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Bacterial Coaggregation and Cohesion Among Isolates From Contact Lens Cases

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Submitted: October 15, 2017
Accepted: April 23, 2018
Citation: Datta A, Stapleton F , Willcox MDP. Bacterial coaggregation and cohesion among isolates from contact lens cases. Invest Ophthalmol Vis Sci. 2018;59:2729–2735. https://doi.org/10.1167/iovs.17-23155

PURPOSE. The aim of this study was to examine cohesion, coaggregation, and coculture between bacteria commonly isolated from contact lens cases.

METHODS. Staphylococcus epidermidis, Staphylococcus baemolyticus, Micrococcus luteus, and Acinetobacter radioresistens (two strains each) isolated from contact lens cases of two asymptomatic wearers were used in this study. In the cohesion assay, bacteria were grown, washed, and examined by incubating lens cases with two different types of bacteria sequentially and assessing the number of adhered cells of each isolate. The ability of isolates to interfere with the growth of other isolates was tested by growing strains in cocultures for 24 hours and determining the numbers of cells of individual strains. For coaggregation, equal proportions of two bacterial suspensions were mixed and allowed to coaggregate for 24 hours. Inhibition of coaggregation was tested by the addition of lactose (0.06 M) or sucrose (0.06 M) or pronase.

RESULTS. The initial adhesion of M. luteus or A. radioresistens significantly \((P < 0.05)\) enhanced the subsequent adhesion of the staphylococci. The addition of A. radioresistens in liquid media significantly \((P < 0.05)\) enhanced the growth of staphylococci. S. baemolyticus or S. epidermidis coaggregated with M. luteus or A. radioresistens. The degree of coaggregation varied between 30% and 54%. The highest coaggregation (54% ± 5%) was seen between A. radioresistens 22-1 and S. epidermidis 22-1, isolated from the same lens case. Only lactose or sucrose treatment of staphylococci could partly inhibit coaggregation of some pairs.

CONCLUSIONS. Coaggregation, cohesion, and growth promotion may facilitate the process of bacterial colonization of contact lens cases.

Keywords: biofilm, coaggregation, cohesion and contact lens case.
Interactions Between Bacteria From Contact Lens Cases

**Methods**

**Microbial Strains**

Strains of *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Micrococcus luteus*, and *Acinetobacter radioresistens* (two strains each) isolated from contact lens cases of two asymptomatic wearers, enrolled in a previous clinical trial, were used in this study (Table 1). *Actinomyces naeslundii ATCC 12104* and *Streptococcus sanguinis CR2B*, isolated from dental plaque were used as positive controls for coaggregation.

**Cohesion Assay**

Bacterial cells were grown in brain heart infusion (BHI; Becton Dickinson, Macquarie Park, Australia) for 18 to 24 hours at 37°C followed by washing and adjusting the optical density (OD) in PBS (pH 7.4 NaCl 8 g L⁻¹, KCl 0.2 g L⁻¹, Na₂HPO₄ 1.15 g L⁻¹, KH₂PO₄ 0.2 g L⁻¹ pH 7.2) to an OD of 0.1 at 660 nm (1 × 10⁸ CFU/mL) using a spectrophotometer (FLUOstar Omega; BMG Labtech, Ortenberg, Germany). Contact lens cases (ReNu Multiplus; Bausch & Lomb, Rochester, NY, USA) were incubated at 37°C sequentially with two different types of bacteria, each for 24 hours followed by washing the lens cases once with PBS to remove the loosely attached cells. Subsequently, 2 mL of PBS was added to each well of the lens case along with a sterile magnetic stirring bar and the case was vortexed for 1 minute to dislodge the adherent bacterial cells. The recovery of bacterial species was assessed using a selective bacterial growth medium, as described previously. Controls used a single bacterial type incubated for 24 hours followed by the addition of sterile PBS and further incubation of 24 hours.

The total number of adherent bacteria was measured after growth at 37°C for 24 hours on nutrient agar (NA) plates (Thermo Fisher Scientific, Thebarton, Australia). The growth of *Staphylococcus* and *Micrococcus* spp. was estimated by growth for 24 hours at 37°C on the selective medium mannitol salt agar (MSA; Thermo Fisher Scientific). The recovery of *A. radioresistens* was calculated by assessing the difference in the number of bacteria recovered from MSA and NA plates (*A. radioresistens + Staphylococcus or Micrococcus* spp.), as *A. radioresistens* does not grow on MSA plates. The differentiation of *M. luteus* and the staphylococci on MSA or NA plates was performed by evaluating the morphologic appearance of each recovered bacterial colony. *M. luteus* and staphylococci colonies were differentiated by the pattern of pigmentation: golden-brown and bright yellow, respectively. This assay was repeated in duplicate on three different occasions.

