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Microbial contamination of hydrogel contact lenses

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U. GOPINATHAN, F. STAPLETON, S. SHARMA, M.D.P. WILLCOX, D.F. SWEENEY, G.N. RAO AND B.A. HOLDEN. 1997. Bacterial contamination of contact lenses (CLs) may contribute to CL-related corneal infection and inflammation. This study reports CL biota over time during daily and extended wear. Microbial contamination of a 58% water, ionic hydrogel CL and a 38% water, non-ionic hydrogel CL was evaluated in an Australian and an Indian population. Fifty wearers were repeatedly sampled over 18 months. Overnight CL use did not alter the frequency of positive cultures, nor the spectrum of organisms compared with daily CL wear. There were no differences in type and frequency of CL contamination between the CL types. Positive cultures were more frequently recovered from the Indian population compared with the Australian population. *Streptococcus* spp. and *Propionibacterium* spp. were more frequently isolated from the Australian population. Fungi and *Bacillus* spp. were more frequently isolated from the Indian population. Normal CL biota alone cannot explain the increased rate of infection and inflammation in extended wear.

INTRODUCTION

Contact lens (CL) wear causes a diverse spectrum of external ocular disease including rare but severe corneal infections and more common but less severe, corneal inflammation. Both types of disorders occur more commonly in extended wear (EW) compared with daily wear (DW) hydrogel CL use (Schein *et al.* 1989a; Dart *et al.* 1991; Stapleton *et al.* 1993). In CL-related corneal infections (microbial keratitis), bacterial contamination of the CL by the causative organism has been reported (Stapleton *et al.* 1995a). Gram-negative bacteria, particularly *Pseudomonas aeruginosa*, have been strongly implicated as causative agents in up to 70% of culture-proven CL-associated microbial keratitis (Galentine *et al.* 1984; Schein *et al.* 1989b). This is in contrast with non-CL-related microbial keratitis, which is more frequently associated with Gram-positive organisms (Galentine *et al.* 1984). This alteration in the spectrum of organisms in lens-related microbial keratitis may be partly associated with the ability of certain organisms to colonize the CL during wear or storage. In addition, large numbers of Gram-negative bacteria, including *Ps. aeruginosa*, *Serratia marcescens* and *Haemophilus influenzae*, have been recovered from the CLs of wearers with an acute corneal inflammatory response – contact lens-induced acute red eye (CLARE) (Sankaridurg *et al.* 1995; Holden *et al.* 1996).

Previous studies on bacterial contamination of CLs during wear have shown that high numbers of Gram-negative bacteria are rarely isolated from the lenses of asymptomatic wearers following wear. Lens contamination appears to be infrequent and involves small numbers of organisms, typically less than 30 colony-forming units (cfu) per lens (Mowree-McKee *et al.* 1992; Hart *et al.* 1993). Coagulase-negative staphylococci are the commonest organisms isolated from CLs following wear (Hovding 1981; Fleiszig and Efron 1992; Mowree-McKee *et al.* 1992), although occasional isolation of *Staphylococcus aureus*, streptococci and less frequently Gram-negative bacteria (Fleiszig and Efron 1992) have been reported. Contact lens contamination is usually attributed to lens handling (Mowree-McKee *et al.* 1992); however, during uncomplicated CL wear, these organisms appear to be rapidly cleared from the CL surface. Contact lens-associated organisms may also be derived from contaminated storage cases,

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and where CLs have been sampled following storage, higher rates of contamination with Gram-negative organisms have been reported (Lipener *et al.* 1995). The majority of studies on CL contamination have reported sampling on a single occasion only for heterogeneous groups of CL wearers using a range of care systems, lens types and wear schedules. Comparison between studies is often not possible and the effects of care system contamination, lens storage and handling are not easy to differentiate from biota during wear alone.

The aims of this study were to elucidate the impact of daily wear and extended wear on the contamination of CLs over time in a controlled population of neophyte CL wearers. The effect of two different base CL materials on contamination in use was evaluated while controlling for the effects of CL age, care system and wear modality. To establish possible regional and seasonal variations in CL contamination, this study was performed at two centres over an 18-month period.

METHODS

Subjects

Fifty subjects participated in the study, which was conducted at two centres: L.V. Prasad Eye Institute, Hyderabad, India, and the Cornea and Contact Lens Research Unit, School of Optometry, University of New South Wales, Sydney, Australia. Twenty-five neophytes requiring CLs for low myopic correction only were enrolled in the study at each centre. All subjects were free of ocular and systemic pathology and had no previous ocular surgery. Subject details are shown in Table 1.

