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Review

Contact lens hygiene compliance and lens case contamination: A review

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ABSTRACT

A contaminated contact lens case can act as a reservoir for microorganisms that could potentially compromise contact lens wear and lead to sight threatening adverse events. The rate, level and profile of microbial contamination in lens cases, compliance and other risk factors associated with lens case contamination, and the challenges currently faced in this field are discussed. The rate of lens case contamination is commonly over 50%. Coagulase-negative Staphylococcus, Bacillus spp., *Pseudomonas aeruginosa* and *Serratia marcescens* are frequently recovered from lens cases. In addition, we provide suggestions regarding how to clean contact lens cases and improve lens wearers' compliance as well as future lens case design for reducing lens case contamination. This review highlights the challenges in reducing the level of microbial contamination which require an industry wide approach.

1. Contact lens related microbial keratitis

Contact lens wear is usually considered a safe and effective means to correct refractive error, however, adverse reactions may occur. The most significant complication is microbial keratitis, a microbial infection which leads to corneal ulceration, a vision threatening condition. Contact lens wear accounts for 65% of all new cases of microbial keratitis in the UK [1]. Similar figures have also been reported in Holland (63%) [2], Taiwan (53%) [3] US (52%) [4] and Japan (55%) [5]. In the late 1990s, the incidence of contact lens related microbial keratitis was estimated to be 2.2–4.1 per 10,000 wearers of soft daily wear lenses and 13.3–20.9 per 10,000 wearers of soft extended wear [6–8]. With contemporary contact lens wear the annualized incidence is essentially unchanged for daily and extended wear use [9].

It has been found that the disease severity is lower in microbial keratitis patients wearing daily disposables than those wearing other modalities [9,10]. This perhaps supports the hypothesis that lens case hygiene still plays an important role in safe contact lens wear and this is shown in recent epidemiological studies [11].

Many epidemiological studies have identified risk factors for contact lens related microbial keratitis. Some of the modifiable behavioral factors include infrequent disinfection of contact lenses [1,12], overnight wear [13–15], smoking [14,16,17,8], inadequate hand washing [8] and poor lens case hygiene [8].

1.1. The relevance of storage case contamination

Colonization of the lens storage cases by pathogenic microorganisms may predispose lens wearers to microbial or sterile keratitis [18–22]. It has also been demonstrated that identical organisms have been identified from both a lens storage case and cornea ulcer [23]. A recent study by Wiley et al. has demonstrated that lens case contamination, in particular biofilm formation may lead to the development of contact lens related microbial keratitis [24]. Further, the study also found that the disease severity correlates with an increase in the diversity of bacterial types found in lens cases [24].

Lens case contamination rate ranges from 18% to 85% (Table 1). The geographical location in which the various studies were conducted, study design, microbiological sampling and methods, subject factors and sample size may account for the wide variation in lens case contamination rates. For example, the study by Simmons et al. looked at the contamination of case wells and lids prior to handling by the subjects, which seems to explain why their contamination rates were low at 18% [25]. Wu et al. has shown that different areas of the same lens case swabbed for bacterial recovery show a different rate and profile of contamination, which may account for microbial recovery discrepancies among reported studies [26]. There are also issues regarding different microbial recovery techniques used amongst studies. *E.g.* viable but non-cultural

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Table 1
Summary of studies estimating lens case contamination rate in contact lens wearers.