The ability of bacteria adhering or cohering to the lens cases to produce biofilms was also evaluated. Extracellular DNA (eDNA) in biofilms was quantified using a previously described protocol using 1 μM SYTOX Green (Invitrogen Australia Pty Limited, Sydney, Australia) at excitation/emission wavelengths of 504/523 nm. The amount of eDNA produced by strains grown individually in wells was measured by incubation in SYTOX Green for 2 to 4 minutes, as was the amount of eDNA produced when strains were grown in the same well sequentially, as per the protocol for cohesion. These experiments were repeated in duplicate on three different occasions.

**Bacterial Growth**

For this assay only, the bacterial pairs that cohered were tested. In the first instance, bacterial cells were grown overnight in BHI and were resuspended to 0.1 OD₆₆₀ in PBS and spread on NA plate using a sterile cotton swab. Subsequently, the other bacteria of the pair was prepared as above and spotted (10 μL) on the bacterial lawn. The plates were then dried and incubated at 37°C for 24 hours. Inhibition of growth was seen as an inhibitory zone around the spotted strain. The zone of inhibition was graded on 1–4 scale, as described previously.

For bacterial growth in nutrient broth, the method of Qin et al. was used with modifications. Overnight cultures in BHI of *A. radioresistens*, *M. luteus* or *Staphylococcus* spp. were centrifuged, washed with PBS, and diluted to OD 0.1 at 660 nm (1 × 10⁸ CFU/mL) in BHI. One mL of one bacterial suspension was added to 50 mL of the other partner bacterial suspension and the cocultures were incubated at 37°C with shaking at 250 rpm. Every 2 hours, the bacterial suspension was diluted 10-fold in PBS and these dilutions were plated onto NA and MSA plates and the number of bacteria was counted after overnight incubation at 37°C. The bacteria on agar plates were identified by evaluating the morphological appearance of each recovered bacterial colony. The experiment was repeated in duplicate on three different occasions.

**Coaggregation Assay**

Bacterial coaggregation was performed as previously described. In brief, cells were grown in BHI for 18 to 24 hours at 37°C followed by washing and adjusting the OD to 1.0 (1 × 10⁹ CFU/mL) in coaggregation buffer (1 mM Tris [hydroxymethyl] amino methane, 0.1 mM CaCl₂, 0.1 mM MgCl₂, 0.15 M NaCl and 3.1 mM NaN₃ at pH 8.0). An equal volume (0.2 mL) of two cell suspensions was mixed (e.g., *A. radioresistens* plus *S. epidermidis*), vortexed, and the OD was measured after 24 hours of static incubation at ambient temperature and the percentage coaggregation was assessed by comparing to single suspensions of each bacteria. A percentage decrease in OD of greater than 30% indicated that coaggregation had occurred. The experiments were performed in duplicate and repeated three times.

**Table 1. Microorganisms Used in This Study**

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>Bacterial Species</th>
<th>Source*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram positive</td>
<td><em>Micrococcus luteus</em> 22-1</td>
<td>Contact lens case of asymptomatic lens wearer</td>
</tr>
<tr>
<td></td>
<td><em>Micrococcus luteus</em> 14-1</td>
<td>Contact lens case of asymptomatic lens wearer</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus epidermidis</em> 22-1</td>
<td>Contact lens case of asymptomatic lens wearer</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus haemolyticus</em> 14-1</td>
<td>Contact lens case of asymptomatic lens wearer</td>
</tr>
<tr>
<td></td>
<td><em>Actinomyces naeslundii ATCC 12104</em></td>
<td>Isolated from dental plaque (positive control)</td>
</tr>
<tr>
<td></td>
<td><em>Streptococcus sanguinis CR2B</em></td>
<td>Isolated from dental plaque (positive control)</td>
</tr>
<tr>
<td>Gram negative</td>
<td><em>Acinetobacter radioresistens</em> 22-1</td>
<td>Contact lens case of asymptomatic lens wearer</td>
</tr>
<tr>
<td></td>
<td><em>Acinetobacter radioresistens</em> 14-1</td>
<td>Contact lens case of asymptomatic lens wearer</td>
</tr>
</tbody>
</table>

