Candida Interface Infections After Descemet Stripping Automated Endothelial Keratoplasty

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Purpose: To describe 2 *Candida* interface keratitis infections occurring in the setting of positive donor rim cultures from precut corneal tissue used for Descemet stripping automated endothelial keratoplasty (DSAEK) and the ensuing public health investigation.

Methods: Following 2 clinical *Candida* interface keratitis infections, patients from 2012 to 2014 in the same surgical center were evaluated for bacterial and fungal rim cultures and subsequent infection. All cases of fungal infections occurring post-DSAEK were analyzed. Data included patient demographics, surgical technique, donor rim cultures, donor mate outcomes, clinical courses, and outcomes. A review of the relevant literature was also undertaken.

Results: From 2012 to 2014, among 99 DSAEK procedures performed, 7 (7.1%) donor rim cultures were positive for fungi. Use of this tissue with positive donor rim cultures resulted in 2 (28.6%) episodes of confirmed fungal interface keratitis, both *Candida* species, and presumptive treatment in an additional 2 patients. An investigation did not identify any breach in sterile technique or procedures by the surgeon or surgery center. Our literature review identified 15 reports of postoperative fungal infection associated with DSAEK, of which 11 involved *Candida* spp.

Conclusions: While postoperative infection remains rare, our 2 additional cases along with those previously reported suggest that DSAEK may be susceptible to infection with *Candida* spp. Furthermore, this report of correlated rim cultures and clinical infection suggests a need for reevaluation of the utility of obtaining routine corneoscleral donor rim fungal culture.

Key Words: Descemet stripping automated endothelial keratoplasty, DSAEK, fungal keratitis, donor rim culture, eye banking, *Candida*, endothelial keratoplasty

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In the past decade, endothelial keratoplasty (EK) has largely replaced penetrating keratoplasty (PK) as the surgical procedure of choice for corneal endothelial failure.[1,2] Compared with PK, the many benefits of EK for patients include preservation of recipient corneal structural integrity and topography resulting in less postoperative astigmatism, faster visual recovery, and elimination of postoperative suture management.[1]

Corneal graft usage data demonstrate this shift in procedure selection. The Eye Bank Association of America (EBAA) reported that in 2014, a total of 47,530 corneal grafts were used domestically in the United States.[2] Of these 25,965 grafts were used in 2014 for EK, representing an increase of 48.6% in EK procedures compared with 2008. In contrast, 19,294 corneas were used domestically for PK in 2014, representing a 40.7% decrease from 2008.[2,3]

The EBAA reported an increasing trend of fungal infection after corneal transplantation over the past 6 years.[4] Although this increase was not statistically significant, the EBAA indicated that fungal keratitis and endophthalmitis occurred almost twice as frequently after EK than after PK from 2007 to 2010.[4] Published reports have also documented fungal interface keratitis and endophthalmitis following Descemet stripping automated endothelial keratoplasty (DSAEK).[4–22]

We describe our experience of *Candida* spp DSAEK interface infections associated with positive donor rim cultures, the ensuing investigation, and a review of the current literature. These cases highlight important considerations regarding eye bank and surgical practices. In particular, we discuss the potential utility of fungal corneoscleral rim cultures, which are performed for only approximately 29% of corneas distributed by eye banks in the United States (Aldave AJ, The Utility of Donor Corneal Rim Cultures: A Report of the EBAA MAB Subcommittee on Fungal Infection Following Corneal Transplantation. EBAA Annual Meeting, June 6, 2015, Georgia).

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METHODS

Investigation

On December 1, 2014, hospital A and its affiliated ambulatory surgery center (ASC A) reported to the New Hampshire (NH) Department of Health and Human Services, Division of Public Health Services (DPHS) an increase of positive donor corneal rim cultures and subsequent clinical fungal infections. The NH DPHS initiated an investigation to determine if the increase represented an outbreak, to identify the mechanism of infection, and to assess ongoing patient risk. The NH DPHS, and hospital A's infection preventionist, reviewed ASC A's practices and procedures for adherence to instrument processing, environmental cleaning, and compliance with aseptic technique. DPHS staff reviewed ASC A data for 2013 and 2014, conducted 2 site visits, reviewed national data, contacted federal partners (Centers for Disease Control and Prevention and US Food and Drug Administration), and conducted additional case finding through notification of professional ophthalmology organizations and other public health jurisdictions. The primary surgeon halted all corneal transplant surgery for 1 month during the investigation to ensure no ongoing risk to patients.

