

EXTENDED REPORT

Survival of *Pseudomonas aeruginosa* in M-K preserved corneas

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Aim: To present seven eyes of suspected donor to host transmitted *Pseudomonas* sp corneal graft infection after corneal and scleral graft leading to corneal melting within 24 hours, in a span of 10 months.**Methods:** Case series. Seven eyes, operated for either penetrating or lamellar keratoplasty or scleral patch graft for different indications and which developed massive corneal/corneoscleral infection within 24 hours, were studied prospectively.**Results:** *Pseudomonas aeruginosa*, resistant to almost all antibiotics except polymyxin B in all and vancomycin in two, was identified as the causative organism from all the specimens obtained from the infected graft.**Conclusion:** Post-keratoplasty infection is a disaster. The source of early infection is invariably iatrogenic. Use of empirical antibiotics in the media is not always sufficient to prevent such infection. Thus, measures must be taken in the form of strict maintenance of asepsis and revision of antibiotics added to the storage medium. Further, early recognition and energetic therapy for such infection could reduce the ophthalmic morbidity.

Graft infection after keratoplasty is a well recognised but infrequent phenomenon.^{1–8} Among bacterial invasions, infection by Gram positive organisms is more frequently reported than that by Gram negative bacteria. Post-transplant *Pseudomonas* infection is a disaster. The source of early infection is usually iatrogenic and donor to host transmission of infection is infrequent.^{9–10} In this report we describe seven eyes with *Pseudomonas* graft infection probably transmitted from contaminated donor tissue and discuss the measures taken to control the infection. Further, the emphasis is given to minimise the possible source of infection.

MATERIALS AND METHODS

The details of seven eyes of seven patients are tabulated (table 1–3) and illustrated (figs 1–6).

DISCUSSION

Donor to host transmission of graft infection was established as early as early as 25 years ago.⁹ A review of the literature since then confirmed isolation of *Pseudomonas* from both the sources in four reports.^{5–8} Of four, two did not mention the sensitivity of gentamicin and the other two confirmed resistance to gentamicin, which is routinely used in the storage medium. The reported cases are concurrent with the latter two studies as organisms were resistant to gentamicin and other antibiotics except polymyxin B, commonly tested for sensitivity in our laboratory. Further, as the culture from the medium used for donor eyes in our study revealed growth of the organism in two, the routine culture specimen testing from the culture medium after corneal button removal in the operating theatre should be adhered to; as well as the first specimen—that is, from the untreated donor eye, a second specimen should be tested after treating the eyeball with broad spectrum antibiotics. Further, the samples collected from the donor eye should be inoculated immediately as the first and second samples from five of the donor eyes obtained in our series were sent for microbiological evaluation after a lapse of some hours. This may help in early diagnosis of graft infection and modulate postoperative therapy. Thus, it also

calls for addition of an extra broad spectrum antibiotic to the storage medium.

Factors responsible for the early graft infection as reported are

- (1) recurrence of host infection such as preoperative herpetic keratitis and/or other infective keratitis
- (2) intraoperative contamination
- (3) use of contaminated donor buttons.

In the present series the first and second factors are ruled out as there was no evidence of any clinical or subclinical preoperative infection and the subsequent surgeries contemplated in the same operating theatre by same surgical team did not reveal any abnormality. The third factor, use of contaminated donor buttons, is more likely to have had a role as all the samples from graft infection and three donor tissues showed the isolation of the same organism. Further, use of cooled donor endothelium on the recipient bed could be another factor.

The analysis of the data for the donor eye used for our patients revealed that eyes were enucleated in the mortuary and processed in the eye bank in a routine environment.

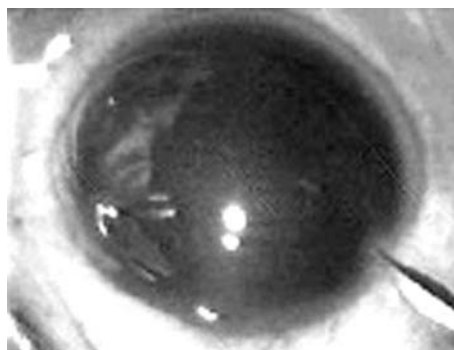


Figure 1 Intraoperative clinical photograph of congenital hereditary endothelial dystrophy showing microvitreal entry.

