Prevalence of bacteria and fungi in samples of cornea preservation fluid

Grażyna Broniek[1], Ewa Langwińska-Wośko[1,2], Małgorzata Sybilska[1], Jerzy Szaflik[1], Jacek P. Szaflik[1,2], Marta Wróblewska[3,4]

[1]SPKSO Ophthalmic University Hospital, Medical University of Warsaw, Warsaw, Poland
[2]Department of Ophthalmology, Medical University of Warsaw, Warsaw, Poland
[3]Department of Dental Microbiology, Medical University of Warsaw, Warsaw, Poland
[4]Department of Microbiology, Central Clinical Hospital, Warsaw, Poland

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Corresponding author:
Marta Wróblewska MD, PhD
Department
of Dental Microbiology
Medical University
of Warsaw
1a Banacha St
02-097 Warsaw, Poland
Phone: +48 22 599 17 77
Fax: +48 22 599 17 78
E-mail: mwroblewska@
wum.edu.pl

Abstract

Introduction: Recipients of corneal transplants are at risk of healthcare-associated infections, which, apart from other causes of surgical site infections, may also occur as a result of the transfer of infected corneal tissue. In this study we assessed the risk of bacterial and fungal infections based on the results of routine microbiological testing of cornea preservation fluid samples.

Material and methods: We examined a total of 725 samples of corneal preservation fluid, obtained during a period of 3 years (2011–2013). Corneal preservation fluid samples were cultured and identified in accordance with standard microbiological methods.

Results: The analysis comprised 725 samples of corneal preservation fluid, of which 32 (4.4%) samples tested positively in microbiological cultures. In total, 34 strains of bacteria and fungi were cultured. Gram-positive bacteria, Gram-negative bacteria and fungi comprised 85.3%, 8.8% and 5.9% of these strains, respectively. Analysis of the susceptibility of the cultured bacterial isolates to gentamicin was also performed, as this antibiotic is present in the composition of corneal preservation fluid. Among the cultured bacterial strains, 10 (33.3%) were resistant to gentamicin. None of the 32 patients who received a cornea stored in preservation fluid contaminated with bacteria and/or fungi demonstrated infectious complications in the surgical site within 1 year following cornea transplantation.

Conclusions: We postulate that perioperative antibiotic prophylaxis in cornea transplant recipients is important in preventing bacterial infections derived from the donor cornea. We believe that the addition of an antifungal agent to commercially available cornea preservation fluids should also be considered.

Key words: cornea transplantation, healthcare-associated infections, antibiotics, cornea preservation media, bacteria, fungi.

Introduction

Recipients of corneal transplants are at risk of healthcare-associated infectious complications from these procedures [1]. The frequency with which postoperative endophthalmitis occurs has remained unchanged over the past 20 years. Intraocular procedures are affected by infectious

complications in around 0.08% of cases, which is relatively low when compared to procedures in other medical fields. This prevalence in ophthalmology depends on the specific surgical procedures and remains at between 0.08% and 0.77% for penetrating keratoplasty (PKP) [2]. Acute cases of postoperative intraocular infections may occur within 6 weeks following the procedure, usually developing within the first few days. Apart from standard causes of infections which apply to all ophthalmological procedures, corneal transplants are also at risk of infection following the transfer of infected corneal tissue [3, 4]. In this study we assessed the risk of developing bacterial and fungal infections based on the results of routine microbiological testing of corneal preservation fluid samples.

Material and methods

The material comprised samples of corneal preservation fluid (Eusol C, Alchimia, Padova, Italy) routinely obtained during consecutive procedures of cornea transplantation, performed at the SPKSO Ophthalmic University Hospital (Medical University of Warsaw, Poland) in the period 2011–2013. Samples were taken during surgery, immediately before transplantation of the cornea, while maintaining all aseptic precautions.

In total, 725 cornea transplant procedures were performed in this 3-year period (2011–2013). Therefore, 725 cornea preservation fluid samples were aseptically obtained during surgery and sent for bacteriological and mycological culture testing.

Samples of the corneal preservation fluid were cultured according to standard microbiological methods. Bacteriological cultures were grown on blood agar, Columbia agar and MacConkey agar, while for mycological cultures, Sabouraud's medium was used. Inoculated plates were incubated at 35°C for 48 h, while mycological cultures were incubated at 30°C and 35°C and observed for 7 days.

Identification and susceptibility testing of the cultured isolates was done using the automated VITEK 2 Compact system (bioMerieux). Additionally, special tests were performed for detection of the following resistance mechanisms: methicillin susceptibility of staphylococci, macrolides, lincosamides, streptogramins B (MLS_B) resistance pattern of Gram-positive cocci, and detection of enzyme production by Gram-negative rods – extended spectrum β-lactamase (ESBL), metallo-β-lactamase (MBL) and *Klebsiella pneumoniae* carbapenemase (KPC). Testing was done according to the recommendations of the Polish National Reference Centre for Susceptibility of Microorganisms (KORLD) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [5].