Statistical Analysis

Data from ASC A for 2012 to 2014 were analyzed to compare the frequency of clinical infections, positive corneoscleral rim cultures, death to preservation time, death to surgery time, procedure types, and source of tissue over time. Data were analyzed using SAS (version 9.3; SAS Institute Inc, Cary, NC). The Fisher exact test was used to compare proportions with 2-tailed Fisher exact P values < 0.05 considered significant.

Clinical Patient Reports

The study was approved by the Institutional Review Board at Concord Hospital and by the Committee for the Protection of Human Subjects at Dartmouth College. We reviewed all corneal transplant procedures from 2012 to 2014 performed by a single surgeon at ASC A. The primary surgeon routinely obtains donor rim cultures on all donor corneal grafts and all cases of positive fungal or bacterial donor rim cultures were recorded. These patients' demographics, clinical courses, management, and outcomes were also recorded.

Literature Search Strategy

PubMed, Ovid MEDLINE, EMBASE, Web of Science, and Cochrane Reviews/Trials Database were searched in May 2015 using a combination of the following keywords: *endothelial keratoplasty, Descemet's stripping automated endothelial keratoplasty, DSAEK, fungal keratitis, fungal endophthalmitis*. The titles and abstracts of all papers available in English or had English translations were reviewed. Study design, patient characteristics, clinical course, and interventions were recorded separately by 2 independent reviewers. Inclusion criteria were retrospective studies containing clinical reports of fungal infections that occurred postoperatively following DSAEK. Cases were separated into those that documented specific clinical courses and those that retrospectively reviewed prevalence on an institution/eye bank-wide level. Conference proceedings were excluded because they may have duplicated published reports.

RESULTS

Investigation

The NH DPHS did not identify any infection control lapses by the surgeon or facility associated with the positive corneal rim cultures and infections. The infections were reported to the US Food and Drug Administration and the eye bank that provided the tissue (eye bank A). The surgeon resumed performing procedures at the completion of the investigation.

Clinical Summary

Table 1 shows the number of positive rim cultures associated with PK and DSAEK procedures performed from 2012 to 2014 at ASC A. There was a nonstatistically significant increasing trend of positive fungal rim cultures associated with precut tissue, with an incidence of 0% (0/25) in 2012, 6.3% (2/32) in 2013, and 11.9% (5/42) in 2014 ($P = 0.218$). All positive fungal donor rim cultures were associated with precut tissue from eye bank A.

Among 99 DSAEKs performed, 7 donor rim cultures were positive for fungi. Two (28.6%) of 7 patients with positive fungal rim cultures went on to develop interface fungal keratitis. Two (66%) of 3 patients with donor rim cultures positive for *Candida* species developed interface keratitis. Both patients had DSAEK; no fungal eye infections occurred among more than 6000 patients undergoing any other type of eye procedure. Death to preservation time and days to surgery were available for 96 of 99 DSAEK procedures. There was no significant difference ($P = 0.436$) in the number of positive fungal rim cultures of grafts that had death to preservation time less than 10 hours (2/47, 2.1%)

TABLE 1. Data From a Single Surgeon for All Corneal Transplants From 2012 to 2014 Demonstrating Type and Number of Procedures Performed, Number of Positive Microbial Cultures, and Resulting Clinical Infections

Year	2012	2013	2014
Total transplants	40	49	59
Number of DSAEK procedures	25	32	42
Number of PK procedures	14	17	17
Number of patch graft procedures	1	0	0
Positive fungal donor rim cultures	0	4	5
Positive bacterial rim cultures*	2	3	7
Clinical infections (bacterial)	0	0	0
Clinical infections (fungal)	0	0	2

*None of the positive bacterial rim cultures resulted in subsequent infection.

