Light-Blocking Infiltrates in Donor Corneas Preserved in Optisol: Infection or Inflammation?

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ABSTRACT

This prospective study examined corneal infiltrates in donor corneas as compared to previously reported isolates to determine if the changing patterns in nosocomial infections, Acinetobacter and *Clostridium difficile* reported in a recent government study was also occurring in the corneal donor population. Corneas from 50 donors, in which tissue was subjected to ocular surface disinfection by standard protocols and found on slit lamp examination to have corneal infiltrates, were included for study. Infiltrates were identified by technicians during slit lamp examination after tissue had been placed in solution containing antibiotics. Fifty of these corneas, in which bilateral infiltrates were present, were submitted for bacteria and fungi culture and histological examination. Of the 50 corneas, 13 (26%) were culture-positive, predominantly *Candida albicans* and *Staphylococcus aureus*, with no shift in the microbial pathogens from previous studies. There were no isolates of *Acinetobacter*. In 21 corneas in both culture and histological examination, organisms were present extracellularly and intracellularly in corneal epithelial cells and infiltrate macrophages. Since many corneal donors are from hospital populations, individuals having undergone mechanical ventilation, it is important to know there has been no shift in microbial pathogen profiles suggested by CDC report for *Acinetobacter/Clostridium difficile*. Importantly, ventilation intervals do not appear predictive for the presence of invasive pathogens in corneas with light blocking infiltrates as had previously been hypothesized. Nevertheless, continuing awareness and monitoring for shifts in hospital microbial infections represent important safeguards in preventing donor related infections as corneal infiltrates cannot be attributed to a singular circumstance; i.e. ventilation.

Key Words: Microbial colonization, nosocomial infections, ventilator support

INTRODUCTION

Following a recent Centers for Disease Control (CDC) report of changing nosocomial flora in seriously ill hospitalized patients, particularly in those who received mechanical ventilatory support and those in intensive care units, a prospective study was undertaken to identify organisms isolated from infiltrates in donor corneas recovered for transplantation. Specific interest was directed toward any change in flora from previous reports,[2] as well as the possible isolation of multiple resistant *Acinetobacter* which the CDC reported as a primary concern along with *Clostridium difficile*. *Acinetobacter* has not been previously associated with corneal infiltrates and its present status in such infiltrates and therefore potential for endophthalmitis is unknown. CDC reports 2% of healthcare associated infections reported to them are Acinetobacter but the proportion is higher among critically ill patients, 7%, for those on mechanical ventilators.[1] The potential for increased and altered flora in corneal donor infiltrates is highly relevant due to the large number of corneal donor transplants which come from patients whose pre-mortem care was given in intensive care units and included treatment with mechanical ventilation. In this study population 36% of ocular donors were from medical examiner cases while 64% were from individuals hospitalized prior to death. Correlation of ventilation and/or hospital ocular care as the predisposing factor for corneal infiltrates has been suspected but not well documented and therefore its influence on the acceptability/suitability of potential donors for ocular tissues is indeterminate.

METHODS

Fifty donor corneas from three eye banks that were originally recovered for transplantation between October, 2013 and June, 2014, and which were found to have corneal infiltrates by screening slit lamp biomicroscopy routinely performed by the eye bank technicians, were submitted for culture for bacteria and yeasts. Where bilateral infiltrates were present, one of the pair was submitted for histology. Culture methods included rinsing and dilution at

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1:50 to remove antibiotics routinely present in the preservation media (e.g., Optisol). Optisol GS contains both Gentamycin sulfate and Streptomycin antibiotics which have demonstrated bactericidal effects on bacteria including *Pseudomonas aeruginosa, Klebsiella, Enterobacter, Escherichia coli* and *Staphylococcus*.[3] Microbiology was performed by the VRL Reference Laboratory, Centennial, Colorado. Culture media was fluid thioglycolate and trypticase soy broth for anaerobic and aerobic isolation, with incubation at 30-35°C and 20-25°C for 7 days. Cultures were performed by the reference laboratory without knowledge of any donor information (i.e., name, history, ventilation). Histology was performed by a separate laboratory, again absent any patient information. Histology sections were stained with hematoxylin and eosin according to standard tissue staining methodology.[4] All corneas included in this study were from donors for whom consent was obtained not only for transplantation but also for research. Since all study material was from deceased individuals, no HIPPA regulations were applicable as use originally intended for transplantation is specifically exempted. It is important to recognize that both pre-recovery povidone-iodine and the presence of antibiotics in the cornea preservation media, even with laboratory dilution post-recovery, could reduce organism recovery rates as the antibiotic concentration in the storage media is 50ug/ml added to storage media for the control of bacteria and the exposure to the antibiotic occurs at procurement preservation prior to the identification of infiltrates or the preparation of the tissue for culture.

