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Detection of Methicillin-Resistant *Staphylococcus Aureus* in Donor Eye Preservation Media by Polymerase Chain Reaction

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Abstract: To examine contamination of donor eye preservation media with methicillin-resistant *Staphylococcus aureus* (MRSA), we studied the media to detect specific genes for MRSA by use of the polymerase chain reaction (PCR) method. The materials were 36 samples of donor eye preservation media (EP-II) in which donor eyes had been stored for keratoplasty. Polymerase chain reaction procedures were carried out to simultaneously detect the *spa* gene, which codes protein A of *S. aureus*, and the *mec*A gene, which codes penicillin-binding protein-2′ contributing resistance to methicillin. Along with PCR analyses, the preservation media was examined by conventional culture methods to determine bacteriologic contamination. The PCR analyses of the 36 samples revealed that both the *spa* and the *mec*A genes were positive in five samples, only the *spa* gene was positive in two, and only the *mec*A gene was positive in two. The conventional culture of the media showed positive for MRSA in 5 samples, methicillin-susceptible *S. aureus* (MSSA) in 2, methicillin-susceptible coagulase-negative staphylococci (MS-CNS) in 4, and methicillin-resistant coagulase-negative staphylococci (MR-CNS) in 2 of the 36 samples. The results of PCR coincided well with those of conventional bacteriologic culture. Polymerase chain reaction analysis for *spa* and *mecA* genes is useful in detecting contamination of donor eye preservation media by MRSA, MSSA, MR-CNS, or MS-CNS in a shorter time than by conventional culture. **Jpn J Ophthalmol 1998;42:352–356** © 1998 Japanese Opthalmological Society

Key Words: Keratoplasty, *mec*A, methicillin-resistant Staphylococcus aureus, polymerase chain reaction, preservation medium, *spa*.

Introduction

The incidence of endophthalmitis after intraocular surgery has been reduced in recent years because of better aseptic techniques, increased understanding of the causes of infection, improved microsurgical techniques, and the use of prophylactic broad-spectrum antibiotics.[1] Although the reported incidence of corneal ulcers or endophthalmitis after keratoplasty has been relatively rare, once it occurred it was often refractory and the prognosis of grafts and visual function was poor.[2,3] Of various postoperative bacterial infections, those caused by methicillin-resistant *Staphylococcus aureus* (MRSA) have increased in recent years. Postoperative MRSA infection is considered difficult to treat and the prognosis is poor.

It has been reported that early postoperative endophthalmitis following penetrating keratoplasty is often related to contamination of donor tissues.[4,5] As donor corneal tissues cannot be disinfected completely, it is critical to detect contamination of the donor tissues before surgery. To prevent postoperative infection through corneal transplantation, serologic examination of donor blood and conventional bacteriologic culture of the donor cornea and/or its preservation media should be part of routine preoperative examinations. However, MRSA may sometimes be hidden by other colonies or be too sparse to grow. Also, it is difficult to determine MRSA contamination when the minimum inhibitory concentra-

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tion (MIC) of methyl-phenyl-isoxazolyl-penicillin (MPIPC) is 2–4 μg/mL. In recent years polymerase chain reaction (PCR) has been applied to detect MRSA.[6–10] To prevent MRSA infection through donor tissues, we used PCR techniques to detect genes in donor eye preservation media that code specific proteins of *S. aureus* and penicillin-binding protein-2′, which is responsible for the methicillin resistance of bacteria.

