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Prevalence of microbial contamination in donor corneas

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Abstract:

BACKGROUND/PURPOSE: Postoperative infection is the most disastrous complication of penetrating keratoplasty (PK). Corneoscleral rim culture provided information regarding subsequent infections. Our aims were to identify the incidence of microbial contamination in donor corneas and to report the recovery of bacteria with two culture methods, i.e., conventional culture media after aerobic/anaerobic cotton swabs and blood culture media (Fastidious Antibiotic Neutralization [FAN]).

MATERIALS AND METHODS: A total of 118 patients underwent PK. Corneoscleral rim cultures were performed using aerobic/anaerobic culture cotton swabs (Transystem™, COPAN, Italia) with subsequent convention media and blood culture media (FAN bottle, BD BACTEC™, USA). The results of the different methods were reported and analyzed.

RESULTS: Microorganisms were recovered from 24 in total 118 cases (20.3%, *n* = 118), 14 from blood culture media (FAN) (11.8%, *n* = 118), 9 from conventional culture media after aerobic/anaerobic cotton swabs (7.63%, *n* = 118), and 2 from fungus culture (1.69%, *n* = 118). The most commonly identified pathogen was coagulase-negative *Staphylococcus* (CoNS) (*n* = 13, 54.2%), and more isolates of CoNS and *staphylococcus aureus* were recovered from blood culture media (FAN) than those from conventional culture media after aerobic/anaerobic cotton swabs (13 vs. 4, *P* = 0.05). Conversely, more nonfermentative Gram-negative bacilli were recovered from conventional culture media after aerobic/anaerobic cotton swabs. None of the 24 cases with positive corneoscleral rim cultures reported ocular infection for the recipients in at least 6 months' follow-up.

CONCLUSION: The conventional culture media after aerobic/anaerobic cotton swabs and blood culture media (FAN) did not yield identical isolates of bacteria. The blood culture media (FAN) could further yield Gram-positive bacteria in addition to those recovered from convention media. It seemed adding gentamicin and streptomycin could achieve bacteriostatic effect instead of the bactericidal effect. The administration of postoperative antibiotic in the recipient was suggested.

Keywords:

Corneoscleral rim cultures, penetrating keratoplasty, postpenetrating keratoplasty infection

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Introduction

Keratoplasty (corneal transplantation) is a surgical procedure where a damaged or diseased cornea is replaced by donated corneal tissue (the graft). Infections, following penetrating keratoplasty (PK), including microbial keratitis and endophthalmitis, are devastating complications and the incidence has been reported to be approximately 0.2%–0.77% for endophthalmitis[1] and 6.5%–10.5% for microbial keratitis.[2-4] It results in further surgery, including therapeutic PK with an incidence ranging from 9.5% to 30.7%; and evisceration from 7.1% to 9% in those infected eyes[2-4] Recently, it is debated for routine culture involving corneoscleral rim during implantation on the basis of cost-effectiveness.[5-10] However, microbiological testing of media and/or remaining scleral rim postoperatively is still recommended by the Eye Bank Association of America and the European Eye Bank Association.[11,12] Since the corneoscleral rim cultures could benefit in earlier infection

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detection, and the better detection rate for aerobic and anaerobic microbes becomes a crucial issue.

In literature, these were inconsistent methods in obtaining microbial culture at cornea transplantation, involving conventional culture media after aerobic/anaerobic cotton swabs or blood culture media. In fact, complex issues including different antibiotic supplementations of the preservation medium and types of culture medium to increase positive detection rate from aerobic and anaerobic microbes were involved in corneoscleral rim cultures. Conventional culture media after aerobic/anaerobic cotton swabs usually were composed of blood agar, chocolate agar, and thioglycolate broth in cultures of the corneoscleral rim[2,3,7,10,13-16] Recently, blood culture media (Fastidious Antibiotic Neutralization [FAN]) which contains agents for neutralization of antibiotic were developed to alleviate the influence of added antibiotics.[17-19] Indeed, the modern cornea storage media such as Optisol GS, which contained gentamicin and streptomycin could possible result in a higher false-negative recovery rate. The current study aimed to report the culture result of corneoscleral rim at transplantation surgery from conventional culture media after aerobic/anaerobic cotton swabs and blood culture media (FAN).