Lenses

The base materials of the lenses used were etafilcon A (Acuvue; Vistakon, Johnson & Johnson, Jacksonville, FL), a 58% water content ionic hydrogel material, and polymacon (SeeQuence 2; Bausch & Lomb, Rochester, NY), a 38% water, non-ionic hydrogel material. A different lens type was worn in each eye and lenses were allocated randomly. Patients wore their lenses initially on a daily wear (DW) basis for an adaptive period of at least 2 weeks. During the DW phase, the lens care regimen consisted of a rub, rinse and disinfection procedure with Bausch & Lomb Renu multipurpose solution with Allergan Lens Plus (Allergan, Irvine, CA) spray saline for rinsing.

After completion of the DW phase, CLs were aseptically removed and subjected to microbial investigation. New lenses were subsequently inserted and the subjects commenced a six night extended wear (EW) schedule for a period of 18 months. Microbial investigation of CLs was also performed following one night (1N), one week (1W) and one month (1M) of extended wear and every 3 months thereafter.

Procedure

The lenses were removed aseptically, placed in sterile vials containing 2 ml of phosphate-buffered saline (PBS) (pH 7·2 ± 0·2) and transported to the microbiology laboratory within 30 min of lens collection. The lens was vortexed in the transport PBS for 30 s and aseptically transferred into 10 ml of molten nutrient agar (45°C). The nutrient agar containing the lens was gently shaken and poured over a chocolate agar plate, while ensuring that the lens was fully unfolded using sterile forceps. Aliquots of 400 μl of PBS from the lens vial were inoculated onto three chocolate agar plates and one Sabouraud's dextrose agar bottle or plate. The incubation conditions are detailed in Table 2.

Microbial growth on various media was enumerated and the number of cfu per whole lens was calculated. Representative bacterial colonies from each plate were Gram stained and identified employing standard microbiological methods (Balows *et al.* 1991) for Gram-positive organisms. Gram-negative organisms were identified using API strips (Vitek BioMerieux, Sydney, Australia) and Biolog assays (Biolog, Hayward, CA).

Data analysis

The incidence rates were compared using the χ^2 test with Yates correction, where appropriate. Numbers of cfu in the different populations were compared using the Mann–Whitney U-test. Regression analysis was performed to determine the trend in lens contamination with time for different lens types and different populations during extended wear.

Table 1 Subject details

Population	*n*	Age (mean years ± S.D.)	Males:Females	Length of extended wear phase (mean months ± S.D.)
Indian	25	23·4 ± 4·9	13:12	12·6 ± 2·5
Australian	25	29·9 ± 8·3	11:14	12·6 ± 2·5

Table 2 Media and incubation conditions for the contact lens and lens vial solution

Material	Media	Atmosphere	Temperature (°C)	Time (d)
Lens	Chocolate agar	5% CO_2	35	2
Lens vial solution	Chocolate agar	5% CO_2	35	2
	Chocolate agar	Aerobic	35	2
	Chocolate agar	95% N_2, 5% CO_2	35	7
	Sabouraud's dextrose agar	Aerobic	25	7

RESULTS

The frequency of any microbial contamination of etafilcon A and polymacon lenses following daily and extended wear in the two populations is shown in Table 3. Differences in the frequency of contamination between the two lens types were not significant in either population for daily or extended wear. In DW, etafilcon A lenses were more frequently contaminated in the Indian population than the Australian ($P < 0{\cdot}05$). In EW, both lens types were more frequently contaminated in the Indian compared with the Australian population ($P < 0{\cdot}0005$). Extended wear was not found to increase the frequency of lens microbial contamination compared with daily wear. No significant change in lens contamination with time occurred for extended wear for either population or lens type.

The types and frequency of organisms isolated during daily wear for each population and lens type are shown in Table 4. The most common organisms isolated were coagulase-negative staphylococci for both populations and lens types. A greater number of species were isolated from lenses worn by the Indian population compared with the Australian popu-

Table 3 Frequency of microbial contamination per sampling occasion for lenses worn on daily and extended wear schedules in Indian and Australian populations

Wear schedule							
Daily wear				Extended wear			
Indians ($n = 25$)		Australians ($n = 25$)		Indians ($n = 180$)		Australians ($n = 174$)	
Etafilcon A	Polymacon	Etafilcon A	Polymacon	Etafilcon A	Polymacon	Etafilcon A	Polymacon
12 (48%)	12 (48%)	4 (16%)	6 (24%)	97 (54%)	95 (53%)	58 (38%)	56 (32%)

Table 4 Frequency of isolation of bacteria and fungi from lenses during daily wear*

	Indians ($n = 25$)		Australians ($n = 25$)	
	Etafilcon A	Polymacon	Etafilcon A	Polymacon
Coagulase-negative staphylococci	6 (24%)	6 (24%)	4 (16%)	6 (24%)
Staphylococcus aureus	3 (12%)	2 (8%)	0 (0%)	0 (0%)
Bacillus spp.	0 (0%)	5 (20%)	0 (0%)	1 (4%)
Micrococcus spp	1 (4%)	2 (8%)	0 (0%)	0 (0%)
Streptococcus spp.	0 (0%)	0 (0%)	0 (0%)	1 (4%)
Corynebacterium spp.	2 (8%)	3 (12%)	0 (0%)	0 (0%)
Pseudomonas spp.	1 (4%)	2 (8%)	0 (0%)	0 (0%)
Fungus	1 (4%)	1 (4%)	0 (0%)	0 (0%)

*Where more than one organism was recovered, all organisms were recorded per sampling occasion.