TABLE 2. Positive Fungal Donor Rim Cultures and Treatments From 2013 to 2014 DSAEK and PK Procedures Performed by Single Surgeon

Patient No.	Surgery Type	Microorganism Isolated from Donor Rim	Clinical Infection	Microorganism Isolated From Removed Donor Lenticule	Surgical Intervention	Donor Age (yrs)	Donor Cause of Death	Donor Ocular History	Procurement/ Processing Technique
1	PK	*Trichophyton*	None	—	—	68	Pneumonia	None	Excision/uncut
2	DSAEK	Dematiaceous	None	—	—	72	Gastrointestinal bleed	Cataract extraction	Excision/precut
3	PK	*Beauveria*	None	—	—	59	Lung cancer	Corrective lenses	Excision/uncut
4	DSAEK	*Cladosporium*	None	—	—	75	Lung cancer	LASIK, cataract extraction	Excision/precut
5	DSAEK	*Aspergillus*	None	—	—	63	Lung cancer	Corrective lenses	Excision/precut
6	DSAEK	*C. albicans*	Keratitis	*C. albicans*	Repeat DSAEK, then PK	60	Cardiovascular disease	None	Excision/precut
7	DSAEK	*C. glabrata*	Keratitis	*C. glabrata*	Repeat DSAEK, then PK	51	Diabetes mellitus	None	Excision/precut
8	DSAEK	*C. glabrata*	None	—	—	51	Diabetes mellitus	None	Excision/precut
9	DSAEK	*Scedosporium* and *Trichoderma*	None	—	Removal of dislocated graft and replacement with new donor lenticule	38	Mixed drug toxicity	Corrective lenses	Excision/precut

Neither positive fungal rim cultures nor fungal infections occurred in 2012. No infection or complication resulted after procedures from donor mates to patients 4, 5, and 6. Patients 7 and 8 received corneal tissue from same donor.

versus grafts that had death to preservation time of 10 hours or more (5/49, 10.2%). There was no significant difference ($P = 0.456$) in number of positive fungal rim cultures of grafts that were used less than 5 days from death to surgery (4/41, 9.8%) versus grafts that were used 5 or more days from death to surgery (3/55, 5.5%).

Table 2 summarizes all PK and DSAEK procedures with positive fungal rim cultures and the associated culture results, postoperative infections, treatments, and tissue donor information. Clinical summaries are provided for the 4 patients with positive fungal donor rim cultures (patients 6, 7, 8, and 9), 2 of whom developed interface fungal keratitis (patients 6 and 7). *Candida albicans* (patient 6) and *Candida glabrata* (patient 7) were the causative organisms in infections, which matched the organisms cultured from the donor rim. Two additional patients are described in whom interventions were undertaken after a positive fungal corneal rim culture was reported.

Patient 6

An 85-year-old woman underwent DSAEK for Fuchs corneal dystrophy using precut corneal tissue that was allowed to warm 2 hours before surgery (consistent among all DSAEK cases performed). The donor cornea was punched with a Hanna suction donor trephine from the endothelial side and an 8.5-mm donor graft was inserted with forceps through a 5-mm scleral tunnel incision. At the time of surgery, the donor rim was cultured and grew *C. albicans*. The graft was clear and attached until the day 20 visit, where 2 white infiltrates with indistinct margins were seen at the inferior edge of the host–donor interface. Administration of topical steroid drops was stopped. Amphotericin B, 5 μg/0.1 mL, was injected into the anterior chamber on day 21. Additionally, the patient was administered oral fluconazole 200 mg daily and topical amphotericin B 0.15% drops every 2 hours. She underwent repeat DSAEK with an anterior chamber washout and an additional injection of amphotericin B into the anterior chamber. The first donor lenticule was cultured, which grew *C. albicans*. By postoperative day 3, 2 small white infiltrates could be seen reforming in the previous area of infection (Fig. 1). Administration of topical steroid drops was stopped and the patient was switched to topical cyclosporine 1% drops 4 times daily and she received repeated injections of amphotericin B into the anterior chamber. The infiltrate appeared to lessen, but new flocculent material could be seen forming in the anterior chamber and inferior angle. She underwent PK and anterior chamber washout, repeat amphotericin B injection into the anterior chamber, and was given 100 mg micafungin intravenously. Aqueous humor cultures from surgery were negative. Her graft remained clear 5 months after her PK with best corrected vision (BCVA) of 20/40.