Year/location	Study	Sample size and type	Lens type used (% of users using each category)	Disinfection systems used (% of users using each category)	Frequency of case contamination (%)	Frequently recovered micro-organisms NR = not reported[a]		
						Bacteria	Fungi	Protozoa
1985 (Canada)	Callender et al. [30] (cross-sectional study)	58 asymptomatic lens wearers	Soft lenses	Various chemical	72%	*S. epidermidis* *Moraxella* spp. *Enterobacter* spp.	NR	NR
1987 (US)	Donzis et al. [31] (cross-sectional study)	100 asymptomatic lens wearers	Soft (62%) Rigid (38%)	Chemical Peroxide Heat	44% 44% 32%	Coagulase-negative Staphylococcus, *Bacillus* spp.	Fusarium	NR
1989 (UK)	Larkin *et al.* [32] (cross-sectional study)	102 asymptomatic lens wearers	Soft (66%) Rigid (34%)	Chemical (61%) Peroxide (20%) Heat (19%)	Overall: 42%	Environmental pseudomonads, Gram-negative bacilli *Serratia marcescens* (range: 0–10^6 CFU)	NR	Acanthamoeba (9%)
1990 (USA)	Simmons et al. [25] (cross-sectional study)	53 lens wearers	Soft lenses	Peroxide (74%) MPS (16%)	18% 21%	*Pseudomonas* spp.	NR	NR
1990 (US)	Wilson et al. [28] (cross-sectional study)	118 asymptomatic lens wearers	Soft Rigid	Chemical Peroxide Saline Miscellaneous	Overall: 54% 11% 8% 40% 61%	*Staphylococcus epidermidis* *Micrococcus* spp. *Serratia marcescens* *Pseudomonas aeruginosa* (range: 0–10^5 CFU)	NR	NR
1992 (UK)	Devonshire et al. [33] (cross-sectional study)	178 asymptomatic lens wearers	Soft (74%) Rigid (26%)	Peroxide (22%) Chemical (42%) Chlorine (30%) Chlorhexidine tablet (3%) Others: Unknown	Overall: 53%	*Serratia marcescens* *Pseudomonas fluorescens* *Acinetobacter* spp.	Yeast	Acanthamoeba (4.5%) Hartmanella (0.75%)
1995 (NZ)	Gray et al. [27] (cross-sectional study)	101 asymptomatic lens wearers	Soft (85%) Rigid (15%)	Chemical (23%) Peroxide (75%)	Overall: 81%	*Pseudomonas* spp. *Serratia* spp. *Diphtheroids* (72% had mixed bacterial contaminations)	*Cladosporiums* spp. *Candida* spp.	*Acanthamoeba* spp. *Naegleria* spp.
1996 (Norway)	Midelfart et al. [34] (cross-sectional study)	21 asymptomatic medical students	Soft (95%) Rigid (5%)	Chemical Peroxide	Overall: 24%	*Xanthomonasmaltophilia* *Pseudomonas cepacia* *Serratia liquefaciens* *Serratia plymuthica*	NR	NR
1996 (Spain)	Velasco et al. [35] (clinical trial)	126 lens cases	Soft	Polyaminopropylbiguanide	Overall: 81%	*Staphylococcus epidermidis* *Staphylococcus aureus* *Streptococcus viridans* *Pseudomonas aeruginosa*	NR	NR
1998 (UK)	McLaughlin-Borlace et al. [36] (cross-sectional study)	20 Microbial keratitis patients	Various	Chlorine based Hydrogen peroxide Thiomersal Polyhexamethylene	Overall: 85%	*Staphylococcus aureus* *Pseudomonas aeruginosa* *Enterbacter*	NR	*Acanthamoeba* spp.
1999 (UK)	Seal et al. [37] (clinical trial)	155 lens wearers	Soft	MPS Peroxide	78% 58%	Gram + Gram − (range: 0–10^4 CFU)	NR	NR
2005 (HK)	Boost et al. [38] (clinical trial)	47 asymptomatic lens wearers	Orthokeratology	Boston Advance and Simplicity	Overall: 70%	*Acinetobacter* spp. *Pseudomonas aeruginosa* *Serratia* *Staphylococcus aureus*	0	0
2007 (HK)	Yung et al. [39] (cross-sectional study)	101 asymptomatic lens wearers	Various	Multipurpose solution	Overall: 34%	*Staphylococcus aureus* *Pseudomonas aeruginosa* *Serratia marcescens*	0	0

Table 1 (*Continued*)

Year/location	Study	Sample size and type	Lens type used (% of users using each category)	Disinfection systems used (% of users using each category)	Frequency of case contamination (%)	Frequently recovered micro-organisms NR = not reported[a]		
						Bacteria	Fungi	Protozoa
2008 (Brazil)	Pens et al. [40] (cross-sectional study)	81 contact lens wearers	NA	NA	Overall: 80%	Gram+ cocci Gram+ rod Gram− rods (range: 0–10^6 CFU)	NR	*Acanthamoeba* spp.
2010 (Australia)	Willcox et al. [41] (clinical trial)	232 asymptomatic lens wearers	Silicone hydrogel lenses	Polyhexanide (35%) Polyquaternium (36%) Hydrogen peroxide (35%)	92% 76% 81%	*Staphylococcus aureus* *Staphylococcus epidermidis* *Staphylococcus saprophyticus* *Streptococcus viridans* *Delftia acidovorans* *Serratia marcescens* *Stenotrophomonas maltophilia*	Overall: 14%	
2010 (Australia)	Wu et al. [26] (cross-sectional study)	64 asymptomatic lens wearers	Soft (95%) Rigid (5%)	RGP disinfecting solution (3%) Hydrogen peroxide (13%) Multipurpose solution (83%)	Overall: 58%	*Bacillus* spp. *Coagulase-negative Staphylococci* *Propionibacterium acnes* *Micrococcus* spp. *Serratia marcescens* *Pseudomonas aeruginosa* *Achromobacter xyloxidans*	Filamentary fungi	0
2012 (USA)	Wiley et al. [24] (cross-sectional study)	28 microbial keratitis 9 asmptomatic lens wearers	Soft contact lenses	NA	Overall: 61%	*Achromobacter* spp. *Stenotrophomonas* *Delftia* *Enterobacter* *Serratia* *Escherichia* *Ewingella* *Shigella* *Pseudomonas aeruginosa*	NR	NR
2014 (Croatia)	Kuzman et al. [42] (cross-sectional study)	52 asymptomatic lens wearers	Soft (48%) Rigid (52%)	Polyhexanide (33%) Polyquaternium (8%) Hydrogen peroxide (8%) RGP disinfecting solution (45%) Unknown (6%)	Overall: 58%	*Coagulase-negative Staphylococci* *Bacillus* spp. *Proteus mirabilis* *Enterobacter* sp. *Acinetobacter* spp. *Serratia* spp. *Staphylococcus aureus* *Corynebacterium* spp. *Klebsiella pneumonia*	*Chrysosporium* sp. *Penicillium* spp. *Candida parapsilosis*	0
2014 (Australia)	Wu et al. [43] (cross-sectional study)	119 asymptomatic lens wearers	Soft (92%) Rigid (8%)	Polyhexanide (43%) Polyquaternium (33%) Hydrogen peroxide (8%) RGP disinfecting solution (8%) Unknown (8%)	Overall: 66%	*Coagulase negative staphylococci* *Bacillus* spp. *Micrococcus* spp. *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, *Delftia acidovorans*, *Serratia marcescens*	Molds Yeast	NR