Table 5 Frequency of isolation of bacteria and fungi from lenses during extended wear*

	Indians ($n = 180$)		Australians ($n = 174$)	
	Etafilcon A	Polymacon	Etafilcon A	Polymacon
Coagulase-negative staphylococci	67 (37%)	76 (42%)	44 (25%)	38 (21%)
Staphylococcus aureus	14 (8%)	11 (6%)	2 (1%)	0 (0%)
Streptococcus spp.	2 (1%)	0 (0%)	5 (3%)	5 (3%)
Corynebacterium spp.	11 (6%)	14 (8%)	2 (1%)	0 (0%)
Bacillus spp.	11 (6%)	13 (7%)	2 (1%)	2 (1%)
Propionibacterium spp.	0 (0%)	0 (0%)	16 (9%)	19 (11%)
Pseudomonas spp.	5 (3%)	2 (1%)	0 (0%)	2 (1%)
Other Gram negatives	4 (2%)	2 (1%)	4 (2%)	2 (1%)
Fungus	7 (4%)	13 (7%)	0 (0%)	4 (2%)

*Where more than one organism was recovered, all organisms were recorded per sampling occasion.

lation; however, population and lens differences for individual species were not significant.

The types and frequency of organisms isolated during extended wear for each population and lens type are shown in Table 5. The most common organisms isolated were coagulase-negative staphylococci. Compared with daily wear, wearing lenses on an extended wear schedule neither significantly increased nor decreased the frequency of isolation of any single organism. Isolation rates were higher in the Indian population, with coagulase-negative staphylococci, *Staph. aureus*, *Corynebacterium* spp., *Bacillus* spp. and fungi isolated more frequently ($P < 0{\cdot}05$) for both etafilcon A and polymacon lenses, compared with the Australian population. Conversely, *Propionibacterium* spp. were more frequently isolated from Australian lenses compared with Indian for both lens types ($P < 0{\cdot}001$). *Streptococcus* spp. were isolated more frequently from Australian lenses compared with Indian for polymacon lenses only ($P < 0{\cdot}05$).

There was no significant increase in total lens contamination over time for either population. There were also no significant differences between lens types in total contamination over time. Table 6 shows the median and range of cfu recovered for both lens types in each population for daily and extended wear. There were no significant differences in the numbers of cfu recovered from etafilcon A and polymacon lens types. However, higher numbers of cfu were consistently recovered from Indian lenses compared with Australian lenses ($P < 0{\cdot}0001$–$P < 0{\cdot}02$).

DISCUSSION

This study reports for the first time the frequency and type of lens contamination in daily and extended lens wear in a

Table 6 Median (range) of cfu recovered for all sampling occasions for lenses worn on daily and extended wear schedules in Indian and Australian populations

Wear schedule							
Daily wear				Extended wear			
Indians ($n = 25$)		Australians ($n = 25$)		Indians ($n = 180$)		Australians ($n = 174$)	
Etafilcon A	Polymacon	Etafilcon A	Polymacon	Etafilcon A	Polymacon	Etafilcon A	Polymacon
1 (0–400)*	1 (0–700)†	0 (0–22)*	0 (0–21)†	2 (0–797)†	2 (0–700)§	0 (0–120)‡	0 (0–60)§

Comparison between numbers of cfu recovered from Indian and Australian lenses were made using the Mann–Whitney U-test.
*$P < 0{\cdot}02$.
†$P < 0{\cdot}01$.
‡$P < 0{\cdot}0001$.
§$P < 0{\cdot}0001$.

population of neophyte lens wearers. The effects of two different types of disposable lenses and regional variation on lens contamination are also reported.

Compared with daily wear use, no significant alteration in the frequency of lens contamination was observed with extended wear for both lens types at both centres. There was also no significant difference between the frequency of isolation of any single organism in daily and extended wear at both centres. In both populations, a greater number of species were recovered during extended wear compared with daily wear, although this may simply reflect greater numbers of sampling occasions during extended wear.

