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A Cluster of Corneal Donor Rim Cultures Positive for *Achromobacter* Species Associated With Contaminated Eye Solution

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Abstract

Purpose: To investigate a cluster of corneoscleral rim cultures positive for *Achromobacter* species over a 6-month period at Massachusetts Eye and Ear.

Methods: An increased rate of positive corneal donor rim cultures was noted at Massachusetts Eye and Ear between July and December 2017. Positive cultures were subjected to identification and antimicrobial susceptibility testing by phenotypic (MicroScan WalkAway) and genotypic (16S rDNA sequencing) methods. Samples of the eye wash solution (GeriCare) used in the eye bank were also evaluated. Antimicrobial activity of Optical-GS against *Achromobacter* spp. at 4°C and 37°C was assessed by time-kill kinetics assay.

Results: Of 99 donor rims cultured, 14 (14.1%) grew bacteria with 11 (78.6%) due to uncommon nonfermenting Gram-negative bacilli. These had been identified by standard automated methods as *Achromobacter* (n = 3), *Alcaligenes* (n = 3), *Ralstonia* (n = 2), *Pseudomonas* (n = 2), and *Stenotrophomonas* (n = 1). Eight of these 11 isolates were subsequently available for molecular identification, and all were identified as *Achromobacter* spp. Six bottles of eyewash solution were evaluated and were positive for abundant *Achromobacter* spp. ($3.4 \times 10^5 \pm 1.1$ CFU/mL). Optisol-GS had no bactericidal activity against *Achromobacter* spp. at 4°C after 24-hour incubation but was bactericidal at 37°C. None of the patients who had received the contaminated corneas developed postoperative infection.

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Conclusions: An eyewash solution arising from a single lot was implicated in the contamination of donor rims by *Achromobacter* spp. The isolates were able to survive in the Optisol-GS medium at the recommended storage temperature. This highlights the need to continue improving protocols for tissue preparation and storage.

Keywords

endophthalmitis; keratitis; corneoscleral rims; *Achromobacter* spp

Postoperative keratitis and endophthalmitis are uncommon yet feared complications of corneal transplantation which can result in severe ocular morbidity.¹ In some cases, the source of the infecting organism is contamination from the donor cornea.^{2–4} The Eye Bank Association of America (EBAA) standards include recommendations to decrease the contamination rates during tissue preparation and storage.⁵ Despite many modifications made by EBAA in the 1990s to better control donor tissue microbial contamination, this problem has not been completely eliminated.

Although the prognostic value of routine surveillance cultures has been debated, microbiological cultures of tissue medium and the remaining scleral rims are often performed.⁶ Bacterial contamination is most common, with rates ranging from 4% to 14%, and *Staphylococcus* spp., *Propionibacterium acnes*, *Streptococcus* spp., and *Pseudomonas aeruginosa* are the species most commonly identified.^{6–9} Fungal contamination rates range from 1.2% to 8.6%, with *Candida* spp. frequently identified.^{10,11} There are many potential contamination sources other than the donor eyes, particularly at the tissue-processing and storage levels.^{12–14} In this study, we investigated a cluster of corneoscleral rims positive for *Achromobacter* spp., nonfermenting Gram-negative bacilli (NF-GNB), identified for more than a 6-month period at the Massachusetts Eye and Ear (MEE). A likely common source of contamination was a particular lot of eyewash solution used to rinse the eye globe during tissue processing in the eye bank.¹⁵

MATERIALS AND METHODS

Corneoscleral Donor Rims and Eyewash Solution Cultures

Bacterial isolates used in this study were routinely collected and frozen in our clinical microbiology laboratory. Protocols for obtaining these isolates were approved by the MEE Institutional Review Board (protocol number: 2019P001001). Isolates were recovered from corneoscleral donor rim cultures performed between July and December 2017. During this period, 99 donor tissues were cultured on chocolate agar and in meat broth for 7 days at 37°C with 5% CO₂. Aliquots of 100 µL collected from 6 bottles of the implicated eyewash solution (GeriCare Eye Wash; Kareway Products, Inc) were cultured on blood agar and chocolate agar and incubated under the above-described similar conditions. To determine the bacterial loads and the stability of the microbial biomass in the contaminated solution stored at room temperature as recommended, we performed quantitative cultures weekly over an 8-week period, by plating serial dilutions of the eyewash solution on blood agar.