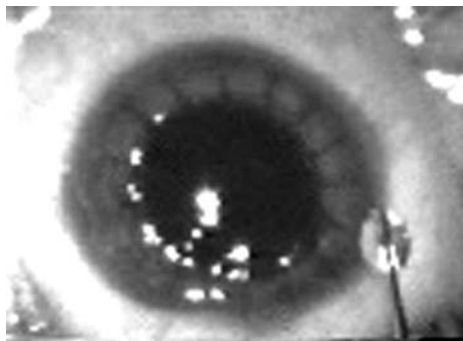


Figure 2 Clinical photograph after completion of keratoplasty.

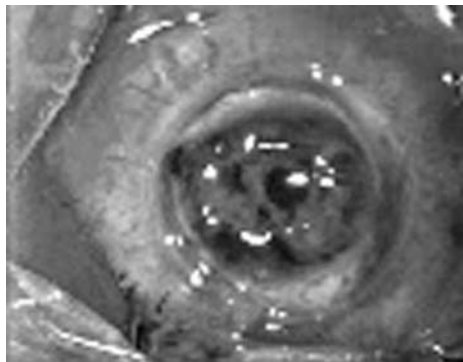


Figure 3 Clinical photograph showing sloughing of the graft and host cornea.

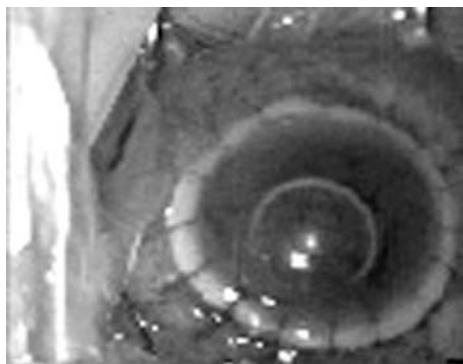


Figure 4 Clinical photograph at the completion of therapeutic keratoplasty for the sloughing cornea.



Figure 5 Clinical photograph on the 10th postoperative day after therapeutic keratoplasty showing an apparently quiet eye.

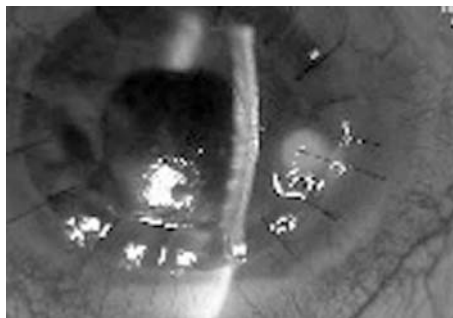


Figure 6 Clinical photograph of case 2 showing an ulcer at the graft-host junction with hypopyon.

Thus, the role of environmental contamination, aseptic surgical process, and endothelial side contamination cannot be ruled out.

Lack of a constant electric supply is also a major factor as the majority of the eye bank refrigerators do not have the provision of a constant electric supply, despite the emphasis given to this issue by the "eye bank standard."¹¹

Insler *et al* in 1985² reported the possibility of donor to host contamination following keratoplasty as:

- (1) a large potential inoculum due to (a) increased length of storage, (b) possibility of tissue culture fluid which encourages growth of micro-organisms
- (2) contamination of endothelial surface of the donor cornea
- (3) the emergence of more antibiotic resistant micro-organisms in antibiotic supplemented media.