RESULTS

Of the 50 corneas submitted for culture, 13 demonstrated culture-positive organisms; predominately Candida albicans and Staphylococcus aureus findings consistent with prior published reports2. Table 1 shows all donor cornea isolate numbers by donor. In five cases, more than one isolate occurred in the same donor tissue, as shown in Table 2.

Histologic sections demonstrated both inflammation and presence of microorganisms, as can be seen in Figures 1, 2 and 3. Figure 1 shows the presence of microorganisms in the microphage cytoplasm, which is consistent with the isolation in culture of *Coagulase*-negative *Staphylococcus*. Figure 2 shows both phagocytosed and extra-cellular organisms, and matched cultures which were positive for both *coagulase*-negative *Staphylococcus* and yeast. Figure 3 demonstrates inflammatory cells, but without readily identifiable microorganisms.

No correlation was found between days of mechanical ventilation and the presence of bacteria or yeast

***Table 1**: Organism Prevalence in 13 Donor Corneas*

Donors	Isolates
3 donors	yeast/*Candida albicans*
2 donors	*Staphylococcus aureus*
2 donors	*Coagulase*-negative *Staphylococcus*
1 donor	Alpha *Streptococcus* (not *Pneumococcus*)
1 donor	*Clostridium perfringens*
2 donors	*Propionibacterium* species
1 donor	*Proteus mirabilis, Enterobacter*
1 donor	*Escherichia coli, Pseudomonas, Enterobacter aerogenes*

***Table 2**: Colonization Pattern in Donor Corneas*

DONORS	ISOLATES
Donor a	*Proteus mirabilis, Enterobacter aerogenes*
Donor b	*Escherichia coli, Pseudomonas aeruginosa, Enterobacter aerogenes*
Donor c	*Candida albicans*
Donor d	*Staphylococcus aureus*
Donor e	*Staphylococcus aureus, Propionibacterium* species
Donor f	*Staphylococcus aureus*
Donor g	*Propionibacterium* species
Donor h	alpha *hemolytic Streptococcus*
Donor i	*Clostridium perfringens* and *Coagulase*-negative *Staphylococcus*
Donor j	*Coagulase*-negative *Staphylococcus, Candida albicans*
Donor k	*Candida albicans*

