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Microbial Flora in Eyes of Current and Former Contact Lens Wearers

SUZANNE M. J. FLEISZIG†* AND NATHAN EFRON‡

Corneal Biophysics Laboratory, Department of Optometry, University of Melbourne, Parkville 3052, Victoria, Australia

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Microbial flora from the right eye conjunctival sac of 84 consecutively presenting contact lens patients were compared with cultures from both surfaces of their lens after aseptic removal and with the flora of their storage cases. Similar results were obtained from contact lens and conjunctival cultures of each individual; however, there was no correlation between storage case isolates and lens or conjunctival flora, suggesting that in uncomplicated lens wear, the eye is highly efficient in eradicating microorganisms introduced via handling. Conjunctival flora during daily contact lens wear was similar to the conjunctival flora of a matched control group of non-lens wearers. However, bacteria that are considered to be part of the normal ocular flora were isolated significantly more often from former contact lens wearers. The data also indicated that the use of nonperoxide chemical lens disinfection was associated with a higher proportion of positive cultures for pathogenic microorganisms than the use of other forms of disinfection, for both current and former contact lens wearers. The isolation of potential pathogens was particularly common among elderly subjects using thick contact lenses for extended wear. These changes to conjunctival flora may contribute to the increased risk of ocular infection associated with contact lens wear.

Contact lens wear has been associated with an increased risk of infectious keratitis (26). Most varieties of bacteria do not infect the intact healthy cornea (23, 29); however, there are changes to the cornea during contact lens wear that could contribute to the development of infection (7). A thorough knowledge of conjunctival and lens storage case flora during contact lens wear is necessary in order to understand how potentially pathogenic organisms may gain access to and colonize the anterior eye prior to the development of infection. In addition, this information may assist in determining how the precorneal defense mechanisms against these microorganisms are impaired by the presence of a contact lens. The effect of contact lens wear on normal ocular bacterial flora is also an important consideration, as these organisms produce antimicrobial factors which play a role in defense of the ocular tissues from infection (10, 14).

It is thought that short-term daily wear of hard (polymethyl methacrylate) contact lenses does not alter the normal bacterial flora of the eye (34). However, the results of one study suggested that there is an increase in the quantity of bacteria in the eye during hard lens daily wear, but only after 6 months of use (16).

Normal conjunctival flora has been found to be preserved during therapeutic soft lens extended wear for the treatment of corneal disease (2, 13, 25). With cosmetic soft lens wear there has been controversy as to the nature of changes to conjunctival flora. Investigators have reported no change (30), reductions (9, 12, 19, 28), and increases (3) in the quantity of bacteria isolated from the conjunctival sac during this form of lens wear. One report described a decrease in the early stages of lens wear followed by a return to baseline levels (24), while another study found changes to the variety of microorganisms present during lens wear (12). The latter finding consisted of a decrease in gram-positive normal bacterial flora and an increase in nonpathogenic gram-negative species.

Interpretation of the results of the studies described above is complicated by inconsistencies in microbiological sampling techniques, subject selection, and other aspects of methodology.

The aim of this study was to compare microbial flora of the conjunctiva during contact lens wear with the conjunctival flora of control eyes and with that of the lens storage case. In an attempt to explain the inconsistencies noted by previous investigators, the effect of various modes of lens wear and care, time factors, subject age, and previous lens wear in control non-lens wearing subjects were considered. Anaerobic bacteria, which have also been found to be associated with ocular disease (20), along with both normal and pathogenic aerobic flora, were identified.

The results of this study are the first to describe changes to conjunctival flora as a result of previous wear and to demonstrate that changes to conjunctival flora, which are found in certain groups of contact lens wearers, may not be predicted by lens storage case contamination.

MATERIALS AND METHODS

Subjects. Eighty-four consecutive healthy contact lens-wearing subjects, presenting to the Contact Lens Clinic at the Department of Optometry in The University of Melbourne, were included for investigation. Subjects had worn contact lenses for a total of between 1 week and 14 years, and microbiological samples were collected at least 4 h following lens insertion. The control group consisted of 84 individuals not wearing contact lenses (32 previous contact lens wearers and 52 subjects who had never worn lenses), who had presented to the clinic for the fitting or delivery of

* Corresponding author.
† Present address: Channing Laboratory, Department of Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, MA 02115.
‡ Present address: Department of Optometry and Vision Sciences, University of Manchester Institute of Science and Technology, Manchester M60 1QD, United Kingdom.

contact lenses. This mode of selection was adopted to ensure that control subjects were well matched to the contact lens-wearing group. Among previous lens wearers, the mean number of months since lenses had been worn was 10.8 (±21.9), and the mean total period of lens wear was 23.2 (±27.5) months.