* Isolates with the same strain numbers were isolated from the same contact lens case.
Inhibition of Bacterial Coaggregation

Lactose (0.06 M) and sucrose (0.06 M) were used to inhibit the coaggregation reactions as described previously.46,47 Additionally, the inhibition of bacterial coaggregation was assessed after incubating bacterial strains in protease (from Streptomyces griseus, P-5130; Sigma-Aldrich Corp., Castle Hill, Australia).42 The bacterial strains were incubated for 2 hours in lactose, sucrose, or pronase at ambient temperature and then the cell suspensions were washed three times with coaggregation buffer to remove unbound inhibitors. Then, the treated bacteria were added to their nontreated bacterial partners and the percentage of coaggregation was recorded after 24 hours of incubation at ambient temperature as described previously.42 A solution of 0.05% (wt/vol) Tween-20, 0.2 M NaCl was used as a negative control, to control for nonspecific bacterial interactions such as those associated with hydrophobicity and ionicity. Only if there was a greater inhibition than this control was inhibition of coaggregation considered to have occurred.37,46 The experiments were performed in duplicate and repeated three times.

Statistical Analysis

Data analysis was performed using spreadsheet and statistical software (Excel 2010; Microsoft Corp, Redmond, WA, USA; and

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**Table 2.** Bacterial Cohesion on Contact Lens Cases Between the Strains of *Staphylococcus* spp., *Micrococcus* spp., and *Acinetobacter* spp., That Were Isolated From the Same Contact Lens Case

<table>
<thead>
<tr>
<th>Primary Adherer</th>
<th>Secondary Colonizer†</th>
<th>Numbers of Primary Adherer</th>
<th>Numbers of Secondary Colonizer</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. radioresistens</em> 22-1</td>
<td><em>S. epidermidis</em> 22-1</td>
<td>3.20 ± 0.4*</td>
<td>4.53 ± 0.3*</td>
</tr>
<tr>
<td><em>M. luteus</em> 22-1</td>
<td>None</td>
<td>2.96 ± 0.3</td>
<td>1.78 ± 0.3</td>
</tr>
<tr>
<td><em>A. radioresistens</em> 14-1</td>
<td><em>S. baemolyticus</em> 14-1</td>
<td>2.25 ± 0.2</td>
<td>3.46 ± 3*</td>
</tr>
<tr>
<td><em>M. luteus</em> 14-1</td>
<td>None</td>
<td>3.68 ± 0.6*</td>
<td>2.20 ± 0.2</td>
</tr>
<tr>
<td><em>M. luteus</em> 22-1</td>
<td><em>S. epidermidis</em> 22-1</td>
<td>3.49 ± 0.3*</td>
<td>4.05 ± 0.4*</td>
</tr>
<tr>
<td><em>A. radioresistens</em> 22-1</td>
<td>None</td>
<td>1.60 ± 0.5</td>
<td>3.11 ± 0.5*</td>
</tr>
<tr>
<td><em>S. epidermidis</em> 22-1</td>
<td><em>A. radioresistens</em> 22-1</td>
<td>2.60 ± 0.4</td>
<td>-</td>
</tr>
<tr>
<td><em>S. baemolyticus</em> 14-1</td>
<td><em>A. radioresistens</em> 22-1</td>
<td>3.50 ± 0.5*</td>
<td>4.01 ± 0.3*</td>
</tr>
<tr>
<td><em>A. radioresistens</em> 22-1</td>
<td>None</td>
<td>2.25 ± 0.4</td>
<td>3.50 ± 0.4*</td>
</tr>
<tr>
<td><em>S. epidermidis</em> 22-1</td>
<td><em>A. radioresistens</em> 22-1</td>
<td>2.70 ± 0.4</td>
<td>3.60 ± 0.3*</td>
</tr>
<tr>
<td><em>M. luteus</em> 22-1</td>
<td>None</td>
<td>3.70 ± 0.4*</td>
<td>2.60 ± 0.4</td>
</tr>
<tr>
<td><em>A. radioresistens</em> 22-1</td>
<td>None</td>
<td>2.70 ± 0.4</td>
<td>-</td>
</tr>
<tr>
<td><em>S. baemolyticus</em> 14-1</td>
<td><em>A. radioresistens</em> 22-1</td>
<td>2.74 ± 0.2</td>
<td>3.00 ± 0.3*</td>
</tr>
<tr>
<td><em>M. luteus</em> 14-1</td>
<td>None</td>
<td>3.68 ± 0.2*</td>
<td>2.78 ± 0.2</td>
</tr>
<tr>
<td><em>A. radioresistens</em> 14-1</td>
<td>None</td>
<td>3.11 ± 0.4*</td>
<td>-</td>
</tr>
</tbody>
</table>

* Statistically significant (*P < 0.05*) difference in the bacterial adhesion.
† None represents when PBS was added as secondary suspension = control condition.