Adapted from Szczotka-Flynn et al. [29].

[a] For illustration of basket lens cases, please refer to Wu et al. [26] (Fig. 2).

bacteria. A recent study adopted a culture-independent method to investigate lens case contamination using 16S rRNA gene sequencing, such a method may enhance the likelihood of characterizing the bacteria in the future [24]. In terms of study design, cross-sectional studies that included lens wearers from the population tend to involve different types of contact lens materials including rigid lenses as well as hydrogel lenses [27,28]. Table 1 summarizes studies estimating lens case contamination in contact lens wearers and stratified by the year of the study.

A study by Yung et al. examined the levels of microbial contamination in contact lenses, disinfecting solution and contact lens cases. The authors found that nearly all subjects with contaminated lens cases also had either contaminated contact lenses or disinfecting solutions [39]. This suggests that there is cross contamination between the lens cases and the contact lenses and/or between lens cases and lens care solutions. In contrast to contamination of the contact lens which is usually exclusively bacterial [44,45], contamination of contact lens cases tends to be a mixture of different types of organisms, including bacteria, viruses, fungi and protozoa [27,29,32,41]. In addition, Gray et al. found that approximately 72% of cases demonstrated mixed bacterial contamination [27]. The lens case may be a more advantageous environment for biofilm formation as the biofilms on lens cases were also thicker than those on contact lenses [36]. While there seem to be an association between the frequency of isolation of Gram-negative bacteria from contact lenses cases and corneal infiltrative events [46], no clinical studies have established a safe threshold level of microbial exposure, *i.e.* the level of microbial recovery (CFU), below which the risk of microbial and/or sterile keratitis is decreased. Elucidation of this threshold and the key hygiene/compliance steps in reducing contamination would be a major advance in lens wear safety. At the same time, the variety of bacteria recovered from lens cases seems to play a role in the disease severity. A study by Wiley et al. [47] has found that the bacterial diversity from contact lens cases of symptomatic lens wearers was correlated with severity of disease and presenting visual acuity, and the bacterial diversity was greater than that of asymptomatic controls. This suggests that not only the level of microbial load is critical in disease initiation, but also that the diversity of the contamination might also be a critical risk factor for lens related adverse events.

1.2. The profile of microbial contamination in lens cases

The most commonly recovered bacteria from corneal scrapes in contact lens related microbial keratitis include *Pseudomonas*, *Serratia* and *Staphylococcus* spp. [3,5,11,13–15]. *Pseudomonas* spp. and *Serratia* spp. are both virulent Gram negative bacteria that can cause corneal inflammation [48–50] and infection [13,18,36,51]. *Pseudomonas aeruginosa* is a Gram negative bacteria and one of the most virulent and common bacterial causes of microbial keratitis in contact lens wearers [27,52]. It is considered a versatile organism due to its ability to survive at the ocular surface, form biofilm, adapt to different environments and energy sources and can also exist in the natural environment [27,52,53]. Further, it may have or acquire resistance to contact lens disinfecting solutions [54]. *Staphylococcus* is a Gram positive bacterium that is also often recovered from contact lenses and lens cases of asymptomatic wearers [26,55–57]. *Staphylococcus* spp. identified in contact lens cases are commonly found as part of the normal external ocular microbiota and elsewhere on the human body [58,59]. For example, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus capitis* and *Staphylococcus warneri* can be found in large amounts on the skin particularly in the areas covering the upper body [58,59]. Staphylococci are associated with conjunctivitis, blepharitis [60] and contact lens induced peripheral ulcer [61,62]. *Staphylococcus* spp. from contact lens cases can be derived from the normal facial microbiota or from hands *via* the lens to the contact lens case [58] and the frequency of recovery from lens storage cases perhaps suggests a "carry over effect" from worn contact lenses to the lens storage case or *vice versa.*

A study by Lakkis et al. has found that lens cases can develop moderate (100–300 colony forming unit, CFU) or heavy contamination (>300 CFU) (particularly by Gram negative bacteria) after two weeks of use [63], and the total level of lens case contamination can be as high as 10^6 colony forming units.[32,40,41] As pathogenic micro-organisms in lens cases may colonize contact lenses and be transmitted into the eyes [64], and disinfecting solutions do not necessarily eradicate all microbes even when used properly [36,65,66], a reasonable goal would be to develop cleaning methods that can reduce the level of lens case contamination as much as possible, and to encourage frequent replacement of lens cases to minimize the exposure of the eyes to pathogenic microbes. The use of antimicrobial technologies may assist in limiting case contamination and reducing the risks of microbially driven adverse events.