Culture technique. A sterile urethral swab (Disposable Products, Melbourne, Australia) was premoistened in sterile saline, and the right lower conjunctival sac of each subject was sampled by rolling the swab against the conjunctiva. For lens wearers, the conjunctiva was sampled whilst the lens was in the eye.

Attempts to remove soft lenses aseptically with rubber gloves failed because of a lack of grip. Thus, the lenses were removed with bare fingers after a finger wash with a 100% alcohol swab. To monitor residual contamination with viable microorganisms from the experimenter, the fingers were allowed to dry and were then wiped on a chocolate agar plate, immediately before lens removal. On no occasion did the test plate reveal the presence of viable microorganisms. Hard contact lenses were removed by using a sterile suction cap specifically designed for this purpose. Sterility of plungers was verified by culturing the device prior to lens removal.

Following removal from the eye, both surfaces of the contact lens were cultured separately by vigorous rubbing of the surface with a sterile swab soaked in saline in order to isolate microorganisms that were reversibly associated with the contact lens surface.

The interior surface and contents of patients' contact lens storage cases were sampled with a moistened swab by using a firm rubbing action against the wall of the container.

Microbiological identification. Two horse blood agar plates and one chocolate agar plate were inoculated with each sample. One horse blood agar plate was incubated in an anaerobic jar. The remaining plates and the anaerobic jar were placed in an incubator adjusted to 35°C with 5% CO_2. All plates were observed daily for the formation of microbial colonies for a total of 7 days. Gram-negative bacteria, *Streptococcus* species, and anaerobic bacteria were identified with API 20E (or 20NE for nonfermentative gram-negative varieties), APIS, and APIA identification kits, respectively (Analytab Products, Inc., Plainview, N.Y.), in conjunction with appropriate supplementary tests (17). Other gram-positive species and fungi were identified by using standard microbiological techniques, as previously described (17).

Since the swabbing process is unlikely to remove all resident microorganisms, the results of any quantification procedures may not accurately reflect the total number of microbes residing in the conjunctival sac, on the lens, or in the lens storage case. For this reason, only the species and frequency of isolation are reported.

Data analysis. Bacteria considered to be normal flora for the purposes of this investigation, included coagulase-negative *Staphylococcus* species, *Corynebacterium* species (except *Corynebacterium diphtheriticum*), *Propionibacterium acnes*, *Micrococcus* species, *Bacillus* species, and *Peptostreptococcus* species. This classification was based on observations that these microorganisms have been isolated from at least 5% of healthy eyes (20, 22). In instances when other microorganisms were isolated, the sample was considered to contain potentially pathogenic species. A list of these organisms and the location from which each was isolated are presented in Table 1. Negative cultures included only those samples from which no microorganisms were isolated by the methods described above.

TABLE 1. Isolation and location of microorganisms considered to be potential pathogens

Microorganism	No. of subjects positive for isolate				
	Lens wearers				Control (conjunctiva)
	Conjunctiva	Lens front	Lens back	Lens case	
Gram-negative bacteria					
Acinetobacter anitratus		4			
Acinetobacter lwoffi	1	2			
Actinobacillus lignieresii					1
Aeromonas hydrophilia				1	
Alcaligenes xylosoxidans subsp. *dentrificans*			1		1
Comamonas acidovorans	1	1	1	1	
Comamonas testosteroni				1	
Enterobacter cloacae		1		3	
Escherichia coli			1		
Flavobacterium indologenes				2	
Klebsiella oxytoca			1	1	
Klebsiella ozaenae				1	
Klebsiella planticola				1	
Klebsiella pneumoniae				2	
Moraxella atlantae				1	
Moraxella lacunata				1	
Moraxella species				1	
Ochrobacterum anthropi				1	
Pasteurella multocida	1		1		
Proteus species				1	
Providencia species				1	
Pseudomonas alcaligenes				1	
Pseudomonas cepacia				1	
Pseudomonas paucimobilis				3	
Pseudomonas putida				1	
Serratia liquefaciens			1	1	
Serratia rubidaea		1			
Shingobacterium multivorum				2	
Unidentifiable gram-negative rod					2
Other microorganisms					
Aerococcus viridans	1		2		
Eikenella corrodens				1	
Enterococcus faecium			1		
Erysipelothrix species				1	
Staphylococcus aureus	1	2	1	1	1
Streptococcus microaerophilic				1	
"*Streptococcus milleri*" group		1	1	1	1
Streptococcus mitis			1		
Streptococcus pneumoniae	1	1	1		1
Streptococcus salivarus		1	1		
Streptococcus sanguis	1	1	1	1	
Viridans streptococcus			1		
Nocardia species				1	
Candida albicans	1		1	1	