Materials and Methods

Materials and Backgrounds of Donors and Recipients

The materials were 36 samples of eye preservation media that had been used to store eyes enucleated from 24 donors for keratoplasty. The mean age of the donors was 67.4 ± 17.7 (SD) years, ranging from 22–92 years. The causes of death are listed in Table 1. The donor eyes were enucleated aseptically using sterilized surgical instruments and drapes. The enucleated eyes were irrigated with physiologic saline and then stored in preservation media. The eye preservation medium was composed of glucose phosphate Ringer's solution with dextran 70 added at a concentration of 3.5% (EP-II, Kaken Pharmaceuticals, Tokyo). The media contained fosfomycin calcium (1 mg/mL) in 10 samples or fosfomycin calcium (FOM) (1 mg/mL) and gentamicin sulfate (GM) (0.1 mg/mL) in 26 samples. The donor eyes and the media were stored at 4°C until surgery. The *average* time from death to enucleation was 6.1 ± 2.8 (SD) hours, ranging from 1.4–12.9 hours and the average time from enucleation to surgery was 19.2 ± 9.7 (SD) hours, ranging from 5.7–44.7 hours. The sample media were collected at surgery in the operating room. Of the 36 donor corneas, 5 corneas could not be used for transplantation due to corneal opacity or edema.

Table 1. Cause of Donor Death

Causes of Death	Number of Donors
Heart failure	5
Myocardial infarction	4
Pneumonia	3
Cerebral infarction	2
Brain tumor	1
Lung cancer	1
Esophageal cancer	1
Stomach cancer	1
Colon cancer	1
Thyroid crisis	1
Liver failure	1
Breast cancer	1
Rupture of abdominal aortic aneurysm	1
Senility	1

Table 2. Clinical Indications for Keratoplasty

Indications	Number of Eyes
Bullous keratopathy	9
Keratoconus	6
Corneal opacity	4
Chemical burn	3
Herpetic keratitis	2
Stromal keratitis	2
Graft failure	1
Corneal injury	1
Mooren's ulcer	1
Gelatinous drop-like dystrophy	1
Perforation	1

The age of the recipients averaged 56.8 ± 18.1 years, ranging from 19–82 years. The indications for keratoplasty and surgical methods are shown in Tables 2 and 3, respectively.

Polymerase Chain Reaction

Using centrifuged eye preservation media (0.1 mL), PCR analysis was carried out for simultaneous detection of the *spa* gene, which determines synthesis of protein A specific to *S. aureus*, and the *mec*A gene, which determines the synthesis of penicillin-binding protein (PBP)-2′.

The primers for amplification of the *spa* and the *mec*A genes are shown in Table 4. The PCR solution contained 50 mmol/L Tris-HC1 (pH 8.3), 50 mmol/L KC1, 3.6 mmol/L $MgCl_2$, 0.2 mmol/L dATP, dGTP, dCTP, 0.4 mmol/L dUTP, 2.5 U *Taq* polymerase, and 0.5 U UNG. The PCR procedure was performed as follows: incubation at 50°C for 5 minutes and subsequently at 60°C for 5 minutes. The conditions for the PCR were shown as follows: denaturation at 92°C for 15 seconds, annealing at 65°C for 15 seconds, and extension at 72°C for 15 seconds (10 cycles

Table 3. Surgical Methods of Keratoplasty

Methods of Keratoplasty	Number of Eyes
Penetrating keratoplasty (PKP)	
PKP alone	23
PKP + vitrectomy	3
PKP + ECCE + IOL	1
Lamellar keratoplasty (LKP)	
LKP alone	2
LKP + ECCE + IOL	2

ECCE: extracapsular cataract extraction, IOL: intraocular lens.

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Table 4. Primers for Amplification of *spa* and *mec*A Genes

spa gene primers
5′ -biotin-TACATGTCGTTAAACCTGGTG-3′
5′ -biotin-TACAGTTGTACCGATGAATGG-3′
*mec*A gene primers
5′ -biotin-AGAAATGACTGAACGTCCG-3′
5′ -biotin-GCGATCAATGTTACCGTAG-3′

Table 5. Structure of Probes for *spa* and *mec*A Genes

spa gene capture probe
5′ -TAAGAAGCAACCAGCA-3′
*mec*A gene capture probe
5′ -TTGAGCATCTACTCGTT-3′

of PCR), and denaturation of 92°C for 15 seconds, annealing at 60°C for 15 seconds, and extension at 72°C for 15 seconds (30 cycles of PCR). The reacted solutions were hybridized with capture probes for the *spa* and *mec*A genes in microplates. The structures of the probes for the *spa* and *mec*A genes are shown in Table 5.