2. Risk factors associated with lens case contamination

Lens cases receive the least cleaning attention and are the most frequently and heavily contaminated items compared to other lens care accessories [39,67]. It is thought that poor adherence to the recommended cleaning regimen is likely to be the main cause of lens case contamination. Yet, studies have shown that even carrying out the recommended instructions does not necessarily guarantee contamination-free lens cases [21,28,36,68]. These data suggest that factors other than hygiene behavior may be responsible for microbial contamination. Indeed, *in vivo* and *in vitro* studies have suggested microbial factors such as biofilm formation [27,28,36] and microbial resistance [65] may be associated with persistent microbial contamination of contact lens storage cases.

2.1. Biofilm formation in lens cases

Bacterial biofilm can be defined as a structured community of bacterial cells. It is possible that during lens insertion and removal, bacteria may be transferred into the lens storage cases *via* fingers. Once bacteria enter the lens storage case, they may adhere to the surface of the well and switch from a planktonic (free-floating) phenotype to a sessile biofilm phenotype in response to a low-nutrient environment [69]. Micro-organisms in the sessile form become embedded in a polymetric matrix [69,70]. Bacteria initially adhere to a surface by weak, reversible bonds called van der Waals forces. Initially, biofilm can be easily removed due to the loose attachment of cells. However, the adhesion between microbial cells and the contact lens case surface becomes more persistent over time. These bacteria facilitate attachment of other pathogens by anchoring using cell adhesion protein molecules on their surfaces. They also begin to produce matrix that holds the biofilm together. It has been proposed that low metabolism of biofilm bacteria contributes to increased biofilm resistance to antibiotics and disinfectants [71,72].

Bacterial biofilm forming in contact lens storage cases has been well documented [28,36,73,74]. The use of electron microscopy and light microscopy techniques have confirmed that biofilm formation takes place in lens storage cases from patients with microbial keratitis [36]. A lens storage case provides an environment favorable for static biofilm growth [36]. Infrequent lens case replacement schedules [75,76] may allow biofilms to mature and subsequently become more resistant to antimicrobial agents (disinfecting solutions) than planktonic cells [65,74]. These biofilms might also

support free-living protozoa, such as *Acanthamoeba* spp., which may feed on other micro-organisms in the biofilm [69].

The finding of persistent case contamination with good adherence to hygiene instruction raises an issue for the licensing and testing of lens care systems. Although contact lens multipurpose solutions meet the international ISO 14729 [77] and FDA 510(k) standard [78] for adequate antimicrobial efficacy, they are only subjective to assessment against selected reference strains of planktonic bacteria and fungi [77,78]. Antimicrobial activity against attenuated laboratory strains does not ensure efficacy against clinical strains. In addition, commercially available disinfecting solutions may be ineffective against biofilm *in vitro* [28,65,79–81] and *in vivo* [28,40].

2.2. Contact lens care disinfecting systems

Historically, contact lens care systems had multiple steps, for example separate cleaning and disinfecting solution or may have included unpreserved saline used either as a lens rinsing agent or used in conjunction with a chlorine releasing tablet. Since the introduction of multipurpose disinfecting solutions, disinfecting regimens have been simplified to all-in-one multipurpose bottles. Multipurpose solutions account for over 90% of disinfection solution types in the UK [82] and Australia [83]. The most commonly used disinfecting systems currently are hydrogen peroxide, and multipurpose solution – polyhexamethylene biguanide (PHMB) or polyquaternium-1 (polyquad)/myristamidopropyl dimethylamine (ALDOX) based systems [82]. Theoretically, formulation of multipurpose disinfecting solution rather than exclusively disinfection solutions may improve user compliance, and the contamination rate of lens cases may be expected to decrease. However, the rate of lens case contamination has remained consistent (Table 1).

2.2.1. Hydrogen peroxide

Hydrogen peroxide has always been thought of as a powerful method of disinfection of contact lenses due to its broad microbial activity [28,32,84]. *In vitro* formed biofilms of *P. aeruginosa* in contact lens cases have been reported to be more effectively killed by hydrogen peroxide than PHMB [79]. A clinical study by McKenney et al. also supports such finding [73].

While the above studies supported the efficacy of hydrogen peroxide systems, others have found that peroxide care systems were associated with greater case contamination [27,56]. Certain microorganisms found in lens cases, such as Staphylococci are a common contaminant of storage cases and produce catalase which enzymatically degrades hydrogen peroxide to hydrogen and water, reducing the available concentration of peroxide. The repeated use of peroxide care systems can select for a naturally resistant population of microbes adapted to survive in such an environment [27]. However, two studies found that the level and type of contamination with a hydrogen peroxide system was similar to use of PHMB or Polyquad/Aldox-containing solutions [25,41]. Gray et al. suggested that a two step system in which incorporates a properly timed chemical neutralizing agent performs better than a single step system where the neutralizer, a platinum catalyst, is present throughout use [27].