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**Table 3.** Estimation of the Amount of Biofilm Formation (eDNA) Produced by Strains Alone or in Combination

<table>
<thead>
<tr>
<th>Primary Adherer</th>
<th>Secondary Colonizer†</th>
<th>Amount of eDNA</th>
<th>Estimated Amount of eDNA*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. radioresistens</em> 22-1</td>
<td><em>S. epidermidis</em> 22-1</td>
<td>140 ± 22</td>
<td>(93 + 91) = 184</td>
</tr>
<tr>
<td><em>M. luteus</em> 22-1</td>
<td>None</td>
<td>125 ± 17</td>
<td>(93 + 64) = 157</td>
</tr>
<tr>
<td><em>A. radioresistens</em> 14-1</td>
<td><em>S. baemolyticus</em> 14-1</td>
<td>218 ± 24</td>
<td>(56 + 194) = 250</td>
</tr>
<tr>
<td><em>M. luteus</em> 14-1</td>
<td>None</td>
<td>117 ± 15</td>
<td>(56 + 43) = 99</td>
</tr>
<tr>
<td><em>M. luteus</em> 22-1</td>
<td><em>S. epidermidis</em> 22-1</td>
<td>152 ± 22</td>
<td>(64 + 91) = 155</td>
</tr>
<tr>
<td><em>A. radioresistens</em> 22-1</td>
<td>None</td>
<td>124 ± 18</td>
<td>(64 + 95) = 157</td>
</tr>
<tr>
<td><em>M. luteus</em> 14-1</td>
<td><em>S. baemolyticus</em> 14-1</td>
<td>261 ± 22</td>
<td>(43 + 194) = 237</td>
</tr>
<tr>
<td><em>A. radioresistens</em> 14-1</td>
<td>None</td>
<td>115 ± 12</td>
<td>(43 + 56) = 99</td>
</tr>
<tr>
<td><em>S. epidermidis</em> 22-1</td>
<td><em>A. radioresistens</em> 22-1</td>
<td>140 ± 18</td>
<td>(91 + 93) = 184</td>
</tr>
<tr>
<td><em>M. luteus</em> 22-1</td>
<td>None</td>
<td>156 ± 15</td>
<td>(91 + 64) = 155</td>
</tr>
<tr>
<td><em>S. baemolyticus</em> 14-1</td>
<td><em>A. radioresistens</em> 14-1</td>
<td>220 ± 11</td>
<td>(194 + 56) = 250</td>
</tr>
<tr>
<td><em>M. luteus</em> 14-1</td>
<td>None</td>
<td>266 ± 19</td>
<td>(194 + 43) = 237</td>
</tr>
<tr>
<td><em>A. radioresistens</em> 14-1</td>
<td>None</td>
<td>194 ± 12</td>
<td>-</td>
</tr>
</tbody>
</table>

* The estimated amount of biofilm was calculated by adding the amount of eDNA produced by strains when incubated alone.
† None represents when PBS was added as secondary suspension.
Interactions Between Bacteria From Contact Lens Cases

SPSS Statistics, version 20.0; SPSS, Inc., Chicago, IL, USA). The recovery of bacteria after cohesion or coculture was compared a 2-tailed Student’s t-test and using repeated measures ANOVA (for different time points). The percentage bacterial coaggregation between different pairs and the inhibition of bacterial coaggregation with the treatment of different inhibitory substances were compared using ANOVA with Bonferroni correction for post-hoc multiple comparisons. Statistical significance level was set at \( P < 0.05 \).