To this we add another four factors which are also pertinent as a source of infection that includes:

- (1) the environment and quality of surgical preparation at the site of removal of donor corneal rim during processing
- (2) inconsistent electric supply for the refrigerator where the donor eyes were stored
- (3) immediate use of donor eye after removal from storage at 4°C, as warming the donor tissue to room temperature before use is not strictly adhered to by most surgeons
- (4) insufficient antibiotics in storage medium which is emphasised time and again in the literature but cannot be overemphasised. However, the aspect of "insufficient antibiotics" is not certain in this study, but it is high time to evaluate the storage media experimentally.

In the literature, two aspects have been emphasised for management of donor to host bacterial contamination: (1) parenteral antibiotics for 72 hours postoperatively until the culture report is available; and (2) routine culture of donor rim at the time of surgery.

The first factor appears to be impractical for all patients but the second factor is quite justified and should be adhered to routinely. On the basis of our data and data reported in literature, we think the presumptive factors as suggested by us are more meaningful for our set-up and therefore should be looked into.

Persistence of a white spot on a grafted cornea should arouse the suspicion of the post-keratoplasty infection and broad spectrum antibiotics should be started immediately after specimen collection for smear and culture sensitivity. However, occasionally this conventional therapy also fails to prevent/control graft infection as has happened here. Therefore, it is essential to consider antibiotic resistance

Table 1 Donor eye details

Eye No	Age/sex	Cause of death	ETD	Tissue retrieval and, grade of donor eye	Storage medium	Duration of tissue in MK before use	Duration of tissue at room temperature before use	Size of donor button	Culture report
1	30/M	Burn	4 hours	Enucleation, B+	M-K	27 hours	Immediate	7.5 mm	<i>P aeruginosa</i> , <i>Staph albus</i>
2	23 F	Hanging	10 hours	Enucleation, B-	M-K	19 hours	Immediate	8.25 mm	sterile
3	55/M	Cardiac arrest	3 hours	Enucleation, B+	M-K	10 hours	Immediate	8.0 mm	diphtheroid
4A	39/M	RTA	16 hours	Enucleation, B+	M-K	24 hours	Immediate	8.0 mm	sterile
4B	39/M	RTA	16 hours	Enucleation, B+	M-K	24 hours	1 hour	8.0 mm	diphtheroid
5A	75/M	Cardiac arrest	3 hours	In situ removal, B+	M-K	5 hours	Immediate	8.0 mm	No growth
5B	75/M	Cardiac arrest	3 hours	In situ removal, B+	M-K	6 hours	Immediate	8.0 mm	No growth

RTA, road traffic accident; ETD, estimated time of death.

Table 2 Recipient data (preoperative and intraoperative)

Series No	Age	Diagnosis	Date of surgery	Preoperative medication	Donor eye No	Type of surgery	Course of surgery	Type and No of suture	Subcutaneous injection	Culture from media in OT
1	5/F	CHED	28.6.03	0.3% ciprofloxacin four times daily	1	Optical PK (figs 1, 2)	Uneventful	10.0 MFN 16, interrupted	Dexamethasone and gentamicin	<i>Pseudomonas</i> sensitive to poly B
2	17/F	Keratoconus	30.8.03	0.3% ciprofloxacin four times daily	2	DLK	Uneventful	10.0 MFN 16, interrupted	Dexamethasone and gentamicin	Not sent
3	46/M	Leucomatous corneal opacity	7.11.03	0.3% ciprofloxacin four times daily	3	Optical PK	Uneventful	10.0 MFN 16, interrupted	Dexamethasone and gentamicin	Not sent
4	65/F	Fuchs' endothelial dystrophy	7.4.04	0.3% ciprofloxacin four times daily	4*	Triple procedure	Uneventful	10.0 MFN 16, interrupted	Dexamethasone and gentamicin	<i>Pseudomonas</i> sensitive to poly B
5	39/F	Post-ptyerygium surgery, scleral melting	7.4.04	0.3% ciprofloxacin four times daily	4*	Scleral patch graft	Uneventful	10.0 MFN interrupted 5, 8-0 Vicryl scleral suture 6	Dexamethasone and gentamicin	<i>Pseudomonas</i> sensitive to poly B
6	50/M	Leucomatous corneal opacity	21.4.04	0.3% ciprofloxacin four times daily	5A	Triple procedure	Uneventful	10-0 MFN 16 interrupted	Dexamethasone and gentamicin	Not sent
7	55/M	Leucomatous corneal opacity	21.4.04	0.3% ciprofloxacin four times daily	5B	Optical PK	Uneventful	10.0 MFN 16, interrupted	Dexamethasone and gentamicin	Not sent