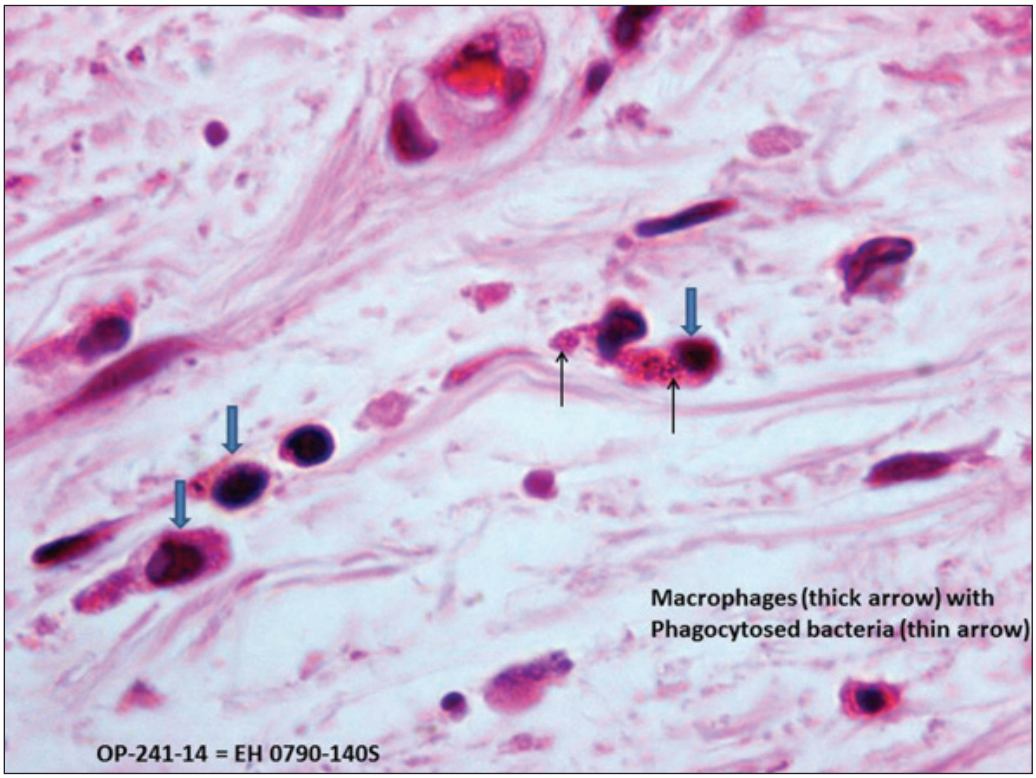

***Figure 1**: Presence of Microorganisms in the Microphage Cytoplasm*

Note: 100 X
Note: the alpha-numeric string in the image is a reference number for the slide.