More frequent lens contamination was encountered in the Indian population compared with the Australian population, for both daily and extended wear modalities. The commonest organisms isolated during both daily and extended lens wear at both centres, were coagulase-negative staphylococci. Higher isolation rates of *Staph. aureus*, *Corynebacterium* spp., *Bacillus* spp. and fungi within the Indian population are likely to reflect environmental differences. Climatic variations have also been shown to influence the conjunctival biota and the spectrum of causative organisms in corneal infections (Ando and Takatori 1982; Singer *et al.* 1988), and may be expected to modulate lens contamination. From a previous study at this centre (CCLRU), it appears that lens biota are reflective of Gram-positive ocular biota, suggesting a potential source of lens contamination via the lid and conjunctival biota (Stapleton *et al.* 1995b). Seasonal variations may also influence both ocular and lens biota; however, individuals were sampled over an 18-month period, minimizing these effects.

In general, small numbers of organisms were recovered from lenses following wear, which concurs with previously reported findings (Mowree-McKee *et al.* 1992; Hart *et al.* 1993). No differences in numbers of cfu were found between the two different lens base materials; however, higher numbers of cfu were consistently recovered from Indian lenses compared with Australian lenses, which may again reflect environmental differences.

Gram-negative bacteria, particularly *Pseudomonas* spp., were isolated infrequently in both daily and extended wear at both centres, despite the known association between *Ps. aeruginosa* and lens-related corneal infections (Galentine *et al.* 1984; Schein *et al.* 1989b). This is consistent with the transient nature of *Pseudomonas* spp. at the ocular surface during asymptomatic lens wear (Stapleton *et al.* 1995b). In lens-related infections, however, it has been suggested that the lens provides a suitable niche for bacterial colonization and growth. Bacteria encased within biofilm on the posterior surface of the contact lens have been demonstrated in wearers with *Ps. aeruginosa* corneal infection (Holland *et al.* 1988; Stapleton and Dart 1995). It has been demonstrated in an animal model that organisms colonizing the contact lens surface are highly resistant to removal by normal host defences (Dart *et al.* 1988). It is possible that in these circumstances the lens acts as a vector for organisms from environmental sources and prolongs the retention time of these organisms at the ocular surface. This may result in an increase in bacterial load at the corneal surface and potential tissue damage may ensue either indirectly due to bacterial toxins or directly by bacterial invasion.

In this study, no significant differences either in the incidence of positive cultures or in the preferential recovery of any particular organism was consistently found for etafilcon A or polymacon base lens materials. The type of lens polymer, water content, ionic charge or lens deposits do not appear to have made either lens type preferentially susceptible to microbial contamination. In addition, no change in either the frequency or type of lens colonization with time was observed for either lens type. This concurs with a previous study of ocular biota in neophyte lens wearers which showed no significant change in either conjunctival or lid biota with increasing wear experience (Stapleton *et al.* 1995b). Asymptomatic lens use appears not to modify the lens biota with time.

The source of lens contaminants is usually attributed to lens handling, although uncomplicated lens wear appears to result in clearing of organisms from the lens surface (Mowree-McKee *et al.* 1992; Hart *et al.* 1993). In wearers with corneal infections, however, the lens storage case has been implicated as a source of causative organisms (Mayo *et al.* 1987). Contaminants from the contact lens storage case have been linked to the ocular biota in lens wearers in one study (Morgan 1979); however, no such association was found in a more recent study of asymptomatic lens users (Fleiszig and Efron 1992). In the current study, disposable extended wear lens users were not using care systems or storage cases, and lens contamination reported here is free of possible influence from this source. Lens contamination may have arisen from transient colonizers of the ocular surface, lens handling or contamination from environmental sources. In the daily wear phase, all wearers used the same multipurpose system for lens care over a 2 week period. Infrequent Gram-negative contamination of daily wear lenses suggests that carry over from lens storage case contaminants is unlikely, since Gram-negative organisms are common contaminants of hydrogel lens storage cases (Larkin and Kilvington 1990). Gram-negative organisms, including *Pseudomonas* spp., have however been recovered in small numbers from multipurpose lens care systems following 2 weeks of use (Collins *et al.* 1994).

In conclusion, these findings may suggest that normal lens biota during asymptomatic wear alone cannot explain the increased rate of infection and inflammation in extended wear. Contamination of human biomaterials by similar Gram-positive organisms, however, frequently causes disease at other body sites. Lens contamination by pathogenic organisms is rare in asymptomatic wearers and low numbers of organisms are recovered. High numbers of Gram-negative

organisms adherent to CLs have been reported during lens-related inflammation and infection and this ability of organisms to colonize lenses in high numbers and form a biofilm may contribute to their ability to resist host defences and cause disease. This phenomenon may be related to the specific properties of the organism; certain species and strains are known to inherently adhere better than others to hydrogel lenses (Klotz *et al.* 1989). Alternatively, this may relate to modified local or systemic host defences preceding an inflammatory or infectious event, allowing lens colonization by opportunistic organisms.

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