CHED, congenital hereditary endothelial dystrophy; PK, penetrating keratoplasty; OT, operating theatre; poly B, polymyxin B.

and use of specific antibiotics against *Pseudomonas* spp such as polymyxin B, which should be added immediately to the treatment regimen if there is progressive graft infection despite appropriate medical therapy against Gram positive and Gram negative bacteria.

On the basis of present series a few important facts are drawn:

- (1) Though *Pseudomonas* infection in a graft is infrequent it is an emergency and requires immediate attention when it occurs.
- (2) Donor predisposing factors such as patients on a ventilator before death, prolonged death enucleation time, and use of compromised cornea may not always be responsible for immediate development of an ulcer as all the donor corneas were obtained from donors ranging in age from 23–75 years; none of them were on a ventilator, and the scheduled death enucleation time and removal

utilisation time were within normal limit in most of the eyes.

- (3) Strict asepsis of corneoscleral tissue removal in an eye bank is mandatory as all the infected donor corneas in the present series underwent corneoscleral tissue removal in a routine environment.
- (4) Graft infection does not necessarily require a recipient eye with ocular surface disorder and compromised cornea as none of the patients had ocular surface disorder.
- (5) Considering a large number of reports that are available on gentamicin resistant organisms and as gentamicin is the only antibiotic commonly used in M-K medium, antibiotic efficacy in M-K medium should be evaluated experimentally.
- (6) Finally, the aspect of warming the donor tissue to room temperature before use, which is not practised most of the time, should be adhered to.

Table 3 Recipient data (postoperative)