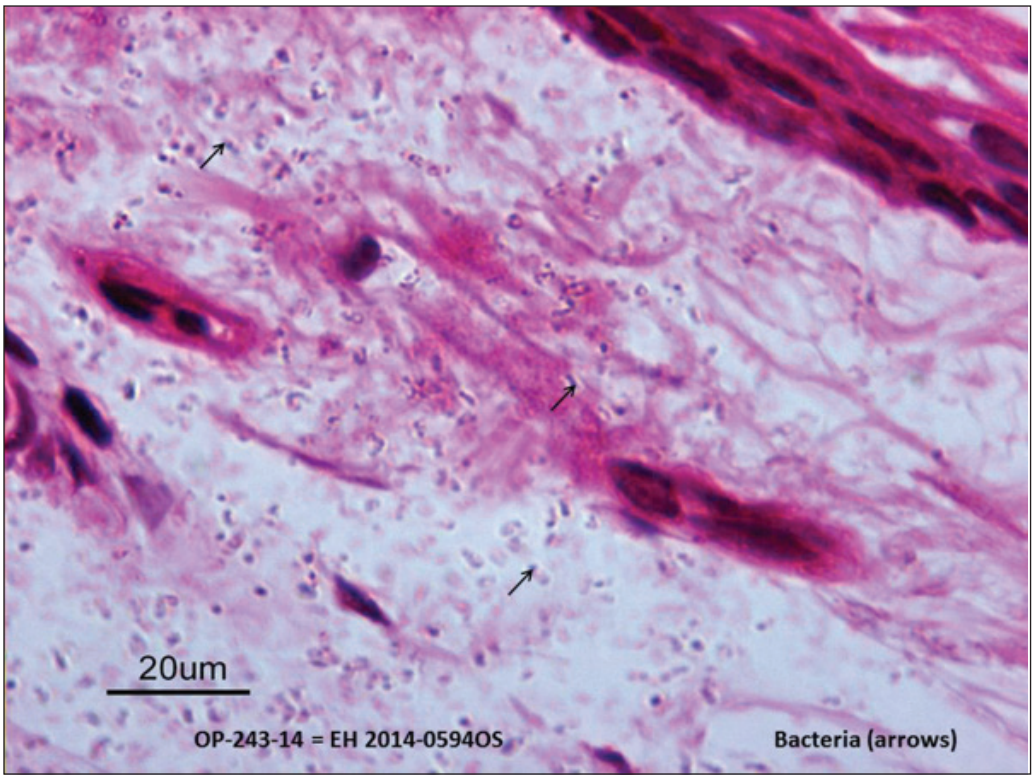

***Figure 2**: Presence of Phagocytosed and Extra-Cellular Organisms*

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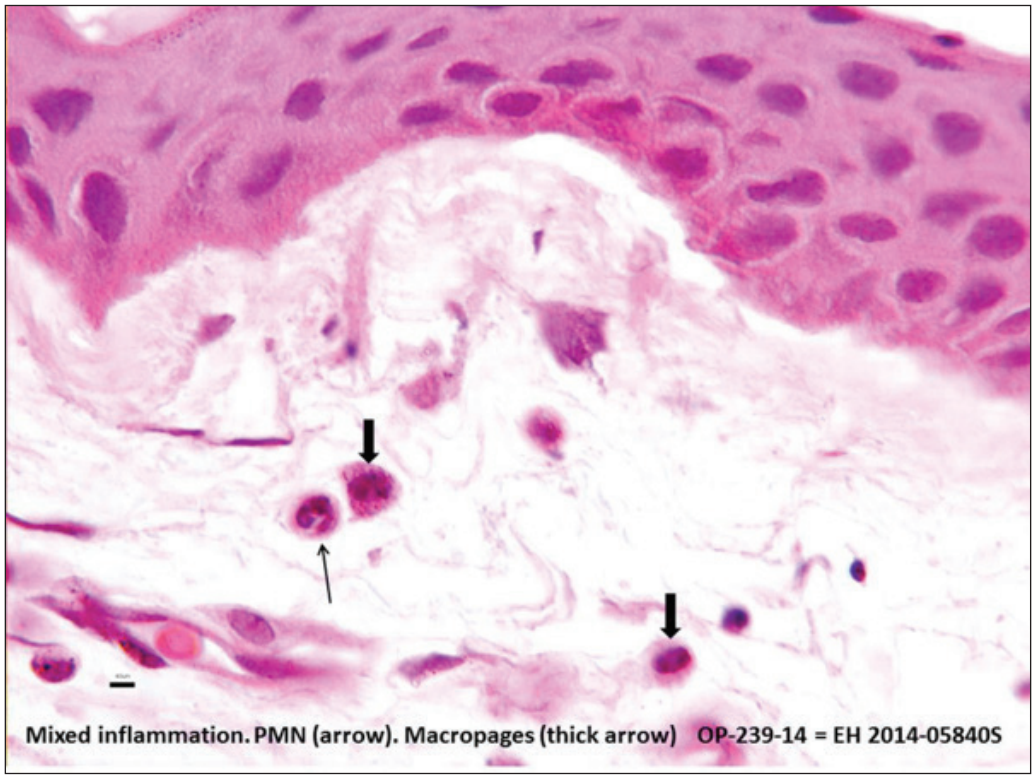

***Figure 3**: Inflammatory Cells with No Readily Identifiable Microorganisms*

Note: 100 X
Abbreviations: PMN, Polymorphic Neutrophils
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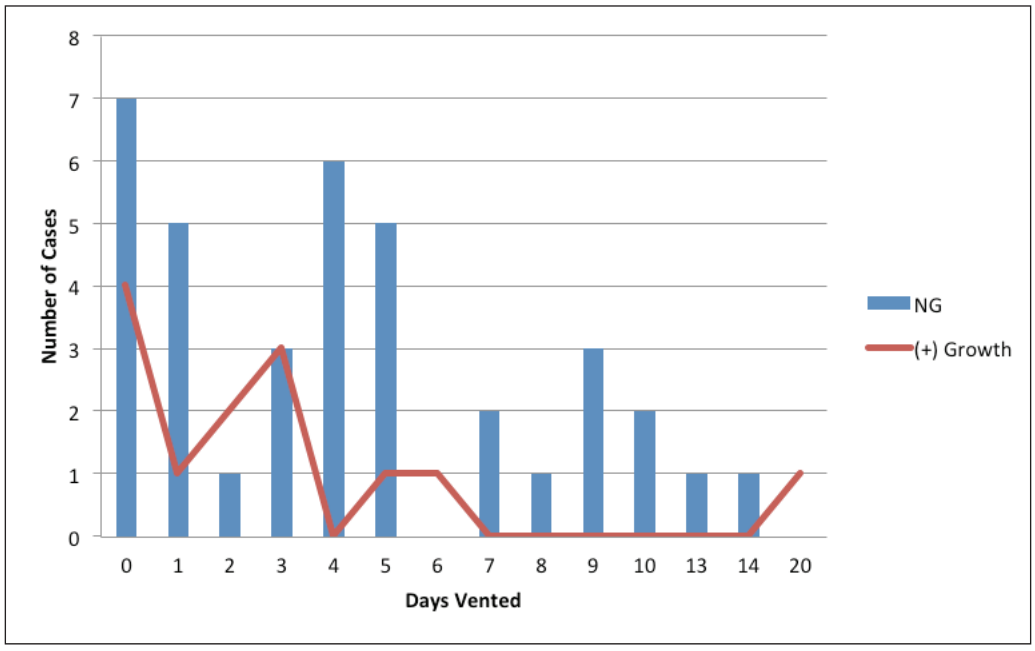
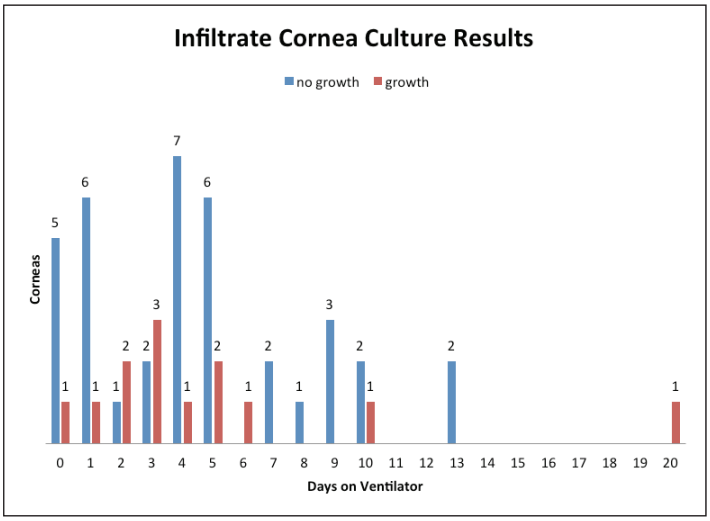