Materials and Methods

The medical charts of 142 patients receiving PK from January 2000 to January 2017 in Chiayi Chang Gung Memorial Hospital were reviewed. All patients receiving PK were prescribed wide spectrum topical antibiotics after surgery. Twenty-four patients were excluded because of lack of both culture methods. One hundred and eighteen ($n = 118$) cases were collected for aerobic/anaerobic culture results by conventional culture media after aerobic/anaerobic cotton swabs (Transystem™, COPAN, Italia) and blood culture media (FAN) (BD BACTEC™, America) [Figure 1]. Convention media included chocolate agar, sheep blood agar, and thioglycolate broth at 37°C. Sabouraud agar plates were also obtained and maintained at 25°C to enhance fungal growth. Positive microbial cultures were defined as growth of the same pathogen on 2 or more culture media. The positive fungal culture was defined on morphology.

The medical charts of patients receiving PK were reviewed.

All the donor corneas were obtained from the National Eye Bank of Taiwan and American Eye Bank. Our study was approved by the Ethics Committee of the Chang Gung Memorial Hospital (IRB: 201600959B0). Clinical information including recipient characteristics, surgical details, and postoperative outcomes were collected and analyzed. Microbiological studies in corneoscleral rim were carried

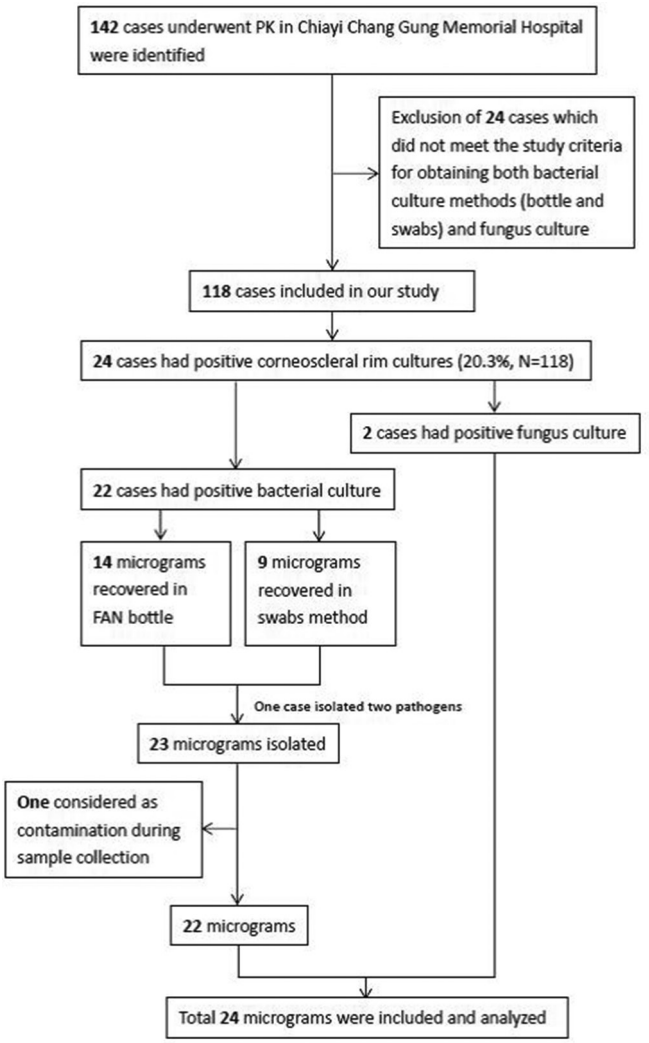

Figure 1: Flowchart of study design

out during operation, using conventional culture media after aerobic/anaerobic cotton swabs and blood culture media (FAN), which were shown in Figure 2. The preserving solution of donor cornea (5 ml) was added to the Blood culture media (FAN). On the other hand, aerobic/anaerobic culture cotton swabs scraped donor corneoscleral rim surface and were soaked in the preserving solution of donor cornea, which further inoculated in conventional culture media. The remaining donor corneoscleral rim tissue was sent for fungus culture afterward.

Contingency tables and mean values of recipient characteristics were analyzed between cases and controls using Chi-square test as described by McNemar for categorical variables. $P < 0.05$ is considered statistically significant.