**RESULTS**

There was cohesion between the pairs of *S. epidermidis* or *S. haemolyticus* and *M. luteus* or *A. radioresistens*. Primary adhesion of *M. luteus* or *A. radioresistens* significantly \( (P = 0.05) \) enhanced the subsequent adhesion of staphylococci \( (P = 0.02; \text{Table 2}) \). The greatest effect was seen with the primary adhesion of *A. radioresistens* 22-1, which increased the secondary colonization of *S. epidermidis* 22-1 by 1.8 ± 0.3 \( \log_{10} \) colony forming units (CFU) compared to the adhesion of *S. epidermidis* 22-1 in isolation \( (P = 0.002; \text{Table 2}) \). Additionally, the primary adhesion of *S. epidermidis* 22-1 increased the secondary colonization of *A. radioresistens* 22-1 by 1.1 ± 0.2 \( \log_{10} \) CFU, compared to adhesion of *A. radioresistens* alone \( (P = 0.008; \text{Table 2}) \). The secondary colonization by *S. epidermidis* 22-1 significantly \( (P = 0.002) \) increased the primary adhesion of *A. radioresistens* 22-1 \( (P = 0.002) \) and *M. luteus* 22-1 \( (P < 0.005) \). The primary adhesion of *M. luteus* 22-1 or *M. luteus* 14-1 significantly increased the secondary adhesion of *A. radioresistens* 22-1 or 14-1, respectively \( (P < 0.005; \text{Table 2}) \). The secondary colonization by *S. haemolyticus* 14-1 significantly \( (P = 0.005) \) increased the primary adhesion of *M. luteus* 14-1 and the secondary colonization *S. epidermidis* 22-1 increased the primary adhesion of *M. luteus* 22-1 \( (P = 0.05; \text{Table 2}) \).

In the eDNA assay, there was evidence of biofilm formation with the largest amount of biofilm for bacteria incubated alone being produced by *S. baemolyticus* 14-1 \( (P < 0.005; \text{Table 3}) \). Where these strains were allowed to cohere, the amount of biofilm appears to closely mirror the amount produced by the strains adhered alone, and the amounts produced when strains were in combination did not differ from the amounts produced alone by more than 2 SDs.

The ability of bacteria to inhibit one another’s growth was first investigated on nutrient agar, but no inhibition with any bacterial pairs was found. The effect of coculturing two different types of bacteria was genus dependent. Incubating staphylococci with *A. radioresistans* 22-1 or 14-1 increased the numbers of both staphylococci \( (P < 0.005; \text{Fig. 1}) \), and *A. radioresistans* 22-1 or 14-1 \( (P < 0.005; \text{Fig. 2}) \) that grew. Conversely, while incubating *S. epidermidis* 22-1 with *M. luteus* 22-1 did not significantly \( (P = 0.59; \text{Fig. 1}) \) increase the numbers of *S. epidermidis*, incubating *S. baemolyticus* 14-1 with *M. luteus* 14-1 reduced the final numbers and the growth rate of *S. baemolyticus* 14-1 \( (P < 0.005; \text{Fig. 1}) \). The coculture of *M. luteus* 22-1 with *S. epidermidis* 22-1 significantly increased the growth of *M. luteus* \( (P = 0.007; \text{Fig. 3}) \). The coculture of *M. luteus* 14-1 with *S. baemolyticus* 14-1 significantly altered the growth kinetics of *M. luteus* \( (P < 0.005; \text{Fig. 3}) \), but approximately the same number of cells of *M. luteus* were produced after 24 hours incubation.
Coaggregation was observed between certain strains of *S. epidermidis* or *S. baemolyticus* with *M. luteus* or *A. radioresistens* (Table 4). The highest coaggregation occurred between *A. radioresistens* 22-1 and *S. epidermidis* 22-1 (54% ± 5%; Table 4). The highest coaggregation between two Gram positive bacteria was 50% ± 3% for the pair *M. luteus* 22-1 and *S. epidermidis* 22-1 (Table 4). The positive control of *A. naeslundii* ATCC 12104 and *S. sanguinis* CR2B showed 92% ± 3% coaggregation after 24 hours.

The results of the inhibition of coaggregation with lactose, sucrose or pronase are shown in Table 5. The negative control solution of Tween-20 and NaCl (that was used to determine any coaggregation as the result of hydrophobic or charge interactions) inhibited up to 38% of coaggregation (data not shown), therefore a significant inhibition in coaggregation for any treatment was considered to be ≥40%. Lactose treated *S. epidermidis* 22-1 showed reduced coaggregation with *M. luteus* 22-1 or *A. radioresistens* 22-1 (45% ± 4% and 41% ± 3%, respectively). Lactose treatment of *S. baemolyticus* 14-1 caused 42% ± 4% reduction in coaggregation with *A. radioresistens* 22-1. Sucrose treatment of *S. epidermidis* 22-1 reduced coaggregation by 40% ± 3% with *A. radioresistens* 22-1 (Table 5) only. Incubation of any strain with pronase did not inhibit coaggregation.