Patient 7

A 71-year-old woman underwent DSAEK for Fuchs corneal dystrophy. Her postoperative course was uneventful until day 6, when the donor rim culture was reported positive for fungal growth and the topical steroid drop was discontinued. On day 9, two small round white infiltrates could be

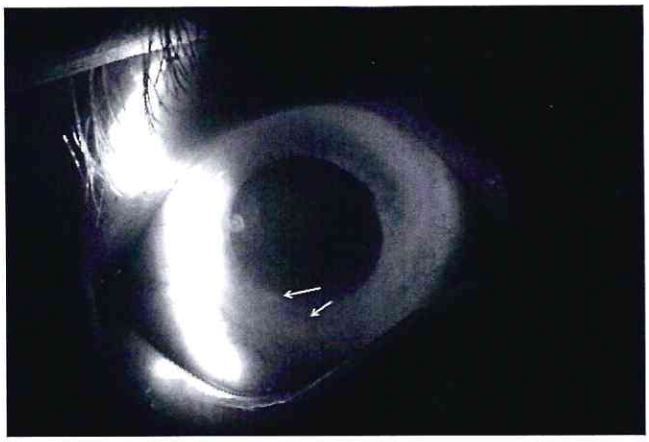

FIGURE 1. Postoperative day 3, anterior segment photograph of patient 6's right eye after initial DSAEK graft was removed and a new graft replaced. Small white infiltrates are seen forming in the previous area of infection on edge of graft (arrow).

seen at the inferior edge of the graft with additional debris seen diffusely in the interface. An aqueous tap was performed for culture and amphotericin B, 5 μg/0.1 mL, was injected into the anterior chamber. The patient was administered oral fluconazole 200 mg daily and topical amphotericin B drops 0.15% every 2 hours as well as topical cyclosporine and ofloxacin drops each 4 times daily. On day 11, the 2 round infiltrates had coalesced into 1 larger irregular white infiltrate at the inferior edge of the DSAEK graft. Additional 5 μg/0.1 mL of amphotericin B was injected into the anterior chamber. The aqueous tap culture was negative and the yeast growing on the donor rim culture remained unidentified. Repeat DSAEK and anterior chamber washout with an injection of amphotericin B into the anterior chamber was performed on day 14. Postoperatively the patient continued topical and oral antifungal medications as well as topical cyclosporine. The original donor rim culture was identified as *C. glabrata*, which also grew from the donor lenticule that was removed from the patient. The patient showed no further signs of fungal infection, although her DSAEK graft did not clear. Her antifungal medication was tapered and then discontinued over the next month. She was administered topical steroid drops 2 months postoperatively because of persistent discomfort. Her graft remained hazy, but no additional fungal infection was seen. She underwent PK 4.5 months after her original DSAEK. No fungal microorganisms were cultured in the second donor lenticule. The patient had a clear PK graft with BCVA of 20/25 at 2.5 months after PK.

Patient 8

The mate to the donor cornea used in patient 7 was used in DSAEK surgery of a 63-year-old man with iridocorneal endothelial syndrome. Although he showed no signs of fungal infection, administration of topical steroid drops was stopped because the donor rim culture was found to be positive for yeast. He was empirically given amphotericin B 5 μg/0.1 mL injection into the anterior chamber on day 9 and administration of topical amphotericin B 0.1%, topical cyclosporine 1%, and oral fluconazole 200 mg daily was started. The donor rim culture was identified as *C. glabrata*. However, his graft remained clear without signs of infection. Antifungal medication was stopped and he was readministered topical steroid drops 3 weeks postoperatively for improvement in comfort and graft clarity. His graft remains clear at 5 months with BCVA of 20/40.

Patient 9

A 65-year-old man underwent DSAEK for Fuchs corneal dystrophy. Three days after surgery, the donor corneal rim culture was reported positive for fungus. Additionally, the graft was dislocated and required repositioning. Due to the recent increase in positive fungal donor rim cultures in concert with his need to undergo a second procedure, the patient underwent removal and replacement of his donor lenticule 11 days after his first surgery. The donor lenticule was cultured and did not show growth of microorganisms. The original donor rim culture grew *Scedosporium* and *Trichoderma* spp. The patient did not receive any antifungal medication. His graft remained attached without signs of fungal infection at 6 months after surgery with BCVA of 20/50. His vision is limited by amblyopia.