The chi-square test was used to determine the statistical significance of any differences in data, when there were five or more datum points in at least 80% of cells (27). Otherwise, Fisher's exact test was employed.

RESULTS

Exclusion criteria. Infectious keratitis in young myopic patients wearing contact lenses for cosmetic reasons is most often due to gram-negative bacteria, particularly *Pseudomo-*

TABLE 2. Subject details[a]

Subject group (no. in group)	Sex		Age (yr)[b]	Time worn (h)[b,c]	Age of lens (mo)[b]	Total lens wear (mo)[b,d]
	F (%)	M (%)				
Current lens wearers (72)	63	38	27.0 ± 9.9	7.8 ± 2.6	8.6 ± 13.9	35.1 ± 46.4
Former lens wearers (23)	65	35	30.1 ± 13.2			
Control (50)	52	48	26.0 ± 12.1			

[a] Following implementation of exclusion criteria.
[b] Mean ± standard deviation.
[c] Hours of lens wear prior to sampling.
[d] Total period of contact lens wear in months prior to sampling.

nas aeruginosa (4). In contrast, older contact lens wearers often suffer from gram-positive bacterial and polymicrobial infection (5, 6), suggesting that the mechanism involved in the development of infectious keratitis in elderly contact lens wearers may differ from the pathogenesis of corneal ulceration in younger subjects. Eyes of subjects wearing high-power (thick) lenses and those wearing lenses on an extended-wear basis are subjected to greater physiological stress than when low-power (thinner) lenses are worn or when lenses are used on a daily-wear basis. Twelve of the contact lens-wearing subjects were over 69 years of age, wore extended-wear lenses, or wore contact lenses with power of greater than ±10.00 diopters. Cultures positive for potential pathogens were isolated from the lens or conjunctival environment of 8 of these 12 subjects: 4 of the 5 elderly subjects, 8 of the 11 subjects using thick lenses, and 5 of the 6 subjects wearing extended-wear lenses (seven subjects belonged to more than one of these categories). This represented a significantly higher isolation rate for potential pathogens than for other contact lens wearers in the study group (10%) ($\chi^2 = 7.00$; $0.01 > P > 0.001$). For these reasons, subjects over the age of 69, subjects wearing extended-wear lenses, or subjects with a refractive error of ±10.00 diopters or greater were excluded from further analysis. There were insufficient data to separately determine the effect of age, extended wear, or lens thickness on lens or conjunctival flora for contact lens wearers. Members of the control groups who were over the age of 69 or had worn contact lenses within the previous 3 weeks were also excluded.

The remaining contact lens-wearing group consisted of 59 soft contact lens wearers and 13 hard contact lens wearers. One soft contact lens wearer did not disinfect lenses. Thermal, hydrogen peroxide (3%), and nonperoxide chemical disinfection was used by 34, 9, and 15 soft lens wearers, respectively. All hard contact lens wearers used nonperoxide chemical disinfection. Control subjects comprised 23 former contact lens wearers and 50 individuals who had never worn contact lenses. Other subject details are outlined in Table 2. A summary of the results of conjunctival, contact lens, and lens storage case cultures are presented in Tables 3 and 4.

Conjunctival flora of lens wearers versus that of control eyes. There was no significant difference in the proportion of positive cultures obtained from lens wearers and subjects who had never worn lenses ($\chi^2 = 3.1$; $0.1 > P > 0.05$). However, positive conjunctival cultures were isolated more frequently from members of the control group who were former lens wearers compared with both control subjects who had never worn lenses ($\chi^2 = 14.8$; $P < 0.001$) and current lens wearers ($\chi^2 = 6.39$; $0.02 > P > 0.01$). These data suggested that lens wear produced an increase in conjunctival flora which was apparent only following the cessation of lens wear.