Results

The mean age of our patients was 68 years old (range 7–89 years), and of them, 74 were male (62.7%), 44

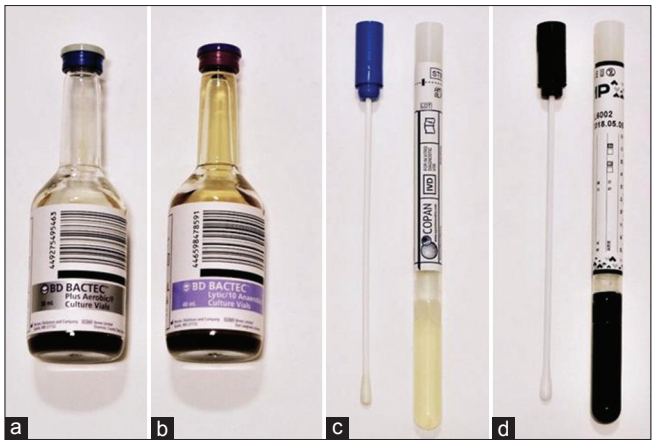

Figure 2: Culture media used in corneoscleral rim culture: (a) Aerobic blood culture media, (b) anaerobic blood culture media, (c) aerobic culture cotton swab, (d) anaerobic culture cotton swab

were female (37.3%). The laterality was equally distributed with 61 right eyes (51.7%) and 57 left eyes (48.3%). The leading indication for PK in our patients were postinfectious corneal scarring (n = 58, 49.2%), refractory corneal ulcers (n = 20, 16.9%), regraft (n = 16, 13.6%), aphakic or pseudophakic bullous keratopathy (n = 11, 9.3%), and traumatic corneal scarring (n = 7, 5.9%). The patient's characteristics of age, gender, diagnosis, and source of cornea are identified as shown in Table 1.

Microorganisms were recovered from 24 in total 118 cases (20.3%, n = 118), 14 by blood culture media (FAN) (11.8%, n = 118), 9 by conventional culture media after aerobic/anaerobic cotton swabs (7.63%, n = 118), and 2 in fungus culture (1.69%, n = 118). Pathogens identified and the culture methods used are shown in Table 2. Interestingly, pathogen recovered from blood culture system (FAN) was not identical to those recovered from swab culture system.

Among 24 positive corneoscleral rim cultures, the most common pathogen identified was coagulase-negative *Staphylococcus* (CoNS) (n = 13, 54.2%), with decreasing frequency as follows: *Staphylococcus epidermidis* (n = 2, 8.3%), *Stenotrophomonas maltophilia* (n = 2, 8.3%), *Staphylococcus aureus* (n = 1, 4.2%), *Viridans streptococcus* (n = 1, 4.2%), *Staphylococcus haemolyticus* (n = 1, 4.2%), *Nocardia* spp. (n = 1, 4.2%), Gram-positive bacilli (n = 1, 4.2%), *Acremonium* (n = 1, 4.2%), and *Candida* spp. (n = 1, 4.2%). The microorganisms identified in our study were shown in Table 3.

One case isolated two pathogens, CoNS in blood culture media (FAN) and *Nocardia* spp. in conventional culture media after aerobic/anaerobic cotton swabs, respectively. Otherwise, there was no pathogen identified in both methods. There was no correlation between these two methods using Chi-square test (χ^2 = 0.005, P = 0.648).

As compared with conventional culture media after aerobic/anaerobic cotton swabs, more isolates of CoNS and *S. aureus* (13 vs. 4, P = 0.052, Odds Ratio: 3.25) and *V. streptococcus* (1 vs. 0) were recovered from blood culture media (FAN). Conversely, more nonfermentative Gram-negative bacilli *S. maltophilia* (2 vs. 0) and Gram-positive bacilli (1 vs. 0) were recovered from conventional culture media after aerobic/anaerobic cotton swabs. None of the 24 cases with positive corneoscleral rim cultures resulted in ocular infection of the recipient for at least 6 months' follow-up.

