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Pseudomonas aeruginosa Endophthalmitis after Penetrating Keratoplasty Transmitted from the Same Donor to Two Recipients Confirmed by Pulsed-Field Gel Electrophoresis[▽]

Ana Paula Miyagusko Taba Oguido,[1]* Antonio Marcelo Barbante Casella,[1] Ana Luisa Hofling-Lima,[2,3] Sergio Arruda Pacheco,[1] Paulo José Martins Bispo,[2] and Fernanda Marques[2]

Department of Ophthalmology of the Universidade Estadual de Londrina, Londrina, Brazil[1]; Laboratório Especial de Microbiologia Clínica of the Universidade Federal de São Paulo-UNIFESP, São Paulo, Brazil[2]; and Department of Ophthalmology of the Universidade Federal de São Paulo, São Paulo, Brazil[3]

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Two devastating cases of multidrug-resistant *Pseudomonas aeruginosa* endophthalmitis after keratoplasty as the result of transmission from the same donor were confirmed by pulsed-field gel electrophoresis. Strategies for preventing donor-to-host transmission, such as the use of antimicrobial agents of greater efficacy and better methods for detecting microorganisms in preservation medium, could minimize this type of transmission.

In this paper, we describe two cases of early multidrug-resistant (MDR) *Pseudomonas aeruginosa* endophthalmitis after penetrating keratoplasty as the result of transmission from the same donor. The cornea donor was a 38-year-old patient who died of cirrhosis 1 day before the surgeries. Donated corneas were collected with a death-to-preservation time of 2 h 45 min and were immersed in the preservative medium Optisol-GS (Bausch + Lomb, Irvine, CA). The donor was treated under conditions that included mechanical ventilation and was not septic. Before his death, previously collected blood and urine for cultures gave negative results. The recipients were (i) a 76-year-old man presenting with bullous keratopathy who underwent an uneventful penetrating keratoplasty on his right eye in August 2009, and (ii) a 14-year-old male with keratoconus who underwent an uneventful penetrating keratoplasty on the right eye in August, 2009. On the first postoperative day, both recipients showed signs of infection in the donor corneal button. For both patients, treatment consisting of intravenous therapy with ceftazidime (2 g three times a day) and levofloxacin (400 mg once a day) and topical therapy with moxifloxacin (0.5%), cefazolin (50 mg/ml), and gentamicin (14 mg/ml) administered every hour following an initial loading dose of 1 to 2 drops every 5 min for the first 30 min was initiated on the first postoperative day. Subconjunctival injection of ceftazidime (200 mg) and vancomycin (20 mg) was performed. On the second postoperative day, intravenous therapy with imipenen (400 mg four times a day) replaced the ceftazidime therapy. Intravitreous injection of ceftazidime (10 mg/0.5 ml) and vancomycin (1 mg/1 ml) was also performed. Therapy using cefazolin eye drops was replaced by administration of imipenem (50 mg/ml). Despite prompt initiation of massive antimicrobial therapy, *P. aeruginosa* endophthalmitis was devastating to the infected eyes. In recipient 1, on the third postoperative day, visual acuity consisted of no light perception, leading to unavoidable evisceration of this eye; in recipient 2, a second keratoplasty and a vitrectomy were performed, and by postoperative day 20, visual acuity consisted of no light perception, leading to eye atrophy.

The corneoscleral rim tissues were sent to the laboratory and cultured in Sabouraud medium (kept at room temperature for 30 days) and in thioglycolate medium (kept in an incubator at 37°C for 24 h) as part of our standard immediate post-corneal-transplant procedure. Afterward, Gram staining was done on the thioglycolate culture. In the presence of Gram-negative rods (grown also in MacConkey medium), *P. aeruginosa* was identified. The susceptibility profile was determined by the Kirby-Bauer disk-diffusion method. Both isolates were resistant to aminoglycosides, cephalosporins, fluoroquinolones, (extended-spectrum) penicillin, and monobactam and susceptible only to carbapenems and polymyxins. Multidrug-resistant *P. aeruginosa* was also isolated from the corneal scrapings of the recipients. Bacterial isolates recovered from both the corneoscleral rim and infected grafted donor corneas were sent to the Laboratório Especial de Microbiologia Clínica of the Universidade Federal de São Paulo for further molecular characterization. Pulsed-field gel electrophoresis (PFGE) was performed using the CHEF-DR II system (Bio-Rad, Richmond, CA) with 0.5× TBE (50 mM Tris, 50 mM boric acid, 0.5 mM EDTA) and 1% agarose at 13°C and 200 V for 24 h, with a switch time of 5 to 90 s, as previously described by Pfaller et al. (7). Digestion of total DNA was achieved using SpeI. The PFGE patterns were analyzed using BioNumerics analysis software (Applied Maths, Kortrijk, Belgium), which revealed identical macrorestriction patterns obtained from the donor corneas in all samples, proving that the donor was the source of both recipient infections, as shown in the dendrogram in Fig. 1.