The captured DNAs were reacted with streptabizin and alkaline phosphatase and the absorption degree was measured at 415 nni. When the absorption degree was equal to or more than 0.2, it was judged positive. The correlation between the results of PCR analysis and the corresponding conventional bacteria culture is shown in Table 6.

Conventional Culture

The preservation media were cultured on blood agar (Nissui, Tokyo), chocolate agar (BBL, Tokyo), and Candida GS media (Eiken, Tokyo) at 35°C with 5% CO_2 for 48–72 hours. Thioglycolate (Nissui) and modified GAM anaerobe media (Kojin, Tokyo) were also used if necessary. If the first culture was positive, the samples were cultured again for identification using blood agar medium, and the kinds of bacteria were determined for each colony. Methicillin resistance was determined when the MIC of MPIPC was over 4 μg/mL.

Results

PCR Analysis

The number of samples that were positive for both *spa* and *mec*A (*spa* + *mec*A+) was 5 of 36 samples (13.8%). The number of samples that were positive for either *spa* only (*spa*+ *mec*A-) or *mec*A only (*spa*-*mec*A+) were 2 each, and the number of samples that were negative for both *spa* and *mec*A (*spa*-*mec*A−) was 27. It took about 3.5 hours to complete the PCR procedures and obtain the results.

Culture

By conventional culture, 13 of the 36 preservation media samples (36.1%) were found contaminated with bacteria. Seven kinds of bacteria and 18 strains were isolated. Two kinds of bacteria or more were isolated from 5 of the 13 contaminated samples.

Methicillin-resistant *Staphylococcus aureus* were positive in 5 of the 36 samples. Methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant coagulase-negative staphylococci (MR-CNS) were positive in two samples each. Methicillin-susceptible coagulase-negative staphylococci (MS-CNS) were positive in four samples. *Corynebacterium* sp. and *Pseudomonas aeruginosa* were positive in two samples each. *E. cloacae* was positive in one sample.

We must be aware of *P. aeruginosa*, which may cause severe infection after keratoplasty. Generally, *P. aeruginosa* is sensitive to gentamicin sulfate (GM), but not to fosfomycin calcium (FOM). In this study, *P. aeruginosa* was found positive by conventional culture in 2-10 media to which only FOM was added as antibiotic agent. On the contrary, there was no positive case in the 26 media to which both FOM and GM were added. For this reason, GM should be added to eye preservation media that are stored at 4°C.[24]

The number of colonies found by conventional culture is shown in Table 7. Correlation between the results of PCR analysis and conventional culture is shown in Table 8. It took approximately 48–72 hours to obtain results with conventional culture.

Table 6. Correlation Between Results of Polymerase Chain Reaction and Conventional Bacteriological Culture

spa	*mec*A	Kinds of Bacteria
(+)	(+)	MRSA
(+)	(−)	MSSA
(−)	(+)	MR-CNS
(−)	(−)	MS-CNS
		Others or no bacteria

MR-CNS: methicillin-resistant coagulase-negative staphylococci; MRSA: methicillin-resistant *Staphylococcus aureus*; MS-CNS: Methicillin-susceptible coagulase-negative staphylococci; MSSA: methicillin-susceptible *S. aureus.*

Table 7. Number of Colonies in Conventional Culture

Kinds of Bacteria	Number of Colonies
MRSA	2 colonies/10μL, +, +++, +++, +++
MSSA	+, +
MR-CNS	4 colonies/10μL, +
MS-CNS	1 colony/10μL, 3 colonies/10μL, 4 colonies/10μL, +
Corynebacterium sp.	+, +
Pseudomonas aeruginosa	+, +
E. cloacae	+

MR-CNS: methicillin-resistant coagulase-negative staphylococci; MRSA: methicillin-resistant *Staphylococcus aureus*: MS-CNS: methicillin-susceptible coagulase-negative staphylococci; MSSA: methicillin-suseptible *S. aureus*.
+:10–50 colonies/10 μL, ++:50–500 colonies/10 μL, +++:500 colonies/10 μL.