Literature Review

Table 3 summarizes the 15 case reports (review of retrospective reports in literature intrinsically do not allow accurate delineation of unique cases owing to reporting of fungal infections in case reports, single-institution reviews of prevalence, and/or eye bank reports) of fungal keratitis or endophthalmitis associated with DSAEK that we identified. Of the cases that specified a causative microorganism, 11 reported *Candida* spp as the causative microorganism,[5–10,13–16] and there was 1 case of *Aspergillus spp*[11] and 1 case of budding yeast.[8] The 2 additional cases were empirically diagnosed and treated as fungal infection.[12] Of the 13 cases for which donor rim cultures were obtained, 61.5% (8/13) were positive for *Candida* spp.[5,6,8,10,13,14,16] The cultures in the remaining 5 cases for which donor rim cultures were obtained remained negative.[7,8,11,12] The median time to clinical infection was postoperative day 36 (range 1–1000 days).

Table 4 summarizes studies evaluating the proportion of fungal infection after corneal transplant. Single-institution studies with consecutive case experience report that 0.28% to 0.79% of DSAEK cases developed postoperative fungal infections.[19–21] In contrast, single-institution investigations of consecutive *failed* DSAEK grafts reported that fungal keratitis was the primary reason for graft failure in 4.3% to 7.7% of cases.[17,18] Last, studies from American eye banks providing corneas for EK report that the proportion of postoperative fungal infections ranged from 0.022% to 0.72%.[4,22]

DISCUSSION

We have presented a cluster of positive fungal corneal rim cultures and interface infections occurring between 2012

TABLE 3. Literature Review of Clinical Case Reports and Case Series of Postoperative Fungal Infections Following DSAEK

Author	Age (yrs), Gender	Donor Cornea Preparation by Eye Bank	Donor Rim Cultured	Microorganism Isolated From Donor Rim	Presentation Postoperative Day	Clinical Infection	Clinical Culture Location	Microorganism Isolated From Clinical Specimen	Surgical Intervention
Kitzmann et al[5]	80, F	Precut*	Yes	*C. albicans, C. glabrata,* and β-hemolytic group B *Streptococcus*	39	Keratitis	Interface infiltrates cultured at time of repeat DSAEK	*C. albicans*	Repeat DSAEK
	80, F	Precut*	Yes	*C. albicans* and *C. glabrata,* β-hemolytic group B *Streptococcus*	41	Keratitis	Anterior corneal infiltrate culture, (anterior chamber paracentesis was negative)	*C. albicans*	Patch graft
Koenig et al[6]	80, F	Uncut	Yes	*C. albicans*	7	Keratitis	Donor lenticule	*C. albicans*	Donor lenticule removal, then PK, then enucleation
Chew et al†·[7]	72, F	Uncut	Yes	No growth	2	Endophthalmitis	Anterior chamber and vitreous humor	*C. parapsilosis*	PK
Lee et al[8]	81, M	Precut	Yes	*C. glabrata*	30	Keratitis	Donor lenticule	Histology showed yeast	PK
	76, F	Uncut	Yes	No growth	21	Keratitis	Corneal scraping, eyelid, conjunctival cultures	*C. albicans*	PK
Ortiz-Gomariz et al[9]	76, F	Uncut	No	n/a	90	Keratitis, endophthalmitis	Aqueous, vitreous, donor lenticule	*C. albicans*	PK
Yamazoe et al[10]	74, M	NR	Yes	*C. albicans*‡	36	Keratitis	Anterior chamber aqueous, donor lenticule	*C. albicans*	PK
Sharma et al[11]	62, M	NR	Yes	No growth	120	Keratitis	Posterior lamellar disk (corneal scrapings were negative)	*Aspergillus fumigatus*	PK
Tu and Hou[12]	66, M	NR	Yes	No growth	90	Keratitis	NR	n/a	No surgery
	70, M	NR	Yes	No growth	49	Keratitis	NR	n/a	No surgery
Villarrubia and Cano-Ortiz[13]	73, F	Uncut	Yes	*C. albicans*	10	Keratitis	Corneal specimen cultured at time of subsequent PK	*C. albicans*	PK
Hsu et al[14]	45, F	NR	Yes	*C. albicans*	1	Keratitis, endophthalmitis	Anterior chamber paracentesis, corneal scraping	*C. albicans*	PK

(*Continued*)

TABLE 3. (*Continued*) Literature Review of Clinical Case Reports and Case Series of Postoperative Fungal Infections Following DSAEK