Microbial Characterization

Positive cultures were speciated, and antimicrobial susceptibility was determined using the MicroScan WalkAway system (Beckman Coulter, Brea, CA) following the manufacture's protocol. Species identification was validated and refined by sequencing the entire 16S rDNA gene. To do this, the 16S sequence was amplified in a 25- μ L reaction using the Kapa HiFi HotStart Ready Mix (Roche, Indiana, IN) and primers 16S-8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 16-1493R (5'-ACGGCTACCTTGTTACGACTT-3'). In addition, primers 16S-743F (5'-CGAAGACTGACGCTCAG-3') and 16S-777R (5'-GGGTATCTAATCCTGTTTGC-3') were used for sequencing. DNA molecules were sequenced by the Sanger method by Genewiz, Inc (South Plainfield, NJ).

Minimum Inhibitory Concentration

Minimum inhibitory concentrations (MICs) for gentamicin (EMD, Inc, Darmstadt, Germany) and streptomycin (Amresco, Solon, OH) were determined by broth microdilution according to the Clinical and Laboratory Standards Institute (CLSI).¹⁶ A control strain of *P. aeruginosa*, ATCC 27853, was included as a control. Data interpretation was based on CLSI 2018 clinical breakpoints.¹⁷

Time-Kill Assay in Optisol-GS

Time-kill kinetics was determined for a representative *Achromobacter* spp. strain isolated from the contaminated eyewash solution. The isolate was incubated in brain heart infusion broth (Difco Laboratories, Detroit, MI) overnight at 37°C with shaking (200 rpm). This solution was diluted 1:100 in fresh brain heart infusion broth and cultured to mid-log phase ($OD_{600} = 0.5$), washed 3 times, and resuspended in phosphate-buffered saline. The inoculum was resuspended in pure Optisol-GS solution (Bausch & Lomb, Inc, Rochester, NY) to a final concentration of approximately 1 to 2×10^6 CFU/mL and incubated at 37°C and 4°C (to mimic the storage conditions). Serial dilutions were plated at 0, 2, 4, 18, and 24 hours after incubation.

RESULTS

The peripheral corneal donor tissue that remains after trephination of the central cornea is commonly referred to as corneal “donor rims.”¹⁸ It is standard practice at MEE to culture the donor rims. The MEE clinical microbiology laboratory reported more culture-positive donor rims between July and December 2017, although there were no patient infections. During this period, 99 corneal donor rims were routinely cultured for bacteria and fungi, with 14 (14.1%) yielding at least 1 organism (Fig. 1). In the preceding 6 months (January through June 2017), the rate of culture positive corneal rims was 1.9%. Culture-positive corneal rims peaked in October of 2017. Unexpectedly, organisms growing from these rims (11/14, 78.6%) included uncommon NF-GNB phenotypically identified as closely related bacteria belonging to the genera *Achromobacter* ($n = 3$), *Alcaligenes* ($n = 3$), *Ralstonia* ($n = 2$), and *Stenotrophomonas* ($n = 1$), and *Pseudomonas* ($n = 2$). The remaining 3 positive donor rims grew α -hemolytic streptococci ($n = 2$) and an unidentified yeast (n

= 1). Molecular identification performed on 8 (of 11) available NF-GNB isolates proved that all belonged to the genus *Achromobacter* (Table 1).

When the increased frequency of NF-GNB species was noted at MEE, the results were reported to the eye bank that had supplied the corneal tissue. At approximately the same time, in October 2017, a different eye bank had experienced a cluster of contamination similar to MEE. Their observations were reported to the EBAA, and an alert was issued by EBAA to all eye banks. An eyewash solution used to irrigate corneas before their storage in Optisol-GS was identified by the eye bank as a possible source of the contamination. Samples of the eyewash were cultured by an independent commercial laboratory that detected a contamination caused by NF-GNB organisms identified as *Achromobacter* spp. (data not shown). The packing company provided to the eye bank the pack lot numbers that contained the implicated eyewash lot. All of those implicated packs were removed immediately from the market on identification.¹⁵ The eye bank sent 6 bottles of the implicated eyewash solution to the MEE microbiology laboratory for additional analysis. These bottles also grew *Achromobacter* spp. (identification confirmed molecularly) at unexpectedly high concentration ($3.4 \times 10^5 \pm 1.1$ CFU/mL). The bacterial loads were stable for 2 months in solutions stored at room temperature (data not shown).