Clinical Results of Keratoplasty

All keratoplasty was carried out without complications. The patients were treated with ofloxacin and 0.1% betamethasone eyedrops 4 to 6 times/day for 3–6 months after surgery, and were followed up for 14.5 ± 4.1 months (mean ± SD, range 6–22 months). No case suffered from postoperative infection. *P. aeruginosa* was positive in media to which only FOM was added.

Discussion

Postoperative infection is one of the most severe complications after keratoplasty. To prevent postoperative infection after keratoplasty, it is a prerequisite that the donor is free of transmittable diseases. All corneal donors should be examined serologically for viral hepatitis, syphilis, and human immunodeficiency virus. Tuberville and Wood[11] reported that the rate of corneal ulcers after keratoplasty was 4.9%. The incidence of endophthalmitis after keratoplasty has been reported to range from 0.11–2.47%.[1,2,5,12] Culture of donor corneal rims has revealed that 14.1–45.2% of donor corneas were contaminated with bacteria or fungi[13–16] Studies of the bacteriological contamination of eye preservation media have demonstrated that 4.2–32.1% of the media were found positive by culture.[17,18]

In this study, PCR analysis demonstrated that 9 of 36 preservation media samples (25.0%) were contaminated with MRSA, MSSA, or MR-CNS; 13 of 36 samples of preservation media (36.1%) were found contaminated with bacteria by conventional culture.

Contamination of the eye preservation media with MRSA was found in 5 of 36 media samples (13.8%) both by PCR analysis and by conventional culture in this series. Polymerase chain reaction analysis studies of the *mec*A gene have been reported to detect MRSA[6–8,10,19] However, in PCR analysis for the *mec*A gene alone it is not possible to clearly distinguish between MRSA and MR-CNS. So we applied PCR analysis for simultaneous detection of the *mec*A and the *spa* genes to detect MRSA more specifically. The correlation between the results of PCR analyses and conventional culture indicated the relevance of this technique. We believe PCR analysis for simultaneous detection of the *mec*A and *spa* genes can be one of the methods to prevent bacterial infection after keratoplasty. This PCR method can be applied to detect other bacteria with high sensitivity and specificity by using appropriate primers.

Polymerase chain reaction analysis for simultaneous detection of the *spa* and *mec*A genes clearly demonstrates the existence of staphylococci that are resistant to methicillin. The time required for the PCR analysis is about 3.5 hours, which is much

Table 8. Correlation Between Results of Polymerase Chain Reaction Analysis and Conventional Culture

	Culture			
PCR Analysis	MRSA	MSSA	MR-CNS	Others or Negative
spa+ *mec*A+	5			
spa+ *mec*A−		2		
spa− *mec*A+			2	
spa− *mec*A−				27

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shorter than that required for conventional culture methods.

On the other hand, simultaneous PCR analysis for *spa* and *mec*A genes has some problems, as follows. (1) There can sometimes be false positive cases of MRSA, when both MSSA and MR-CNS are in the medium. So we should culture the medium to distinguish the true MRSA contamination from the false positive one. Once MRSA genes are found positive by PCR analysis, treatment with antibiotic agents that are effective for MRSA should be started. Usually, the antibiotic agents to which MRSA is sensitive are also effective for MSSA and MR-CNS. (2) Although the positive PCR results for *spa* and *mec*A genes indicate the existence of MRSA, they do not necessarily mean they are in an infectious state. And even though they are infectious, there might be too few bacteria to cause clinical infection. In our PCR analysis and culture, 9 of 36 media (25.0%) were bacteriologically positive, but we had no clinical infections after keratoplasty. Washing donor eyes with sterile saline solution and adding antibiotics to the preservation media, and postoperative treatment with antibiotics can contribute to preventing infection after keratoplasty.[3,20–23]

In this study we demonstrated that simultaneous PCR analysis for *spa* and *mec*A genes was useful to detect MRSA contamination in donor corneas within a shorter time than by conventional culture. Earlier diagnosis and treatment of MRSA infection after keratoplasty will help improve the prognosis for postoperative MRSA infection.

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