***Figure 4**: Growth vs Ventilation Days*

in infiltrates (t=.582, p=.254 CI 95%). Some corneas showed infiltrates with growth after 1 to 2 days of ventilation as seen in Table 3; however, by contrast, others showed infiltrates with growth from donors who had not undergone ventilator support, while others showed no growth in infiltrates even after 13 days of ventilation. It is important to recognize that the presence of antibiotics in the cornea preservation media, even with laboratory dilution post-recovery, could reduce organism recovery rates. The pre-mortem clinical culture results of blood, wounds and sputum can provide valuable screening information in determining donor suitability for transplantation. However, prerecovery cornea cultures are not part of standard practice as no correlation between those results and post-transplant endophthalmitis has been definitively established[8]. Standard antibiotic concentration in ocular storage media is 50ug/ml of gentamycin added for the control of bacteria and may be bactericidal but could be only bacteriostatic as identification of microorganisms in these infiltrates might suggest.

***Table 3**: Growth of microorganisms not consistent with days post ventilation*



DISCUSSION

No isolates of *Acinetobacter or Clostridium* difficile were identified although other organisms were isolated. C*lostridium perfringens* was isolated in one pair of infiltrates. This was an unexpected finding, but when this result is compared with concurrent tissue bank cultures from bone[i] and skin,[ii] *Clostridium perfringens* had also been recovered from these sites. *Clostridium* has also been previously reported[5] in a post-transplant endophthalmitis, which given the finding reported here, suggests that the presence of this anaerobic organism could be clinically significant. Contrary to the recent CDC report of increasing *Acinetobacter* or *Clostridium difficile*, no significant shift in infiltrate colonization was seen in these corneas. Candida and Staphylococcus were the most frequent isolates in this study population. The apparent lack of correlation between ventilator interval and colonization, however, is an important finding which re-emphasizes the need for multifactor donor screening including looking closely at all microbiology reporting with or without ventilator support where organism colonization may affect tissue suitability not only in ocular infiltrates but also as an indication of risk of sepsis. This includes, but may not be limited to, reports of blood, sputum and wound cultures. Cornea scleral rim cultures have not been definitively shown to be predictive of or correlated with, recipient endophthalmitis[7,8] although if present should be evaluated by the medical director and/or implanting physician.

CONCLUSION

The presence of microorganisms in 26% of infiltrates reinforces strongly the continuing need for diligence by eye bank professionals and transplanting surgeons in evaluating the clinical significance of light-blocking corneal defects in prospective donors. With the increasing rise in hospital-acquired infections coupled with accelerating antibiotic resistance and increased "handling-time" for specialized DSAEK and DMEK donor tissue, it remains critical for eye bank professionals to identify and remove from potential transplantation use all donor corneal tissue that demonstrates light-blocking infiltrates.

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i Musculoskeletal Tissue Foundation, Edison, NJ

ii Adam Wilson-VRL Reference Laboratory, Centennial Colorado

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