**DISCUSSION**

The current study was designed to understand how multispecies biofilms might form in contact lens cases. Biofilms may be produced by a number of mechanisms and cohesions, coaggregation and stimulation of growth may be important aspects. The present study demonstrated for the first time that *A. radioresistens*, *M. luteus*, and *Staphylococcus* spp. could cohere. Additionally, the presence of *A. radioresistens* increased the growth of *Staphylococcus* spp. Coaggregation could occur between certain strains of *A. radioresistens*, *M. luteus*, *S. epidermidis*, and *S. baemolyticus* that had been isolated from lens cases of asymptomatic wearers.

No coaggregating pairs presented the high coaggregation scores of the positive controls of *A. naeslundii* ATCC 12104 plus *S. sanguinis* CR2B (92% ± 3%). This outcome resembles a previous study finding where the coaggregation between *P. aeruginosa* and *S. aureus* reached only (62% ± 3%). In general, there was a trend for bacteria isolated from the same contact lens cases to coaggregate with each other, which was similar to the coaggregating pair of *P. aeruginosa* and *S. aureus* in the previous study.

The current study also investigated the inhibitory effect of lactose, sucrose, and pronase on coaggregation. Sato et al. demonstrated that coaggregation between actinomycetes and streptococci occurred via lectin-like substances (i.e., substances similar or identical to proteins that bind sugars) on the surface of streptococci. Pretreating *Staphylococcus* spp. with lactose or sucrose inhibited the coaggregation, which indicates the involvement of staphylococcal lectins in coaggregation, consistent with previous studies. However, the selected inhibitory sugars were unable to stop coaggregation between *Micrococcus* spp. and *Acinetobacter* spp. Understanding how bacteria coaggregate and cohere may help to produce strategies to halt biofilm formation. For example, adding inhibitory substances such as sugars to multipurpose disinfecting solutions may be of benefit. Although, it is unlikely that sugars such as lactose and sucrose can be used as they can be the source of nutrition of many types of bacteria.

Cohesion between the strains of *M. luteus* or *A. radioresistens* with *S. epidermidis* or *S. baemolyticus* provides support to the concept that the initial adhesion of bacteria may control the secondary colonization of other types of bacteria (cohesion). *M. luteus* or *A. radioresistens* may form a conditioning film to enhance the adhesion of staphylococci or vice versa. However, the amount of biofilm formed by strains, as measured by estimating the amount of eDNA produced, was not affected by them adhering alone or in pairs. The ability of small numbers of *A. radioresistens* 22-1 or staphylococci to stimulate the growth of the other partner in coculture demonstrates that this can be another factor involved in the cohesion of bacterial cells and hence multispecies biofilm formation. Identifying the factors that are involved in this phenomenon may lead to determining mechanisms to reduce their production or interfere with their mechanism of action, which again could be used to reduce biofilm formation.
In summary, it appears that a complex series of events may take place between bacteria that are involved in multispecies biofilm formation in contact lens cases. Certain bacteria can cohere—that is, the presence of one bacterial type increases the ability of another to attach. This can be facilitated by coaggregation, the direct adhesion between cells of different bacteria. Coaggregation may be involved in the cohesion between stains of *A. radioresistens* or *M. luteus* and staphylococci. The ability of small numbers of *A. radioresistens* or staphylococci to promote each other's growth may also be involved in cohesion and hence biofilm formation. This research will provide a framework for future studies that examine how to reduce biofilm formation in contact lens cases (e.g., adding substances to contact lens disinfecting solutions that can prevent aspects of cohesion or coaggregation). This may then reduce the contamination of contact lens cases during use. The research may also have application in other areas. For example, both *Acinetobacter* and *Staphylococcus* can be found concurrently in bronco-alveolar lavages from people with chronic obstructive pulmonary disease.  

**Acknowledgments**

The authors thank Nancy Briggs, senior statistical consultant from Mark Wainwright Analytical Centre, for assistance with statistical analysis.

Disclosure: **A. Datta**, None; **F. Stapleton**, Alcon (F), Allergan (F), CooperVision (F), Novartis (C); **M.D.P. Willcox**, Alcon (F), Allergan (F), Cochlear (F), CooperVision (C, F, R), Johnson and Johnson Vision Care (C, F, R, S), P  

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