2.2.2. Multipurpose Solutions (PHMB and Polyquad)

An in vivo study by Willcox et al. found that PHMB-based solution was associated with the highest lens case contamination rate (92%) compared to Polyquad/ALDOX and peroxide based solutions in silicone hydrogel lens wearers [41]. Vermelfoort et al. also found Polyquad preserved solutions were more effective in reducing the transfer of bacterial cells from biofilm cases to silicone hydrogel lenses soaked for 8 h within the case when compared with PHMB preserved multipurpose care solutions [85]. Similarly, an *in vitro* study by Wilson et al. confirmed greater anti-biofilm activity for Polyquad preserved solutions with increased soaking time [79]. Stapleton et al. reported that all planktonic isolates of *P. aeruginosa* were susceptible to either PHMB or Polyquad at full concentration, however, the reduction of *P. aeruginosa* biofilm is significantly higher in Polyquad than that of PHMB *in vitro* [81].

It is difficult to determine outright whether one cleaning system performs better than the others as the antimicrobial efficiency of the active ingredients and formulation of each system may be dependent on the microbial strain and phenotype. In addition, Szczotka-Flynn et al. reported that while *P. aeruginosa* and *S. aureus* biofilms were susceptible to hydrogen peroxide and Polyquaternium-preserved care solution, *Serratia marcescens* biofilm was only susceptible to hydrogen peroxide disinfection [65]. Further, the susceptibility of *P. aeruginosa* biofilm cells was considerably reduced compared with *P. aeruginosa* planktonic cells in PHMB and Polyquad based disinfecting solutions, and there was no direct association between planktonic susceptibility and biofilm susceptibility [81]. These differences in susceptibility may not be entirely due to the types of disinfecting system used. The formulation of the solution can also affect the antimicrobial efficiency. For example, Willcox et al. found that given the close similarities in ingredients between two products, containing Polyquad/ALDOX, changes in other components resulted in different bacterial contamination rates [41].

2.3. Age of the lens case and replacement schedule

Many studies have found that prolonged use of lens case is still a common practice in lens wearers [26,75,86,87] A clinical study by Lakkis et al. reported that levels of lens case contamination can develop rapidly even after two weeks of use [88]. Devonshire et al. also found that there was a positive association between age of the contact lens cases and the presence of microbial contamination in a community study [33]. A significant difference between the ages of contaminated and sterile lens cases was found; contaminated cases were likely to be older than sterile cases, with a median age of 6 months for contaminated cases, compared to a median age of 5.5 months for non-contaminated cases [33]. A study by Wu et al. has found that higher proportion of contamination free lens cases were found if used less than 9 months [26], and this indicates the importance of frequent replacement of lens cases. This is consistent with the epidemiological evidence that if lens cases were replaced less frequently than every 6 months, there was a higher risk of moderate to severe microbial keratitis compared with more frequent case replacement [89,90]. Wilson et al. suggested that lens cases should be replaced at each purchase of new disinfecting solutions [28].

2.4. Case design

There has been some debate about whether the design of the lens case itself may render it more prone to contamination. Certain lens case designs, for example, baskets and cases with the ridges as well as the condition of the storage cases, cracks, and surface defects, may provide an ideal niche for bacterial colonization and biofilm formation [36,94]. Wilson et al. and Penley et al. suggested that when using one step hydrogen peroxide disinfection in the presence of a platinum catalyst, due to the instant decomposition of hydrogen peroxide at the site of the neutralizing disk, more viable micro-organisms can be cultured from the neutralizing discs of a hydrogen peroxide system than other parts of the lens case [79,95]. Kanpolat et al. [96] have suggested a lens case should have an easily cleanable design to avoid biofilm formation. Devonshire et al. however, found no significant difference between lens case designs in the level of contamination [33].

Lens cases on the market are mostly two-flat wells with screw tops made up of polyethylene or polypropylene, with smooth or ridged lens wells. Previous studies have shown that there is potential for the lens case to absorb the biocide from the disinfecting solution [97]. Polyquad tends to bind to polypropylene [97], thus, reducing the antimicrobial efficiency during soaking [97,98]. On the other hand, an antimicrobial solution can condition the case surface in which may provide surface antimicrobial effects.