Methicillin susceptibility of the *Staphylococcus* spp. strains was tested using a disk diffusion technique with a cefoxitin (30 µg) disk. Detection of MLS_B resistance mechanism of Gram-positive cocci was performed with a disk diffusion technique, using erythromycin (15 µg) and clindamycin (2 µg) disks. High-level aminoglycoside resistance (HLAR) in enterococci was assessed using gentamicin (30 µg) and streptomycin (300 µg) disks. Detection of ESBL, MBL and KPC enzyme production by cultured Gram-negative rods was done using a double disk diffusion technique (using clavulanic acid (10 µg), 10 µl of 0.5M EDTA and boronic acid (300 µg) as inhibitors of ESBL, MBL and KPC enzymes, respectively).

Isolates of the *Candida* spp. yeast-like fungi were cultured on Sabouraud's agar, and thereafter identified using the automated VITEK 2 Compact (bioMerieux) system. Susceptibility testing of these strains was done using the automated VITEK 2 Compact (bioMerieux) system and commercially available kits based on the microdilution technique – Fungitest (Bio-Rad) and Integral System Yeast Plus (Liofilchem). Moulds of the genus *Aspergillus* were cultured on Sabouraud's agar, incubated at 30°C for 7 days and identified by morphology of the colonies and microscopy.

Medical records of the recipients of the cornea transplants for whom corneal preservation fluid samples obtained during surgery tested positively for bacteria or fungi were analyzed regarding their risk of healthcare-associated infections within at least 1 year after transplantation.

Results

The analysis comprised 725 samples of corneal preservation fluid, obtained in the period 2011–2013, out of which 32 (4.4%) samples tested positively in microbiological cultures. In total, 34 strains of bacteria and fungi were cultured. The results of the microbiological cultures of the samples are shown in Table I.

Among the 34 strains of cultured microorganisms, Gram-positive bacteria comprised 85.3%, Gram-negative bacteria 8.8%, and fungi 5.9% (Table II).

Staphylococci were dominant among the Gram-positive bacteria (18/29 (62.1%) isolates), including coagulase-negative species (14/18 (77.8%)) and *Staphylococcus aureus* (4/18 (22.2%)). Resistance to methicillin was detected in 6/14 (42.9%) isolates of the coagulase-negative staphylococci (MRCNS) and 2/4 (50.0%) of the *S. aureus* (MRSA) strains. The mechanism of resistance MLS_B was detected in 5 of these strains – 4 strains of *Staphylococcus epidermidis* and 1 strain of *Staphylococcus hominis*. Enterococci constituted 8/29 (27.6%) strains of the cultured Gram-positive bacteria. Among these strains, only 1 isolate (*Enterococcus*

Table I. Results of microbiological culture of cornea preservation fluid samples in the period 2011–2013 (n = 725)

Year	Cornea preservation fluid		Total	Percentage of positive culture results (%)
	Number of negative culture results	Number of positive culture results		
2011	266	18	284	18/284 (6.3)
2012	231	8	239	8/239 (3.4)
2013	196	6	202	6/202 (3.0)
Total	693	32	725	32/725 (4.4)

Table II. Bacteria and fungi isolated from samples of cornea preservation fluid (n = 32)

Bacteria/fungi	2011	2012	2013	Total	Percentage
Staphylococcus epidermidis	8		1	9	26.5
Enterococcus faecalis	4	3		7	20.5
Staphylococcus aureus	1	3		4	11.6
Staphylococcus hominis			2	2	5.8
Staphylococcus salivarius			2	2	5.8
Staphylococcus warneri	1			1	3.0
Streptococcus agalactiae	1			1	3.0
Enterococcus avium		1		1	3.0
Corynebacterium spp.	1			1	3.0
Clostridium perfringens		1		1	3.0
Enterobacter cloacae	1			1	3.0
Cronobacter sakazakii			1	1	3.0
Brevundimonas diminuta	1			1	3.0
Candida albicans		1	1	2	5.8
Total	18	9*	7**	34	100.0

**One sample – mixed flora isolated: Clostridium perfringens and Enterococcus avium; **one sample – mixed flora isolated: Staphylococcus hominis and Candida albicans.*

avium) did not show high-level resistance to aminoglycosides. Among the 7 strains of *Enterococcus faecalis*, 5 were HLAR strains and 2 were HLSR (high level streptomycin resistance) strains.

The following Gram-negative rods were cultured from the tested samples (1 isolate of each): *Enterobacter cloacae*, *Cronobacter sakazakii* (previous name *Enterobacter sakazakii*) and *Brevundimonas diminuta* (previous name *Pseudomonas diminuta*), susceptible to tested antimicrobials.