Author	Age (yrs), Gender	Donor Cornea Preparation by Eye Bank	Donor Rim Cultured	Microorganism Isolated From Donor Rim	Presentation Postoperative Day	Clinical Infection	Clinical Culture Location	Microorganism Isolated From Clinical Specimen	Surgical Intervention
Araki-Sasaki et al§,[15]	72, M	NR	NR	n/a	~1000	Keratitis	Corneal scraping	*C. albicans*	PK
Weng et al[16]	Early 80s, M	NR	Yes	*C. glabrata*‖	30	Endophthalmitis	Vitreous humor, donor lenticule	*C. glabrata*	Removal of lenticule, future PK planned

*Kitzmann et al, donor corneas were reported to be precut in the report by Rauen et al.[22]
†Chew et al, suspected corneal venting incisions allowing microorganism entry rather than donor origin.
‡Yamazoe et al, culture medium grew *C. albicans*.
§Araki-Sasaki et al, suspected infection was due to a combination of the immunosuppressive condition related to the prednisolone eye drops, the postoperative condition, and continuous disposable soft contact use, rather than donor origin.
‖Weng et al, culture medium grew *C. glabrata*.
n/a, not applicable; NR, not reported.

and 2014. Over these 3 years, of the 99 precut corneas used for DSAEK, 7 of 99 (9.1%) precut corneas were positive for fungi on rim culture and 2 of 7 went on to develop interface keratitis. Another 2 of 7 patients received empirical treatment, which may have halted progression to clinical infection.

Several possible microbial sources of surgical infection in corneal transplantation may be implicated as the origin of this cluster of positive rim cultures and interface keratitis: patient microbiota, environmental or instrument contamination, transport media contamination, and donor cornea. Each of these potential vectors was considered by the NH DPHS during the investigation, which did not reveal any specific infection prevention breach, improper surgical technique, or mishandling of transplant tissue that was likely to elevate the risk of fungal surgical infections. The NH DPHS was in contact with the eye bank that provided the tissue, but did not directly investigate their facility or practices. A review of all eye surgical procedures performed at the same facility from 2012 to 2014 revealed no additional fungal infections. These procedures included cataracts, corneal transplants, pterygia and other conjunctival cases, laser-assisted in situ keratomileusis (LASIK) and photorefractive keratectomy (PRK), glaucoma procedures, and oculoplastic procedures. Furthermore, both cases were accompanied by positive corneoscleral rim cultures of the same organism. Taken together, these data suggest that the donor corneal tissue is a likely source of the interface keratitis.

In our series, positive fungal donor rim cultures appeared predictive of cases at risk for fungal interface infection. However, the utility of donor rim cultures has been the subject of debate. Because PK has historically been associated with low postoperative infection rates and donor rim cultures have not been found to be highly predictive of clinical infection, routine culture of donor rims is obtained on a minority of patients undergoing corneal transplant.[23,24] In 2013, of the 45,271 corneas distributed by 62 eye banks reported to the EBAA, donor rim cultures were obtained in only 29.2% of cases (Aldave AJ, The Utility of Donor Corneal Rim Cultures: A Report of the EBAA MAB Subcommittee on Fungal Infection Following Corneal Transplantation. EBAA Annual Meeting, June 6, 2015, Georgia). Our data, as well as those of past studies, showed no significant correlation between positive bacterial donor rim cultures and postoperative bacterial infection.[23,24]

However, emerging literature suggests that positive donor fungal rim cultures may be correlated with postoperative fungal infection.[5,6,22] In a pooled analysis of donor rim cultures and post-PK endophthalmitis, Wilhelmus and Hassan[25] demonstrated that fungi, in particular *Candida* spp, may pose a greater risk of progression to clinical infection than bacteria. The odds of fungal endophthalmitis when fungi were isolated from the donor rim was 247 times greater than if no fungus grew, compared with 18 times greater if bacteria were isolated from the donor rim.[25] In our literature review, in DSAEK cases where donor rim cultures were obtained, the rim-cultured organism appeared to be predictive of the organism that grew from clinical culture. Table 2 shows that these were predominantly *C. albicans*. In our series, of the 3 donor rims that grew *Candida* species, 2 went on to clinical infection. Together, these data indicate that rim cultures positive for fungi may be more predictive for development of clinical infection than rim cultures positive for bacteria.