Staphylococcus species were isolated equally as often from all three groups of subjects; however, significantly more diphtheroids (including various *Corynebacterium* species and *Propionibacterium acnes*) were isolated from the eyes of former lens wearers (11 of 23) than control subjects who had never worn lenses (10 of 50) ($\chi^2 = 5.9$; $0.025 > P > 0.01$). The proportion of current lens wearers who were found to harbor diphtheroids in their conjunctival sac (26 of 72) was not significantly different to the proportion of eyes harboring these organisms for either of the two control groups.

There was no statistically significant difference in the prevalence of potentially pathogenic flora between the lens-wearing and control non-lens-wearing groups. However, there were significantly more samples positive for potential pathogens among former contact lens wearers than among control subjects who had never worn lenses ($P = 0.03$). This effect may have applied only to former lens wearers who had used nonperoxide chemical disinfection, since exclusion of data pertaining to these individuals from the analysis resulted in the number of pathogens for the remaining group of former lens wearers being statistically indistinguishable from that of the control group. *P. aeruginosa*, the most common pathogen involved in contact lens-related infectious keratitis (4), was not isolated from any of the subject or control eyes.

Lens isolates versus conjunctival flora. There was no statistical difference in the distribution of microorganisms isolated from the front and back surfaces of hard or soft contact lenses or between either of the lens surfaces and the conjunctiva by the methods employed in this investigation ($\chi^2 = 0.26$; $0.5 > P > 0.7$).

Lens and conjunctival flora during lens wear. The use of nonperoxide chemical contact lens disinfection was associated with an increase in pathogenic flora in the lens or conjunctival environment of soft contact lens wearers (5 of 15) compared with that in subjects who had used only thermal or 3% hydrogen peroxide methods for lens disinfection (1 of 43; $P = 0.003$). Similarly, among former soft lens wearers, the incidence of pathogenic conjunctival flora was significantly higher if subjects had used nonperoxide chemical soft lens disinfection systems (3 of 5) compared with those in subjects who had used only thermal or hydrogen peroxide systems (0 of 14; $P = 0.01$).

Potentially pathogenic microorganisms and normal flora were isolated in similar proportions from subjects wearing hard and soft contact lenses ($\chi^2 = 0.013$; $0.95 > P > 0.90$). There was no correlation between a history of complications related to lens wear and the distribution of microbial flora, nor was flora affected by months of use or the number of hours of continuous wear. The distribution of results was similar for contact lenses that had been used for greater than 6 months compared with newer lenses (used for less than 6 months) ($\chi^2 = 1.205$; $0.3 > P > 0.2$).

Storage case contaminants. Of the 72 contact lens wearers studied, 60% presented to the clinic with their lens storage case. Of these, 71% were contaminated. Potential pathogens were isolated from 66% of contaminated cases; gram-negative bacteria being isolated from all but one of these cases. The most common contaminants were *Pseudomonas* species (14% of all cases) and *Klebsiella* species (11% of all cases). *P. aeruginosa* was not encountered. Microorganisms were isolated from both empty cases and those containing lens care solutions.

The six storage cases belonging to subjects who wore hard contact lenses were all contaminated. All but one of these cases contained solutions that were manufactured for the purpose of soaking and storage of hard contact lenses. Among storage cases from soft contact lens wearers, there were significantly more contaminated cases belonging to subjects who used thermal or peroxide systems for disinfection (19 of 28) compared with those of subjects who used other chemical systems (3 of 10; $P = 0.04$).

Storage case flora versus lens and conjunctival flora. There did not appear to be any correlation between lens storage case and lens or conjunctival flora. Indeed, there were only 11 individuals for whom both the conjunctiva and lens storage case were culture positive for microorganisms, and only in one instance was the same bacterial species found in the eye or lens environment and in the corresponding lens storage case (*Serratia liquefaciens* was isolated from the lens storage case of one subject, and 1 CFU was found on the back surface of the contact lens from this subject.). Interestingly, lens and conjunctival cultures were negative for the remaining 18 patients with contaminated cases. There appeared to be a trend toward negative conjunctival cultures in subjects with contaminated cases, although this was not found to be statistically significant ($0.2 > P > 0.1$).