The susceptibility of the 11 NF-GNB isolates to various antibiotics in vitro is tabulated in Table 1. As tested by automated broth microdilution using the MicroScan WalkAway system, these isolates were nonsusceptible to aminoglycosides gentamicin, amikacin, and tobramycin but susceptible to levofloxacin as defined by CLSI breakpoints.¹⁷ All *Achromobacter* spp. isolates from 6 samples of contaminated eyewash solution had the same antibiotic susceptibility profile. Because Optisol-GS contains 100 µg/mL gentamicin and 200 µg/mL streptomycin, and the *Achromobacter* spp. isolates were able to survive during storage of the corneoscleral rims, we determined the MIC of a representative eyewash solution-derived *Achromobacter* spp. for these 2 antibiotics in Mueller-Hinton broth by microdilution. MIC values were determined to be 4 µg/mL for gentamicin and 16 µg/mL for streptomycin, which suggested that they might be killed during storage in Optisol-GS. We quantified bacterial killing in Optisol-GS at 4°C—the standard temperature used by eye banks to store donor corneas—by inoculating 10^6 CFU/mL in pure Optisol-GS solution. No bacteria were killed 4 hours after addition of bacteria, and there was only a 1-log reduction after 18 and 24 hours. Incubation of this representative *Achromobacter* spp. in Optisol-GS at 37°C, however, resulted in a 3-log reduction in viable cells after 4 hours of incubation and complete killing after 18 hours as shown in Figure 2. This indicates that, in Optisol-GS at 4°C, *Achromobacter* spp. is not in a physiological state where it is readily killed by gentamicin or streptomycin concentrations above the MIC.¹⁹

Patients (n = 11) were transplanted with these corneas after penetrating keratoplasty (PKP) (n = 10) and Descemetstripping automated endothelial keratoplasty (n = 1) surgeries. Despite the high level of contamination found in the eyewash solution and the ability of *Achromobacter* spp. to cause eye infections,²⁰ none of the patients who received donor rim culture-positive corneal tissues developed a postoperative infection caused by this agent. One patient experienced delayed reepithelialization that led to keratitis and culture-

proven acute endophthalmitis (post-PKP) caused by an unrelated organism (α -hemolytic streptococci).

DISCUSSION

Postoperative infections are one the most feared complications of corneal transplant surgery because of the potentially sight-threatening consequences. Contaminated tissue can be the source of the infecting microbe, and this risk cannot be completely eliminated by existing preparation and storage procedures.^{2–4} In an effort to better manage these infections at early stages, many surgeons perform routine surveillance cultures from the remaining corneoscleral rims and/or their storage medium.

Between July and December 2017, the rate of donor rim contamination (14.1%) was much higher than typically seen at MEE, and most of the contaminated rims were positive for uncommon NF-GNB. Through molecular techniques, 8 of these NF-GNB isolates were determined to belong to the genus *Achromobacter*. This differed from the identification made through the automated method (MicroScan) in several cases, the latter identifying the *Achromobacter* as *Alcaligenes*, *Ralstonia*, or *Stenotrophomonas*, all closely related NF-GNBs. Cultures of representative bottles of the same lot number of eyewash used to irrigate donor eyes in the eye bank also grew *Achromobacter* spp. In February 2018, the FDA published a voluntary nationwide recall of the contaminated eyewash solution.¹⁵

Achromobacter spp. are aerobic NF-GNB, closely related to the genus *Pseudomonas*. *Achromobacter* spp. are ubiquitous in aqueous environments (such as well water, tap water, and swimming pools) and can be frequently found as a contaminant of contact lens storage cases and lenses.^{21–23} The species *Achromobacter xylosoxidans* is recognized as an important emerging nosocomial pathogen and has been isolated from multiple types of aqueous solutions in health care settings^{24,25} and from a mechanical ventilator and catheters.^{26,27} It is considered an opportunistic bacterium of limited virulence; however, in immunocompromised patients, it is associated with a wide range of infections including bacteremia, pneumonia, urinary and intraabdominal infections, eye and ear infections, osteomyelitis, and meningitis.^{28,29} In immunocompromised patients, these infections might be life threatening because *Achromobacter* spp. is a hard-to-treat multidrug-resistant organism.²⁸ Strains of this genus might be resistant to most aminoglycosides, ampicillin, aztreonam, and most cephalosporins, whereas expressing variable resistance to quinolones and carbapenems.³⁰ Currently, none of the commercially available eye tissue preservation media sold in North America contains an agent active against Gram-negative bacteria with all of these resistance profiles. That presents as an opportunity for further development of improved solutions with broader spectrum of activity.