Series No	1st day, morning	1st day, evening	2nd day, morning	2nd day, evening	3rd day	4th day	Subsequent follow up	At healing
1	Uneventful, 4-5 suture abscess. Betadine, cleaning, routine medication GC-4 + VA, 3/60	Intense pain, lid oedema, profuse mucopurulent discharge, more suture abscess. Cleaning, Betadine, cautery	Worsen, hazy cornea. Standard corneal ulcer therapy	Total graft melting. Therapeutic PK planned. Poly B drop 50 000 IU ½ hourly, neosporin ointment 3 times daily	Total therapeutic PK (figs 3, 4). Intracamer VM Slough sent for microbiology	Pseudomonas growth from graft ulcer specimen, sensitive to poly B. Resistant to all tested antibiotics. Standard therapy tapered poly B drop 50 000 IU ½ hourly Neosporin ointment 3 times daily	Pseudomonas growth from infected button, sensitive to poly B. Resistant to all tested antibiotics. Therapy continued, gradual remission (fig 5)	Visual acuity counting fingers close to face. Leucomatous corneal opacity
2	Uneventful, 4-5 suture abscess. Betadine cleaning, routine medication GC-4 + VA-6/24P	Intense pain, lid oedema, profuse mucopurulent discharge, 2 mm graft ulcer, hypopyon (fig 6). Cleaning, sample for microbiology, Betadine, cautery, standard corneal ulcer therapy	Total graft hazy, poly B drop 50 000 IU ½ hourly neosporin ointment 3 times daily added	Graft melting. Therapy continued	Graft removal, VM wash, slough for microbiology. Standard therapy tapered, poly B drop and neosporin ointment continued	Pseudomonas growth from graft ulcer specimen, sensitive to poly B. Resistant to all tested antibiotics, poly B drop and neosporin ointment continued	Gradual remission	Visual acuity 1/60. Leucomatous corneal opacity
3	Intense pain, lid oedema, profuse mucopurulent discharge, multiple suture abscess. Cleaning, sample for microbiology, Betadine, cautery, routine post-keratoplasty therapy	Worsen, hazy cornea, hypopyon + standard corneal ulcer therapy, poly B drop 50 000 IU ½ hourly, neosporin ointment 3 times daily	Status quo	Status quo. Therapy continued	Partial keratectomy, Betadine cautery	Pseudomonas growth from graft ulcer specimen, sensitive to poly B. Resistant to all tested antibiotics. Standard therapy tapered poly B drop Neosporin ointment continued	Gradual remission	Visual acuity, defective PR. Leucomatous corneal opacity
4	Intense pain, 4-5 suture abscess. Betadine, cleaning, routine medication VA-6/12	Intense pain, lid oedema, profuse mucopurulent discharge, more suture abscess. Cleaning, sample for microbiology, Betadine cautery. Standard therapy for corneal ulcer	Hypopyon reduced	Status quo	Graft melting, PR inaccurate	Pseudomonas growth from graft ulcer specimen, sensitive to poly B. Resistant to all tested antibiotics. Standard therapy tapered poly B drop Neosporin ointment continued	Gradual remission	Visual acuity hand movement close to face. Leucomatous corneal opacity
5	Uneventful, 4-5 suture abscess. Betadine, cleaning, routine medication VA-6/12	Intense pain, lid oedema, profuse mucopurulent discharge, more suture abscess. Cleaning, sample for microbiology, Betadine cautery	Worsen, hazy cornea. Standard corneal ulcer therapy poly B drop 50 000 IU ½ hourly Neosporin ointment 3 times daily	Graft removal, VM wash, slough for microbiology. Standard therapy tapered, poly B drop 50 000 IU ½ hourly, neosporin ointment 3 times daily	Remission of pain, lid oedema, discharge	Pseudomonas growth from graft ulcer specimen, sensitive to poly B. Resistant to all tested antibiotics. Standard therapy tapered poly B drop 50 000 IU ½ hourly Neosporin ointment 3 times daily	Gradual remission	Visual acuity 6/9. Clear cornea

Table 3 Continued

Series No	1st day, morning	1st day, evening	2nd day, morning	2nd day, evening	3rd day	4th day	Subsequent follow up	At healing
6	Multiple suture abscess. Betadine cleaning, routine medication GC-2+VA-1/60 KP ++cells in AC+	Intense pain, lid oedema, profuse mucopurulent discharge, more suture abscess. Cleaning, sample for microbiology, Betadine cautery	Worsen, hazy cornea. Standard corneal ulcer therapy poly B drop 50 000 IU ½ hourly Neosporin ointment 3 times daily		KP + cells in AC occasional	Pseudomonas growth from graft ulcer specimen, sensitive to poly B. Resistant to all tested antibiotics. Standard therapy tapered poly B drop 50 000 IU ½ hourly Neosporin ointment 3 times daily	Gradual remission	Visual acuity 1/60. Leucomatous corneal opacity
7	Multiple suture abscess. Betadine, cleaning, routine medication GC-2 + VA-1/60	Intense pain, lid oedema, profuse mucopurulent discharge, more suture abscess. Cleaning, sample for microbiology, Betadine cautery	Worsen, hazy cornea. Standard corneal ulcer therapy poly B drop 50 000 IU ½ hourly Neosporin ointment 3 times daily		Graft melting, therapeutic PK	Pseudomonas growth from graft ulcer specimen, sensitive to poly B. Resistant to all tested antibiotics. Standard therapy tapered poly B drop 50 000 IU ½ hourly Neosporin ointment 3 times daily	Pseudomonas growth from infected button, sensitive to poly B. Resistant to all tested antibiotics. Therapy continued. Eye became phthisical	Visual acuity nil (phthisis bulbi)

VA, visual acuity; GC, gentamicin; VM, vancomycin; KP, keratoprecipitates; AC, anterior chamber; PK, penetrating keratoplasty; poly B, polymyxin B.

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