Two strains of yeast-like fungi (both *Candida albicans*) were cultured, both susceptible to fluconazole. Two samples revealed mixed cultures – *Clostridium perfringens* and *Enterococcus avium*, *Staphylococcus hominis* and *Candida albicans*.

Analysis of the susceptibility of cultured bacterial isolates to gentamicin was performed due to the presence of this antibiotic in the composition of corneal preservation fluid (Eusol C). The results are shown in Table III. Susceptibility to gentamicin was not tested for 2 cultured bacterial strains (*Streptococcus agalactiae* and *Clostridium perfringens*) for which the testing of gentamicin susceptibility is not justified therapeutically. Among the cultured bacterial strains, 20 (66.7%) were susceptible to gentamicin (including 2 strains of *Enterococcus faecalis* HLSR), and 10 (33.3%) were resistant to gentamicin.

The mean time period from the death of the donor to procurement of the cornea, in the cases with positive results for microbiological cultures of the corneal preservation fluid samples, was 9 h 7 min, with a very wide range (from 15 min to 18 h 20 min).

None of the 32 patients who received a cornea stored in preservation fluid contaminated with bacteria and/or fungi experienced infectious complications in the surgical site within 1 year after cornea transplantation.

Table III. Susceptibility to gentamicin of bacteria isolated from samples of cornea preservation fluid (n = 30)

Bacteria/fungi	Gentamicin	
	Susceptibility	Resistance
Staphylococcus aureus MSSA	2	0
Staphylococcus aureus MRSA	2	0
Staphylococcus epidermidis MSCNS	3	0
Staphylococcus epidermidis MSCNS, MLSB	1	0
Staphylococcus epidermidis MRCNS	1	1
Staphylococcus epidermidis MRCNS, MLS_B	1	2
Staphylococcus hominis MSCNS	1	0
Staphylococcus hominis MRCNS, MLS_B	0	1
Staphylococcus salivarius MSCNS	2	0
Staphylococcus warneri MSCNS	1	0
Enterococcus avium	1	0
Enterococcus faecalis HLAR	0	5
Enterococcus faecalis HLSR	2	0
Corynebacterium spp.	1	0
Enterobacter cloacae	0	1
Cronobacter sakazakii	1	0
Brevundimonas diminuta	1	0
Total	20	10

Discussion

Cornea transplantation entails the problem of cornea storage by guaranteeing viability and consistency of the corneal epithelium and at the same time minimizing the risk of bacterial or fungal contamination. The first reported cornea transplantation was performed in 1906; however, the development of eye tissue banks only became possible in the 1950s when antibiotics became available [6]. During the 1960s, corneas destined for transplantation were stored in so-called humid chambers for a period no longer than 2 days. Only 10 years later were cornea storage methods that are currently employed implemented – storage in a liquid medium kept at fridge temperature [6]. Low temperatures and the addition of an antibiotic (gentamicin ± streptomycin) allow for the storage of corneas for up to 2 weeks, from the moment of their procurement from a donor to their transplantation to the recipient.

Cornea preservation fluids contain many nutrients, such as glucose, amino acids, mineral salts and vitamins, which protect corneal cells. However, such a rich composition of these fluids also favors their contamination by bacteria and/or fungi. In recent years, multidrug-resistant (MDR) strains of microorganisms have been increasingly isolated from both hospitalized patients, as well as patients with no history of hospital stay. This means that among the bacteria contaminating cornea preservation fluids, there might be strains resistant to gentamicin, which is added to these compounds (e.g. Eusol C – 0.1 mg/ml). Baer *et al*. showed that the addition of gentamicin into corneal preservation fluid might not be an effective method for inhibition of the replication of *Streptococcus* viridans [7]. They described 3 cases of endophthalmitis in patients who received corneas stored in fluid contaminated with such bacteria, despite gentamicin being added to the storage medium [7]. Similarly, Fong *et al*. noticed that despite the fact that the addition of gentamicin caused a drop in the frequency of contamination of corneal preservation fluids from 43% to 13%, it did not eliminate the contamination of these media with staphylococci, streptococci and fungi [8].

Amongst bacteria, the most common causes of infectious complications following cornea transplantation are Gram-positive cocci (staphylococci, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, *Streptococcus* viridans and enterococci) [8–11].

