in contrast, Wilson et al reported a lower incidence of contamination with the use of peroxide-based solutions.²⁶ This increased microbial contamination of cases from hydrogen-peroxide based solutions has been attributed to 'catalase', an enzyme found in the intracellular contents of certain microorganisms, which breaks down hydrogen peroxide to water and oxygen, potentially limiting the activity of 3% hydrogen peroxide.²⁸ Although the levels of Gram negative bacterial contamination varied significantly between different CL care solutions ($p=0.013$), the magnitude of the difference was less than 1.0 log and this may not be clinically relevant. Further, the statistical significance of visit for Gram-negative bacterial contamination was limited to main effects only. The positive findings for Gram-negative bacteria contamination had maximum observations at visit 9 and this may be attributed to diminished compliance. However, this study was not designed to evaluate compliance and this is a limitation of the current study. As all the CL care solutions were randomly assigned, the sequences of CL care solutions were unbiased across all the visits.

In the current study, the bacteria recovered from storage cases were categorized based on the Gram-staining technique only, which is a limitation of this study. Given the relevance of Gram-negative bacteria in the occurrence of CL-related microbial keratitis, for future studies, it would also be worthwhile to consider and incorporate the identification of specific bacterial species of each recovered microorganism, including screening for *Acanthamoeba* species and exploring the effects of age and gender on case contamination.

In the current study, biofilm formation within the flat storage cases was investigated using an established crystal-violet staining technique.^{30, 35} Biofilms were evident (Figure 4), particularly in storage cases maintained with OPTI-FREE PureMoist, which demonstrated significantly higher biofilm formation on the

case surface compared to those maintained with Biotrue (p=0.028). The scientific/clinical relevance of the biofilm levels observed in this study are yet to be established; however, biofilms are common in CL storage cases that exhibit persistent microbial contamination.^{10, 17, 31} In the sessile form, microbial cells are more resistant to care solutions in-vivo²⁶ and in-vitro⁴² compared to their planktonic phenotype.⁴³ It is interesting to note that the current recommendations from ISO14729⁴⁴ and the FDA 510(k) document for premarket approval of CL care solutions⁴⁵ are limited to the evaluation of antimicrobial efficacy against selective reference strains of planktonic bacteria and fungi only. As previously identified in some in-vitro studies,^{42, 43} the data from this in-vivo study also suggests that there is a need to consider the inclusion of some form of assessment of solution activity against microbes encapsulated in a biofilm, or adoption of other strategies to reduce CL case contamination, such as antimicrobial lens cases or increased case replacement frequency.

A recent clinical study demonstrated that enhanced verbal and written advice on the importance of appropriate storage case hygiene can improve patient compliance.⁴⁶ Additionally, in-vitro⁴⁷ and clinical^{36, 48} studies have suggested that incorporation of additional steps compared to those traditionally recommended by the manufacturers such as rubbing, rinsing with the care solution and tissue-wiping during the daily maintenance of storage cases can substantially decrease bacterial loads, including biofilms.^{47, 49, 50} Thus, future studies may consider factors such as practitioners' instructions on care regimen use,^{46, 48, 51} subjects' behavioral patterns towards CL care and maintenance^{36, 52, 52} and environmental conditions, to acquire a comprehensive understanding of the multifactorial processes involved in the microbial contamination of storage cases.

It is important to note that these findings are based on a two-week period only and further studies are necessary to establish the rates and degree of contamination associated with longer term exposure. In addition, the association between microbial contamination and the occurrence of adverse events during CL wear is worthy of future study, along with the potential role that self-limiting "disposable" cases and/or antibacterial cases may have on such adverse events.

In conclusion, despite the recent introduction of new care systems into the market, data from this study would suggest that there has been little impact on the microbial contamination rate of CL storage cases, as

the percentage of cases that were contaminated was at least 80%, even with contemporary MPDSs with dual disinfection systems. The levels of microbial contamination ranged widely, from 0 to 6.4 Log CFU/storage case well, which varied significantly between care solutions, with predominant recovery of gram-positive bacteria. Contamination rates were unrelated to the CL material worn.

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408

FIGURE 1.

Microbial contamination of storage cases used with different CL care solutions in this study.

FIGURE 2.

Levels of microbial contamination in storage cases (mean of Log CFU/ storage case well $\pm$ SD) maintained with different CL care solutions.

FIGURE 3.

Frequency of bacterial and fungal contamination in storage cases maintained with different care CL solutions.

TABLE 1.

Contact lens care solutions used in this study

	Biotrue (North America and UK)	OPTI-FREE PureMoist (North America and UK)	RevitaLens OcuTec (North America) Complete RevitaLens (UK)	CLEAR CARE (North America) AO Sept Plus (UK)
Manufacturer	BAUSCH + LOMB	ALCON Inc	Abbott Medical Optics	ALCON Inc
Disinfectant agent	polyaminopropyl biguanide 0.00013% w/v, polyquaternium 0.0001% w/v	POLYQUAD (polyquaternium-1) 0.001%, ALDOX (myristamidopropyl dimethylamine) 0.0006%	Polyquaternium-1 0.0003%, Alexidine 0.00016%	hydrogen peroxide 3% (w/v), sodium chloride 0.79%
Buffer	boric acid, sodium borate, sodium chloride	boric acid, sorbitol, sodium chloride	boric acid, sodium borate decahydrate, trisodium citrate dehydrate, sodium chloride	phosphate buffer, phosphonic acid
Chelating agent	edetate disodium	disodium EDTA	edetate disodium (EDTA)	
Surfactant	poloxamine	sodium citrate	Tetronic 904	Pluronic 17R4
Conditioning/ Wetting agent	hyaluronan	TETRONIC 1304 and HydraGlyde Moisture Matrix [EOBO-41-polyoxyethylene-polyoxybutylene]		
Others	sulfobetaine	aminomethyl propanol	purified water	

TABLE 2.

Frequency of microbial contamination in CL storage cases with the use of different care solutions, for a given CL material (each subject used one CL material and all four CL care solutions in random order)

Care solution	CL material					
	etafilcon A (n=13)		galyfilcon A (n=12)		senofilcon A (n=13)	
	Yes* (%)	No# (%)	Yes* (%)	No# (%)	Yes* (%)	No# (%)
Biotrue	79	21	83	17	85	15
OPTI-FREE PureMoist	100	0	100	0	93	7
RevitaLens OcuTec	69	31	92	8	81	19
CLEAR CARE	85	15	92	8	100	0

*contaminated storage cases; #uncontaminated storage cases

TABLE 3.

Comparison of levels of Gram-positive bacteria recovered from different storage cases

Care solution	Log CFU/ storage case well (mean ± SD)	p values for pairwise comparisons using Bonferroni correction			
		Biotrue	OPTI-FREE PureMoist	RevitaLens OcuTec	CLEAR CARE
Biotrue	1.6 ± 0.5	-	1.000	1.000	0.001
OPTI-FREE PureMoist	1.8 ± 0.9	1.000	-	1.000	0.000
RevitaLens OcuTec	1.5 ± 0.5	1.000	1.000	-	0.000
CLEAR CARE	2.3 ± 0.8	0.001	0.000	0.000	-

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