2.4.1. Silver-impregnated lens cases

Other approaches reducing lens case contamination have been explored, such as using silver compounds that have broad biocidal activity against bacteria and low toxicity to mammalian cells [99]. Silver is known to affect bacteria upon contact by interference with DNA, cellular respiration, sulphydryl groups and enzyme conformation [100,101], and the impregnation of silver into lens case materials has recently been developed. The silver-impregnated lens case contains ionic silver which is incorporated during the injection molding process so it cannot be worn away [100]. At the time of writing, there are three commercially available silver-impregnated cases: MicroBlock (CIBA Vision, Atlanta GA), i-clean (Sauflon Pharmaceuticals Ltd., London, UK) and Nanocase (Marietta Vision, Marietta, GA). Of note, different types of silver-impregnated lens cases may have different modes of action. Microblock cases demonstrated robust *in vitro* activity against most Gram-negative bacteria, whereas i-clean cases were more effective against *S. aureus* [102]. Overall, Vermelfoortet al. and Dantam et al. found that silver impregnated cases showed effective antimicrobial action against *P. aeruginosa in vitro* [85,102]. Clinical trials conducted by Dantam et al. [103] and Amos et al. [100] showed that silver-impregnated lens cases had a statistically significantly lower rate of bacterial contamination (the latter study showed that the contamination was reduced by approx. 40%) than other standard lens cases. While these antimicrobial lens cases inhibit biofilm formation, it is also important to ensure that such an action will not further enhance microbe virulence and that toxic factors (silver ions) do not have unwanted ocular or other side effects.

2.4.2. Selenium lens cases

Recently, the antimicrobial effect of selenium has also been investigated. Research has shown that selenium covalently incorporated into polypropylene polymer contact lens cases could inhibit *S. aureus* biofilm formation [104]. Selenium, which is not yet commercially available, kills bacteria by the catalytic formation of superoxide radicals without having to leach out from the material to be active. In addition, it is less expensive than silver and less likely to cause allergies [104]. A recent review of antimicrobial contact lenses and cases assesses aspects of various antimicrobial strategies [105] and further studies are needed to establish the effectiveness of silver-impregnated/selenium incorporated cases in conjunction with multipurpose solution and contact lenses to better reflect the in use situation, especially the impact of non-compliance.

2.5. Wearing schedule and contact lens modality

Occasional wearers store contact lens in their cases for a period of time without regular disinfection, which may favor the development of biofilm in lens cases. This is particularly true for one step peroxide solutions, where the lens is stored in unpreserved saline following neutralization [95]. A study by Devonshire et al. found that the amount of time elapsed since the solution from the contact lens cases had been changed was significantly longer in contaminated than in sterile cases [33]. The authors did not find any significant difference in the level of lens case contamination between conventional and disposable lenses wearers and lens wearing time (h/day) [33]. Yung et al. examined the microbial contamination of lens accessories and found that lenses used by occasional wearers were more likely to be associated with pathogenic micro-organisms and that lens contamination was a predictor of whether a lens case will be contaminated [39]. The use of different contact lens materials does not appear to affect contamination rate or the types of microbes isolated from cases [43].

2.6. Contact lens wearing experience

A study by Yung et al. found no difference in the level of lens case contamination between groups having different wearing experiences [39]. However, it has been hypothesized that the extent of contact lens wearing experience can influence the level of adherence to hygiene practices and theoretically the level of lens case contamination [76]. Experienced lens wearers might reduce lens handling time, and hence, concomitantly reduce the potential for contamination [76]. Conversely, experienced wearers may become less vigilant with hygiene practice over time [106]. A study of a university population also found a strong association between compliance and the length of contact lens wearing experience [107]. A rapid decrease in compliance occurs within the first two years, and a slower rate of deterioration in hygiene compliance thereafter [75]. A recent study also supported that lens wearers who had more than two years of wearing experience had higher levels of contamination than those who had less than two years wearing experience [43]. Nevertheless, re-educating lens wearers regarding hygiene practices increased daily lens case cleaning [108] and other general hygiene compliance [75].

2.7. Gender

Males have been shown to have lower adherence to hygiene practices [33,76,109], and independently have a higher risk of microbial keratitis compared to women. One study has shown that males are more likely to discontinue contact lens wear [110]. While it is unclear whether discontinuation may be due to greater non-compliant behaviors leading to problems with continued successful contact lens wear, males do seem to have a higher risk taking propensity compared with females [111]. Consequently, gender may impact rate and level of lens case contamination. A small prospective randomized clinical study assessing the time course of lens case contamination development found that male gender was a significant risk factor for case contamination [88]. However, this has not been confirmed in other studies [33,112].

2.8. Hand washing

Poor hand washing is common in contact lens wearers, occurring in 14–50% of wears [75,76,86]. Two studies by Mowrey-McKee et al. demonstrated that microbial contamination occurs when handling lenses [113,114], therefore, hand-washing with soap is recommended to minimize contamination [115]. Consistently, lens wearers who failed to wash hands prior to handling lenses were 5 times more likely to have contact lens and case contamination [88]. This is further supported by epidemiological studies that poor-hand washing is a risk factor for microbial keratitis [8,116]. In contrast, a study has demonstrated that hand washing with plain water or regular soap actually increases the amount of contaminants transferred from the hands to a hydrogel lens than not washing hands and only the use of an alcohol wipe after hand washing is beneficial [117]. This finding is controversial. A possible explanation would be that hand washing with water and soap may detach and release micro-organisms from fingernails or palm where high amounts of micro-organisms are concentrated [118]. Thus, these contaminants are transferred onto other surfaces that come in