In our study, 32/725 (4.4%) of the corneal preservation fluid (Eusol C) samples tested positively for bacteriological or mycological cultures. During the period of 3 years we gradually observed a decrease in this occurrence – 6.3% of positive culture results were found in 2011, 3.4% in 2012 and 3.0% in 2013. No possible cause for this difference could be identified however. Overall, Gram-positive bacteria comprised over 85% of the isolated strains, while Gram-negative bacteria comprised almost 9% and fungi close to 6%. This means that gentamicin was not effective in the inhibition of bacterial replication in these samples during cornea storage, which is in agreement with other reports in the literature [7, 8]. Contamination of these samples during their sampling for bacteriological tests is highly unlikely, as this was performed in the operating theatre during surgery, with adherence to all aseptic precautions. Among those isolates 14/34 (41.2%) showed natural or acquired resistance to gentamicin (e.g. *Strepto-*

coccus agalactiae, *Clostridium perfringens*, *Candida albicans*), while staphylococci and enterococci predominated among the other strains against which aminoglycoside is usually not effective in monotherapy. Strains of enterococci were relatively numerous in our study, comprising 8/34 (23.5%) of all isolates from the corneal preservation fluid samples. It should be emphasized that 5 of these were HLAR strains, resistant to the gentamicin concentration in the Eusol C fluid. This emphasizes the role of antibiotic prophylaxis in cornea transplantation surgery, to minimize the risk of surgical site infections in cornea recipients.

According to the literature, the Gram-negative rods that are most commonly isolated from donor corneas are *Pseudomonas aeruginosa*, *Enterobacter* spp. and *Serratia marcescens* [12–14]. Morel *et al*. described the contamination of 28 corneas (an additional 70 were discarded) because of the contamination of trypan blue solution with a strain of *Burkholderia cepacia* in the eye tissue bank [15].

In our study Gram-negative rods comprised 3 isolates (8.8%). Two strains of *Enterobacter* spp. were cultured – *E. sakazakii* (present name: *Cronobacter sakazakii*) and *E. cloacae* (a strain resistant to gentamicin), as well as 1 strain of *Pseudomonas diminuta* (present name: *Brevundimonas diminuta*). No infectious complications were observed in the recipients of the corneas stored in these fluids.

Other bacteria, which are relatively rarely isolated from donors' corneas, are *Corynebacterium* spp., *Bacillus* spp. and *Neisseria* spp. [14]. In our study a strain of *Corynebacterium* spp. was isolated from 1 sample.

According to the literature, fungal keratitis of transplanted corneas is usually caused by fungi of the genus *Candida* (*C. albicans* and *C. glabrata*), and more rarely by moulds of *Aspergillus* spp. [9, 16, 17]. There are reports of keratitis or endophthalmitis caused by documented transmission of *C. albicans* from a donor to a recipient [8, 18, 19].

Frequency of post-transplantation keratitis caused by yeast-like fungi ranges from 0.6% to as high as 25.0% [20–22]. Ritterband *et al*. demonstrated the presence of fungi in cultures of 7/533 (1.3%) cornea rims [22]. The authors also examined the usefulness of adding voriconazole (0.1 mg/ml) to the corneal preservation fluid. Cultures of the samples of corneal preservation fluid containing this drug were negative, with no observed toxicity for epithelial cells of the corneas stored in these fluids. In our study, 2 strains of *C. albicans* were cultured, comprising 5.9% of the isolates from all culture-positive samples of corneal preservation fluid during the 3-year period. This means that in the analyzed material fungi were rare contaminants of corneal preservation fluid.

It should be noted that apart from bacteria and fungi, other microorganisms may cause postoperative complications, e.g. *Demodex* mites are more prevalent in immunocompromised patients than in healthy persons, and they may be linked to keratitis, but their role in cornea transplant recipients is unknown [23].

Human corneal epithelium contains different populations of antigen-presenting cells (APC), including dendritic cells (DC), which mediate corneal immunity [24]. Infectious agents, including bacteria and fungi, may cause their apoptosis, which in turn will impair their presentation of foreign antigens [25]. This pathomechanism may further contribute to immunosuppression and increased susceptibility of the transplanted cornea to secondary infections [25].

Hassan *et al*. found that in 121 cases of endophthalmitis present in cornea transplant recipients, where cultures were available for both the donor and the recipient, 59 (48.8%) of the cases had identical isolated strains [9]. These results indicate that routine cultures of corneal preservation fluid may be useful in the choice of empiric therapy for patients with infectious complications following cornea transplantation. However, in our study, none of the 32 patients who received a cornea stored in culture-positive contaminated fluid developed any symptoms of surgical site infection during the 1-year observation period. This may be the result of the effectiveness of antibiotic prophylaxis administered locally in these patients. Hopefully, novel techniques for cornea preservation will further decrease the risk of infectious complications in cornea recipients.

In conclusion, we postulate that perioperative antibiotic prophylaxis in cornea transplant recipients is important for the prevention of bacterial infections derived from the donor cornea. However, it was surprising that as many as 33.3% of the isolates are gentamicin-resistant bacterial strains, since corneal preservation fluids contain this antibiotic. This finding may be of clinical importance. Furthermore, we believe that the addition of an antifungal agent in the commercially available corneal preservation fluids should be considered.

Conflict of interest

The authors declare no conflict of interest.

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