DISCUSSION

This report describes a study of conjunctival and lens storage case flora during contact lens wear. The data presented suggest that conjunctival flora is altered by contact lens wear, but only following certain modes of lens wear.

No significant difference was noted between the conjunctival flora of the contact lens-wearing group as a whole and the conjunctival flora of control subjects who had never worn contact lenses. However, previous lens wear was associated with an increase in bacterial flora compared with flora of both lens-wearing eyes and control eyes that had not worn lenses. Excluding subjects who had used nonperoxide chemical systems for disinfection, this phenomenon involved species that are not normally pathogenic to the eye and appeared to be restricted to diphtheroid bacteria.

It is thought that, in the conjunctival sac, there is a synergistic relationship between diphtheroids (coryneform bacteria) and coagulase-negative *Staphylococcus* species. Lens wear has been found to disrupt the balance between these two organisms by reducing the diphtheroidal population and favoring coagulase-negative *Staphylococcus* species habitation of the conjunctiva (24). In contrast, the results of this study demonstrate that the isolation rate for coagulase-negative *Staphylococcus* species was not affected by lens wear, whereas the isolation rate for diphtheroids was increased following the cessation of contact lens wear. Former lens wearers in this study may have been predisposed to changes to the diphtheroidal population of the conjunctival sac for reasons other than contact lens wear, which could explain why these individuals no longer wore contact lenses. Otherwise, it may be that contact lens wear led to long-lasting changes to the eyes of these individuals that altered microbial flora.

TABLE 3. Distribution of flora isolated from conjunctiva[a]

Subject group (no. in group)	No. of subjects (%) with:		
	Positive culture	Normal flora only	Potentially pathogenic species present
Current lens wearers (72)	34 (47)	29 (40)	5 (7)
Former lens wearers (23)	18 (78)	15 (65)	3 (13)
Control (50)	15 (30)	15 (30)	0 (0)

[a] Following implementation of exclusion criteria.

For each lens-wearing subject, the flora of the conjunctiva and the two contact lens surfaces were similar, implying that contact lenses in situ and the conjunctiva share their bacterial flora; thus, there was no evidence to suggest a difference in the efficacy of the pre- and post-lens environment in defense against viable microorganisms.

A wide variety of microorganisms were found in contact lens storage cases (Table 1). *P. aeruginosa* was not isolated from any of these containers, although other studies have found this species to be one of the most common lens care solution contaminants (18). This discrepancy may be due to climatic variation between study sites. Climatic variation, together with the relatively conservative use of extended wear in Australia (11), could explain why the incidence of infection with this pathogen is not reported as often during contact lens wear in Australia as it is in the United States. Moreover, *P. aeruginosa* is most often isolated from home-prepared saline solutions (18), which generally are not used in Australia.

Several contact lens-wearing subjects did not present with lens storage cases; thus, the lens containers that were cultured may belong to a biased group of more compliant patients. Nevertheless, the eyes of subjects that presented with storage cases shared flora similar to that of the eyes of those who did not bring their storage case ($0.3 > P > 0.2$).

There have been previous reports that some microorganisms can survive and grow in preserved solutions (1). In this study, some storage cases containing nonperoxide chemical disinfectants were contaminated. In fact, microorganisms were isolated from all hard contact lens chemical soaking solutions tested. None of these storage cases had been handled within the 4-h period prior to microbiological sampling. These findings support the results of an earlier investigation (33) which revealed that some disinfection solutions may not prevent the survival of microorganisms in contact lens storage cases during normal use, even though all disinfectants are tested with challenge organisms in the laboratory prior to their release on the market.

Microorganisms found to be contaminating lens storage cases in this study must either be survivors of ineffective disinfection or were introduced during removal of the lens from the case several hours prior to culturing. In either circumstance, these microorganisms would then have been exposed to the eye during lens insertion. This investigation found little correlation between lens storage case flora and lens or conjunctival flora, suggesting that the eyes of lens wearers were highly efficient in eradicating foreign microbes introduced during lens insertion.