contact first, instead of being dislodged into the sink or onto the tissue had the rinsing procedure been adequate or hands been dried on clean tissues. However, the authors still recommend hand washing in order to remove environmental contaminants which may have greater pathogenic potential than the common skin bacteria. A recent study by Wu et al. has also found that washing hands with soap and water can reduce lens case contamination [43]. It is difficult to compare suitability of products for hand washing due to differences in methodology and study design. Nevertheless, infection control guidelines for optometrists [119] recommends the most to least effect hand hygiene products as alcohol formulations, chlorhexidine, iodophors, triclosan, and plain soap. Many studies assessing hand washing are conducted in hospital settings where medical personal are tend to have a higher exposure to pathogenic micro-organisms than healthy lens wearers who only carry mostly commensal biota. Therefore, whether an alcohol based formulation is necessary in the usual household environment, and if the residual alcohol on the fingers might be an issue for handling lenses need further investigation. This highlights the importance of educating wearers in appropriate hand washing procedures.

Assessing hand washing behaviors amongst lens wearers is a difficult task as this is often conducted using self-reported questionnaires which have several limitations. Recall bias and potentially over-reporting of socially desirable answers are possible. In addition, the experimental tool (questionnaire) needs to be sensitive enough to capture the scope of hand washing practices. *E.g.* with tap water only or with soap and water. The wording and phrasing need to be precise in specifying the use of soap and/or other detergents to differentiate adequate and poor hand washing and questions need to be time specific. These limitations perhaps explain why the link between poor hand washing and lens case contamination was infrequently reported.

3. How should we clean lens cases to minimize contamination?

Contact lens care instructions are regularly updated from time to time to reflect current cleaning products and new evidence. In 1994, Larragoiti evaluated several measures for cleaning lens cases *in vitro* and reported that rinsing contact lens cases with hot water and allowing them to air dry was the most efficient method in reducing microbial loads [120]. Rinsing cases with water was later discouraged by Larkin [32] as tap water could be a source of *Acanthamoeba* which can cause severe microbial keratitis [19,22,121–123], although the use of water boiled in a kettle and left to cool was recommended by some sources [124]. Topping-off solutions (filling up solution without discarding the old solution) is associated with higher levels of contact lens/case contamination [125] and lens related complications [126]. Therefore the use of fresh disinfecting solution is essential.

Formal guidelines on lens case hygiene have been conflicting. The Food and Drug Administration (FDA) only has limited information on lens case hygiene practice (http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm048893.htm). The FDA advises that lens cases should be rinsed with disinfecting solution, air-dried face down after use and replaced every 3–6 months [93]. A review of the product inserts from different manufacturers revealed a general recommendation that lens cases are rinsed with disinfecting solution and air-dried afterwards without specifying positions [92]. The recommended case replacement schedule from the manufacturers ranges from one to six months. Although most of the two-well flat lens cases need to be air-dried after use, the silver-impregnated lens cases reportedly perform better when recapped [100], but this was not explicitly recommended in the manufacturers' or the FDA's guidelines reviewed recently.

Other strategies have included cleaning cases with a cotton bud [27] or toothbrush [127], heating cases in boiling water [120,128], with a hairdryer [25], or using a microwave oven to limit contamination [11,129]; although none of these methods has been studied in depth. In addition, their applicability across the myriad of care systems and storage case types and designs is unclear, particularly with the advent of antimicrobial storage cases.

3.1. Evidence-based lens case cleaning methods

A series of studies showed that a cleaning protocol incorporating rubbing and tissue-wiping is more effective in removing biofilm than the current manufacturer's guideline regardless of lens case type and rinsing agents [95,130,131]. Soaking and air-drying alone may not be adequate and recapping lids should be discouraged in non-silver impregnated lens cases. Rubbing and wiping, currently not included in the manufacturers' guidelines, proved essential in dislodging biofilm from lens cases and tissue-wiping also helps to remove excess water from the lens case. Drying lens cases with tissue after rinsing also significantly reduces numbers of adherent *Acanthamoeba* [132]. Further, air-drying lens cases face down may minimize the source of air-borne contamination [133]. This finding supports the guidelines from the FDA. Since mechanical friction has been proven to be the most effective, it is expected that the majority of biofilm/contaminants on the lens well and lids can be removed by the cleaning procedures. As the threads of screw tops may be difficult to clean by rubbing with a finger, future study is needed to investigate the effectiveness of the new cleaning guidelines on screw tops where fingers may come in contact when handling contact lenses. Recently, a warming device [134] has been developed for use with contact lens cases. It has demonstrated a total elimination of bacterial biofilm when lens cases are left at 60 °C in the warm contact device [135]. Its ability to reduce biofilm was also shown to be better when compared with the "rub, rinse, wipe and dry" [66,95] method as well as the silver-impregnated lens cases [100,102]. It is essential to investigate whether these changing in cleaning practices, either by the cleaning methods, the use of silver-impregnated lens cases or the warming device have impacted on the rates of microbially driven adverse events in the community. *Acanthamoeba* contamination is often overlooked in the literature, and future studies are warranted to establish the rate of *Acanthamoeba* contamination following the changing hygiene protocols.