One question that arises from the literature is whether lamellar cut of donor corneal tissue in DSAEK increases the risk of infections compared with PK. A study of 629 corneal transplants compared precut with uncut corneal tissue for PK.[22] Although the rate of positive fungal rim cultures was similar between uncut and precut corneal tissue (8/351, 2.5% and 7/278, 2.3% respectively), no infections occurred among the uncut tissue group used for PK with positive fungal cultures (0/8), whereas infections occurred in 2 of 7 (28.6%) of the cases in which the precut donor rim was positive for fungi, both involving *Candida*.[5,22] No infections occurred in cases with negative rim cultures.[22] In a study by the EBAA using data reported into the Online Adverse Reaction Reporting System database from 2007 to 2010, almost twice as many EK cases (0.022%) developed postoperative

TABLE 4. Retrospective Reviews Investigating Histopathology, Microbiology of Corneal Donor Tissue and Removed Specimens, or Eye Bank Reported Data Involving DSAEK Procedures

Author (Year)	Number of Fungal Infections (%)	Number of Positive Fungal Rim Cultures	Microorganism Isolated from Donor Rim	Presentation Postoperative Day	Type of Infection	Clinical Culture Location	Microorganism Isolated From Clinical Specimen	Surgical Intervention
Single-institution studies investigating failed DSAEK grafts								
Zhang et al[17]	2/47 (4.3)	n/a	Case 1: *C. glabrata*	Case 1: NR	Case 1: Keratitis	Case 1: Graft culture	Case 1: *C. glabrata*	Case 1: PK
			Case 2: No growth	Case 2: 21	Case 2: Keratitis	Case 2: Cornea/ eyelid	Case 2: *C. albicans*	Case 2: PK
Alkatan et a[18]	1/13 (7.7)	n/a	NR	NR	Stromal keratitis (host–graft interface)	NR	Histology showing yeast	PK
Single-institution studies investigating all DSAEK grafts								
Nahum et al*,[19]	3/1088 (0.28)	Negative in reported cases	Negative in reported cases	Case 1: 112 (1 mo after relaxing incision)	Case 1: Keratitis	Case 1: NR	Case 1: *C. parapsilosis*	Case 1: PK
				Case 2: 21	Case 2: Keratitis	Case 2: Positive cultures from scraping of the posterior recipient stroma (cultures of the corneal surface scrapings were negative)	Case 2: *C. parapsilosis*	Case 2: Repeat DSAEK, then PK
				Case 3: 28	Case 3: Keratitis	Case 3: Positive cultures from excised tissue (cultures of the scrapings obtained from the corneal surface were negative)	Case 3: *C. albicans*	Case 3: PK
Garg et al[20]	1/127 (0.79)†	NR	*C. albicans*	28	Interface keratitis	Donor lenticule	*C. albicans*	PK
Basak[21]	2/430 (0.47)‡	NR	NR	Case 1: within 2 mo	Keratitis	NR	Case 1: *Fusarium* spp	Case 1: PK
				Case 2: after 2 mo	Keratitis		Case 2: *Aspergillus*§	Case 2: Medical management
Studies reviewing eye bank fungal cultures								
Rauen et al[22]	DSAEK group n = 2/278 (0.72)¶	Precut DSAEK tissue: 7/ 278 (2.5%)	Case 1: *C. albicans, C. glabrata*	Case 1: 39	Case 1: Keratitis	Case 1: Interface infiltrates cultured at time of repeat DSAEK	Case 1: *C. albicans*	Case 1: Repeat DSAEK
			Case 2: *C. albicans, C. glabrata*	Case 2: 41	Case 2: Keratitis	Case 2: Anterior corneal infiltrate culture	Case 2: *C. albicans*	Case 2: Patch graft
Aldave et al[4]	15/69,007 (0.022)‖	NR	NR	NR	NR	NR	*Candida* spp	NR

*Nahum et al, reported 10 interface infections post-DSAEK, of which 3 were fungal infections requiring PK.
†One clinically infected DSAEK graft, other positive cultures not specifically stated for DSAEK.
‡All cases were surgeon cut.
§Reported to have foreign body entry in eye.
¶Fungal infections reported prior by Kitzmann et al[5] (Table 1).
‖All EK procedures.
n/a, not applicable; NR, not reported.

endophthalmitis as PK cases (0.012%).[4] Although not statistically significant, the increased trend of infections in EK versus PK raises concern that differences in tissue preparation could lead to infections. In Table 3, 5 of 8 cases in which the cutting technique was reported were uncut tissue provided to the surgeon for DSAEK. Pooling these data with our additional 2 cases that both used precut tissue, fungal infection following DSAEK is evenly divided between eye bank precut and uncut tissue. However, as is the nature of case reports, there may be a selection bias in the reported cases. More large studies are needed to determine if there is an increased risk of postoperative infections, in particular fungal infections, associated with DSAEK whether cut by an eye bank or the surgeon.