The use of cold nonperoxide chemical disinfectants (which

TABLE 4. Distribution of flora isolated from contact lenses and lens storage cases[a]

Surface	No. of samples collected	No. of subjects (%) with:		
		Positive culture	Normal flora only	Potentially pathogenic species present
Lens front	72	35 (49)	29 (40)	6 (8)
Lens back	72	31 (43)	25 (35)	6 (8)
Lens storage case	41	29 (71)	10 (24)	19 (46)

[a] Following implementation of exclusion criteria.

are rinsed, rather than neutralized away from the contact lens) for both former and current contact lens wearers was associated with an increased prevalence of lens or conjunctival potential pathogens compared with the use of thermal or hydrogen peroxide (3%) disinfection. Since lens storage case contaminants were not found more often in association with nonperoxide chemical disinfection (in fact, contamination was more common with thermal and peroxide methods of disinfection), this effect may not be due to increased exposure to pathogens at the time of lens insertion. A prior investigation reported similar changes to conjunctival flora following the use of nonperoxide chemical disinfection of contact lenses (15). That study also revealed that certain forms of nonperoxide chemical disinfection lead to depletion of immunoglobulin levels in the tears. As the disinfectants tested by those investigators were not able to inactivate immunoglobulins in vitro, it was postulated that some chemicals used in lens disinfection may cause long-term damage to the tissues responsible for immunoglobulin production. It has been suggested that the constant diffusion of toxic preservatives from soft lens materials into the ocular tissues may enable organisms such as *Pseudomonas* species, which are normally of low virulence, to initiate infection (4). This may relate to the higher incidence of infection during soft lens wear compared with the relatively low incidence associated with the use of hard contact lenses (26), which do not absorb significant levels of solution preservatives. Interestingly, Weissman et al. (31) reported that 15 of 18 patients with infectious keratitis related to contact lens wear had used chemical disinfection.

Since both the present study and the previous investigation of chemical disinfection described above (15) included only subjects using older chemical disinfection systems, the effect of the new-generation systems, such as polyquad (Optifree; Alcon Laboratories) or dymed (Multi-Purpose Solution; Bausch and Lomb) on eye flora is not known. However, these more recently developed systems are also likely to interfere with resistance against infection; as recent studies have found that contact lens wear in rabbits, in conjunction with these new generation chemical disinfectants, leads to punctate corneal epithelial defects and enhanced adherence of *P. aeruginosa* to these lesions (32).

Grouping of data from lens wearers to investigate modes of lens wear, lens care, and patient variables, resulted in some small sample sizes. For this reason, only gross differences in conjunctival flora between groups would have been demonstrated statistically. Although disinfection was found to be the only factor to influence conjunctival flora among contact lens wearers, it is possible that other factors may have induced more subtle changes.

The inconsistencies in the results from previous studies of microbial flora during contact lens wear may be related to variation in the mode of selection of control subjects, or in the distribution of disinfection systems used by the sample lens-wearing group. For example, an investigation of cosmetic soft lens extended wear that revealed a decrease in conjunctival flora (28) may have described the effect of age, rather than lens wear, on microbial flora, since the subject group consisted of both postoperative elderly patients and young people wearing lenses for cosmetic purposes, while the control group only included older preoperative patients. Certainly, if former lens wearers are included in a control group, an artifactual decrease in flora in the "matched" lens-wearing population may be observed. Similarly, the proportion of subjects using nonperoxide chemical disinfection in a contact lens-wearing group may influence the outcome of flora studies.

There are two prerequisite steps in the pathogenesis of corneal infection: (i) contamination of the eye and (ii) compromise to corneal defense against infection. The data presented in this report suggest that there are changes to conjunctival flora in some contact lens wearers and that this phenomenon may not necessarily result from increased exposure to contamination during contact lens wear. Rather, changes to conjunctival flora may signal the suppression of factors which normally inactivate or clear foreign microorganisms from the eye. In this respect, the contact lens might act as a physical barrier that prevents blinking and tear flow from removing microorganisms from the ocular surface. In addition, the use of contact lenses and lens care solutions has been associated with a variety of physiological and pathological changes to the ocular surface (5, 8, 21, 32, 35); and these, in turn, could compromise immunological factors that regulate normal conjunctival flora. Changes to conjunctival flora cannot, on their own, lead to infection, nor are they a necessary step in the development of infection. However, in the presence of corneal compromise and microbial contamination, the suppression of mechanisms that normally clear foreign microorganisms from the eye may facilitate the infectious process.

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