4. Improving lens case hygiene practice

Past efforts by practitioners have generally focused on improving hygiene practice through education and by providing information. Despite these efforts, the level of hygiene practice remains low [75,76,91,108,136]. Non-adherence to recommended hygiene regimens in lens wearers ranges from 50% to 79% [136]. The major reported behaviors in lens wearers include not rinsing lens cases with disinfecting solution after use [39,87,91,108,137,138], not air-drying lens cases [39,75,91] and not replacing lens cases regularly [75,86,91,137,139]. Ascertaining the underlying causes of non-adherence to recommendations are complex and the underlying causes may also be diverse. Lens wearers' adherence to hygiene practices is usually assessed subjectively by self-reported questionnaires or interviews, or assessed objectively based on the level of lens case contamination recovered. Nevertheless, these assessments have major drawbacks. Firstly, "adherence" is defined as the extent to which a person's behavior coincides with a given recommendation. Non-adherence then implies that patients do not follow the advice of their health care providers. However, if comprehensive and consistent advice/guidelines are not available in the first place for lens wearers, it is not correct to justify a lens wearer being

non-adherence in the strictest sense. Research has shown that the majority of non-adherence in lens wearers appears to be due to a lack of awareness of adequate cleaning procedures [108] or the importance of lens case cleaning [136]. Secondly, scant information was available to ascertain whether the recommended guidelines were effective in removing biofilm. As a result, it could be very difficult to differentiate whether lens wearers had not carried out a cleaning regimen, or had carried out a cleaning regimen that itself was not effective in removing biofilm.

Therefore, it may be that lens wearers are not receiving adequate instructions from eye care practitioners in the first place [107], are confused by different recommendations from various sources [92], choose not to follow instructions due to their perception of the impact of the risk of non-adherence to recommended hygiene regimen [108]. This highlights the importance of establishing effective lens case cleaning guidelines.

5. Potential improvements in the lens care products

While lens case contamination may predispose lens wearers to adverse events [20,21,47], it is not practical to achieve a lens case that is completely free from microbial contamination due to commensal microbiota on the eyelids that can be transferred into the lens case during lens handling, *via* fingers, despite prior hand cleaning. To date, no clinical research has determined a threshold level of microbial exposure below which the risk of ocular adverse events is decreased. The goal is to develop a protocol that minimizes bacterial contamination of lens cases and thus reduces ocular exposure to opportunistic pathogens. However, this goal is not straightforward as several factors, other than hygiene are usually outside the control of lens wearers and are in the hands of the industry.

First, the regulatory bodies should introduce standardized test methods for assessing care solution efficacy against *Acanthamoeba* or acceptance criteria for trophozoite or cyst kill [140].

Second, in terms of lens case topography, a smooth internal surface is easier to dislodge biofilm from lens cases by shearing force compared with a ridged surface. As the mechanical shearing force has been proven to be the most significant method in removing biofilm, when a lens case is cleaned by the rubbing mechanisms, the antimicrobial efficiency may be less relevant [66].

Lens care systems on the market often have a potent disinfecting solution accompanied with less than ideal lens case designs, or *vice versa.* Wu et al. has found a mismatch between care solution and the respective lens case in approximately 25% of lens wearers [43], and such a mismatch is an independent risk factors for lens case contamination. Further research is needed to design a potent disinfecting solution accompanied with a lens case internal well from which it is easy to dislodge bacteria and of an antimicrobial property, to achieve maximum antimicrobial effect.

As the literature has identified poor hand washing is a risk factor for lens case contamination [88] and microbial keratitis [8], better instruction delivery/informing tools regarding proper hand washing and lens case cleaning steps need to be developed and incorporated into the lens product inserts. These goals require the collaboration of lens care manufacturers, lens care practitioners and the FDA. As a lens wearer may comply with the cleaning regimens to a different degree in different situations and over the course of long-term usage, the next steps will be to determine how these strategies are best combined and delivered to encourage lens hygiene commitment in lens wearers and how this process is to be modified for different populations and products.

This review urges the industry to revisit storage case designs and solution antimicrobial efficiency against biofilm and *Acanthamoeba*, and to provide more comprehensive hygiene information in the products inserts and encourages professional bodies to revise current hygiene guidelines for practitioners and lens wearers.

6. Conclusion

Lens wearers' compliance in lens case hygiene is often a neglected aspects of contact lens maintenance and lens case contamination is common in the community. Poor hygiene practices may be associated with the limited and inconsistent lens case cleaning guidelines in literature and various authoritive advisories. Rinsing, rubbing, tissue-wiping and air-drying face down", an evidence-based lens case cleaning guideline was proven effective in removing biofilm in two flat well lens cases.

The level of lens case contamination is determined by the cleaning procedure used, the antimicrobial efficiency of disinfecting solutions used to rinse and soak the lens case wells, and the internal lens well design. Lens case replacement is also an important aspect of lens care. Even though there are several recommended replacement intervals, limited clinical evidence is available to support these suggestions. Future clinical studies should be set up to establish the optimum lens case replacement frequency to limit contamination and to understand the threshold level of case contamination in adverse responses.

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