In the United States, donor corneas are commonly stored in hypothermic Optisol-GS medium, which contains antibacterial agents (gentamicin sulfate and streptomycin sulfate), but no antifungal agents. Ritterband et al[26] found that adding 100 μg/mL voriconazole to cornea storage medium significantly reduced fungal growth. In contrast, Layer et al,[27] were able to culture *C. albicans* and *C. glabrata* from media inoculated with a lower dose of voricazonole (50 μg/mL). However, in samples supplemented with amphotericin B, there was no growth of fungal organisms except at 0.25× and 0.5× minimum inhibitory concentrations.[27]

Although corneas destined for PK are also stored in Optisol-GS, there are several distinct opportunities for fungal growth and contamination associated with precut tissue processing for DSAEK when compared with PK. After harvesting, precut tissue is placed in Optisol and stored at 4°C. At a later time, the tissue is warmed and then cut in a sterile tissue culture hood, assessed for quality, then returned to Optisol, and maintained at 4°C. Warming and cutting at the eye bank provides an opportunity for fungal growth and possible implantation of fungi in the cut stromal tissue. On the day of surgery, typically, the tissue is warmed to room temperature, usually less than 2 hours, but warming times may vary. Warming before surgery is undertaken because it promotes the antimicrobial effects of the antibiotics in Optisol-GS[28] as well as increases metabolic activity in the corneal endothelial pump, thereby decreasing the likelihood of graft detachment. Furthermore, the interface environment may be vulnerable to fungal infection. It is a sequestered space lacking the multifaceted armamentarium of the innate immune system on the ocular surface. Finally, the relative hypoxia of this space may promote fungal virulence as well as reduced immune responses.[29,30] Study of all these steps in donor tissue preparation is warranted to determine if one is particularly associated with fungal contamination and to provide a basis for recommendations to reduce the risk of infection.

Until more information is available, we concur with the views expressed by Rauen et al[22] with regard to obtaining routine corneoscleral rim culture for all DSAEK cases and promptly initiating antifungal therapy following positive cultures instead of waiting for signs of clinical infection. Antifungal therapy should include topical, intracameral or intravitreal, and possibly systemic treatment. Choice of the specific agent should be guided by the organism isolated and the patient's medical history. After appropriate medical therapy is initiated, consideration can also be given to removal of the donor graft. In the case of a positive fungal donor rim culture in the absence of clinical signs of infection, we would advocate medical therapy along with close monitoring of the patient. However, if signs of infection develop, removal of the DSAEK graft should be strongly considered. Removal of the DSAEK graft allows for cultures of the interface to be obtained, may improve treatment by eliminating persistent fungi associated with the donor graft, and may improve access of antifungal compounds to the host cornea. In some cases, PK may be needed to eradicate infection. In 73% (11/15) of cases in Table 3, PK was performed or planned as part of the management of fungal keratitis indicating that repeat EK may not be successful in a large number of these cases. More prospective data are required to determine the optimal medical management, surgical management, or combination for treatment of fungal infection after DSAEK.

In summary, our series adds to the growing body of literature indicating that DSAEK may be more vulnerable to fungal infections, in particular *Candida* spp, than PK. Engagement of the appropriate public health jurisdiction is indicated when suspected clusters, outbreaks, or unusual occurrence of disease arise. Increased and improved surveillance for these events may prove very useful for future epidemiological investigations, guide clinical recommendations, and shape policy change at the national level. If additional studies confirm an increased risk of fungal infections in DSAEK, it may be important to develop protocols for screening donor tissues for fungal colonization and excluding these from use in lamellar surgery. Eye banks would play a crucial role in developing these protocols. While awaiting additional studies, clinicians should be aware that donor rim cultures positive for fungi may be more predictive of postoperative infection in patients undergoing corneal transplant than positive bacterial cultures and consider presumptively treating these patients with antifungal agents.

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