NF-GNB are particularly capable of contaminating solutions that might come in contact with the eye because of their wide environmental distribution and resistance to antimicrobials. Organisms such as *P. aeruginosa*,³¹ *Stenotrophomonas maltophilia*,²¹ *Burkholderia* spp.,³² *Acinetobacter* spp.,³³ have been implicated in outbreaks that resulted in severe ocular infections where the source was a contaminated eye product. Fortunately, none of the patients in our series developed a postoperative infection caused by the organism

contaminating the transplanted corneas. One patient experienced delayed reepithelialization that led to keratitis and culture-proven acute endophthalmitis (post-PKP) caused by α -hemolytic streptococci not identified in the laboratory as a contaminant of the donor tissue. Patients who undergo corneal transplantation procedures at MEE are routinely prescribed topical fluoroquinolones postoperatively as prophylaxis, and the *Achromobacter* spp. isolates were susceptible to levofloxacin, so this prophylaxis might have been protective. On the other hand, positive corneal donor rim cultures are relatively common at centers across the country, yet postoperative infections are very rare; so, many factors might contribute to the frequent lack of correlation between donor rim cultures and clinical infection.

Corneoscleral donor tissue is stored at 4°C in Optisol-GS containing relatively high levels of gentamicin (100 µg/mL) and streptomycin (200 µg/mL). Based on MIC values determined in bacteriological medium for a representative isolate of *Achromobacter* spp. in this study, Optisol-GS would be expected to control contamination during storage but that was not the case. Antimicrobial agents that target fundamental metabolic processes, such as aminoglycosides, are effective against viable and metabolically active cells in a culture.³⁴ Thus, low nutrient value combined with refrigeration might paradoxically increase the survival of bacteria in solutions containing preservatives or antibiotics.³⁵ Others have observed reduced bactericidal activity of Optisol-GS at low temperature against common microbes of the eye and improved activity at higher temperature,³⁶ as observed in this study. These results highlight a limitation to the effectiveness of Optisol-GS in limiting microbial contamination of donor rims during storage at the recommended temperature.

In conclusion, we found an uncommon Gram-negative pathogen as the major contaminant of donor rims at our institution over a 6-month period. This was probably the result of 2 events that took place during processing and storage. First, the supplying eye bank processed the donor corneas using a particular lot of eyewash solution that served as the common source of contamination. Second, contaminated rims were not decontaminated by Optisol-GS at the low temperature that is typically used for cornea donor tissue storage. Although many advances have been made in protocols for tissue preparation and storage, the risk of potential postoperative infections resulting from contaminated reagents used in processing donor eyes has not been eliminated. Our study demonstrates that processing and storage of corneal rims warrants additional improvements to further minimize the likelihood of corneal donor tissue contamination.

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FIGURE 1.

Positivity of donor corneal rim cultures from July to December 2017. Among the 99 donor corneal rim cultures, 14 donor rims (14/99, 14.1%) were positive with the highest rate in October. (The full color version of this figure can be found online at www.corneajrnl.com.)

FIGURE 2.

Time-kill kinetics of Optisol-GS against *Achromobacter* spp. A suspension of *Achromobacter* spp. in Optisol-GS was incubated for 24 hours at 4°C (blue line) and at 37°C (orange line). A 10-fold serial dilution from 10^{-1} to 10^{-6} was performed and plated for each temperature at 0, 2, 4, 18, and 24 hours after incubation. Each dataset is the average CFU/mL value calculated from a triplicate. (The full color version of this figure can be found online at www.corneajrnl.com.)

TABLE 1.

Identification and Susceptibilities of NF-GNB Growing Corneal Rim Cultures

Case No.	Phenotypic ID	16S rDNA ID	Amikacin	Gentamicin	Tobramycin	Levofloxacin
1	<i>Alcaligenes</i> spp.	<i>Achromobacter</i> spp.	I	R	R	S
2	<i>Stenotrophomonas maltophilia</i>	<i>Achromobacter</i> spp.	ND	ND	ND	S
3	<i>Alcaligenes</i> spp.	<i>Achromobacter</i> spp.	I	R	R	S
4	<i>Alcaligenes</i> spp.	<i>Achromobacter</i> spp.	R	R	R	S
5	<i>Achromobacter xylosoxidans</i>	ND	R	R	R	S
6	<i>Ralstonia pickettii</i>	<i>Achromobacter</i> spp.	R	R	R	S
7	<i>Pseudomonas</i> spp.	ND	R	R	R	S
8	<i>Pseudomonas</i> spp.	ND	R	R	R	S
9	<i>Achromobacter xylosoxidans</i>	<i>Achromobacter</i> spp.	R	R	R	S
10	<i>Ralstonia pickettii</i>	<i>Achromobacter</i> spp.	R	R	R	S
11	<i>Achromobacter xylosoxidans</i>	<i>Achromobacter</i> spp.	R	R	R	S

I, intermediate; ND, not determined; R, resistant; S, susceptible.