Donor-to-host transmission of *P. aeruginosa* is uncommon (6), and transmission of MDR *P. aeruginosa* to two recipients

* Corresponding author. Mailing address: Department of Ophthalmology, Universidade Estadual de Londrina, Robert Kock, 60, 86010 100, Londrina, Parana, Brazil. Phone and fax: 55 43 33217398. E-mail: aoguido@gmail.com.

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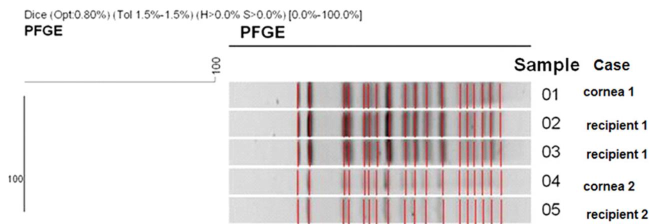

FIG. 1. Dendrogram (UPGMA [unweighted-pair group method using average linkages]) produced using BioNumerics software, showing distances calculated with the Dice similarity index of PFGE SpeI macrorestriction patterns for five strains isolated from the donor corneoscleral rim (cornea 1 and 2) and recipient ($n = 3$) corneal cultures. The similarity of the strains was 100%.

from a single donor after penetrating keratoplasty has not been previously described. The increasing frequency of infection by MDR *P. aeruginosa* strains is alarming, since effective antimicrobial options are severely limited (5). Although donor-to-host transmission has been associated with cancer as a cause of long hospitalization time, mechanical ventilation, and death (3, 4, 6), culturing of this environment is not routinely done. Contamination during corneal harvesting, preparation, and preservation is unlikely, given that different harvesting sets were used and that strict sterile conditions were maintained for preparation (1, 3). Despite the aseptic precautions, the role of environmental contamination cannot be ruled out. Also, contamination during keratoplasty seems unlikely, because it was performed in different operating rooms with another set of instruments, other assistants, and a different surgeon. The solution of Optisol GS (Bausch + Lomb, Irvine, CA) contains 100 μg/ml gentamicin sulfate and 200 μg/ml streptomycin sulfate. The possibility of adding antibiotics with a broader spectrum of activity, such as vancomycin, fluoroquinolones, and penicillin, has been evaluated, although such therapy could be ineffective against MDR pathogens (2, 8). Studies aiming to evaluate the effects of antibiotics from the carbapenem or polymyxin class in preservation medium would be interesting and may represent an alternative method for treatment of multidrug-resistant *P. aeruginosa*. The use of PFGE typing, as well as the routine culture of the corneoscleral rim, was important for confirming that the source of infection was the donor button in these cases. Besides all normal preventive measures, the judicious screening of donors, the use of antimicrobial agents of greater efficacy in preservation media, and the employment of better means of microorganism detection in preservation media before transplantation such as PCR using species-specific primers to identify potential posttransplantation keratitis pathogens, could minimize this type of transmission.

REFERENCES

1. **Builles, N., et al.** 2006. Reducing contamination when removing and storing corneas: a multidisciplinary, transversal, and environmental approach. Cornea **25:**185–192.
2. **Cameron, J. A., S. R. Antonios, J. B. Cotter, and N. R. Habash.** 1991. Endophthalmitis from contaminated donor corneas following penetrating keratoplasty. Arch. Ophthalmol. **109:**54–59.
3. **Hassan, S. S., et al.** 2008. Infectious disease risk factors of corneal graft donors. Arch. Ophthalmol. **126:**235–239.
4. **Karjalainen, K., and A. Vannas.** 1984. Bacterial contamination of donor corneas. Ophthalmic Surg. **15:**770–772.
5. **Obritsch, M. D., D. N. Fish, R. MacLaren, and R. Jung.** 2005. Nosocomial infections due to multidrug-resistant *Pseudomonas aeruginosa*: epidemiology and treatment options. Pharmacotherapy **25:**1353–1364.
6. **Panda, A., G. Satpathy, and H. S. Sethi.** 2005. Survival of Pseudomonas aeruginosa in M-K preserved corneas. Br. J. Ophthalmol. **89:**679–683.
7. **Pfaller, M. A., R. J. Hollis, and H. S. Sader.** 1992. Chromosomal restriction fragment analysis by pulsed-field gel electrophoresis, p. 105. *In* H. D. Isenberg (ed.) Clinical microbiology procedures handbook. ASM Press, Washington, DC.
8. **Ritterband, D. C., et al.** 2006. Efficacy and safety of moxifloxacin as an additive in Optisol-GS a preservation medium for corneal donor tissue. Cornea **25:**1084–1089.