Table 1: Characteristics of recipient and indications for penetrating keratoplasty

Recipient factor	Number of patients (total)	Percentage
Gender of recipient		
Female	44 (118)	37.3
Male	74 (118)	62.7
Recipient age (years)		
0-49	14 (118)	11.9
50-69	54 (118)	45.8
>70	50 (118)	42.4
Diagnosis		
Postinfectious corneal scarring	58 (118)	49.2
Refractory corneal ulcers	20 (118)	16.9
Regraft	16 (118)	13.6
ABK/PBK	11 (118)	9.3
Traumatic corneal scarring	7 (118)	5.9
Other	6 (118)	5.0

ABK=Aphakic bullous keratopathy, PBK=Pseudophakic bullous keratopathy

Table 2: Comparative yields of bacteria in Fastidious Antibiotic Neutralization blood culture bottles and in conventional culture media (Swabs methods)

Microorganism	Number of isolates recovered from			*P*
	Both methods	FAN bottle	Swabs	
Gram-positive *cocci*				
Staphycococcus aureus	0	1	0	N/A
Coagulase-negative *staphylococci*	0	12	4	0.080
Streptococci	0	1	0	N/A
Gram-positive bacilli				
Nocardia	0	0	1	N/A
Other GPB	0	0	1	N/A
Gram-negative bacilli				
Stenotrophomonas maltophilia	0	0	2	N/A
All microorganisms	0	14	8	0.286

Statistical analyses of bottles-to-swabs comparisons made by McNemar's Chi-square test. FAN=Fastidious Antibiotic Neutralization, N/A=Not available, GPB=Georgia public broadcasting

Table 3: Microorganisms identified in corneoscleral rim culture

Microorganism	Number of isolates recovered (*n*=24)	Percentage
Gram-positive *cocci*		
Coagulase-negative staphylococci	13 (24)	54.2
Staphylococcus epidermidis	2 (24)	8.3
Staphylococcus aureus	1 (24)	4.2
Staphylococcus haemolyticus	1 (24)	4.2
Viridans streptococcus	1 (24)	4.2
Gram-positive *bacilli*		
Nocardia	1 (24)	4.2
Other GPB	1 (24)	4.2
Gram-negative *bacilli*		
Stenotrophomonas maltophilia	2 (24)	8.3
Fungus		
Acremonium	1 (24)	4.2
Candida	1 (24)	4.2

GPB=Georgia public broadcasting

Discussion

The most significant finding of the present study was that conventional culture media after aerobic/anaerobic cotton swabs and blood culture media (FAN) did not yield identical isolates of bacteria. That was, either culture system had its own advantages and limitations in yielding the bacteria residing the corneoscleral rim. This issue became complex since evolving techniques, such as adding effective spectrum antibiotics in preservation mediums, were developed. It was reported that the combination of gentamicin and streptomycin in 4°C significantly improve anti-microbial activity against *S. aureus*, *S. epidermidis*, *Propionibacterium acnes, Escherichia coli*, and *Pseudomonas aeruginosa*, which were common pathogens leading to postoperative endophthalmitis.[20] The present study included cases after the year 2000 to meet the current standards as storage media, culture techniques, and transplantation technique.[21] We found that the conventional medium only yield 7.6%, while blood culture media (FAN) recovered 11.8%. With the advances in disinfection techniques such as better iodine sterilization of donor, and adding antibiotics in preservation medium, a lower rate of 7.6% for positive corneoscleral rim culture by conventional media was shown as compared to literature that ranged from 11% to 39%.[22] However, the blood culture media (FAN) could further yield bacterial isolates that were not recovered by convention media. It seemed adding gentamycin could achieve bacteriostatic effect instead of the bactericidal effect. These results supported the continuity of antibiotic usage in the donor until the local immunity was completely recovered.

In literature, direct inoculation and swab with subsequent convention medium have been employed for corneoscleral rim culture.[4,7,10,13,16,23] However, it seemed the positive rate would be underestimated if there were antibiotics in the preservation medium. Blood culture media (FAN), which contains agents for neutralization of antibiotic, seemed more logical for corneoscleral rim culture because of the antibiotics in the preservation medium.[17-19] We did find a further detection of Gram-positive pathogen in this method. More isolates of CoNS and *S. aureus* were recovered in blood culture media (FAN) as compared to conventional culture media after aerobic/anaerobic cotton swabs (13 vs. 4) in the present study. It has been reported that the medium contained in FAN bottles have been shown superior recovery for *Staphylococci*.[17] In spite of the limited case number, it was noteworthy that using blood culture media (FAN) could recover more CoNS and *S. aureus* in corneoscleral rim cultures which was reported clinically significant species following keratoplasty. Besides, discrepancies of recovered microbial pattern between these two methods were probably partially explained by lower oxygen content in FAN bottles. For example, *S. maltophilia*, which is strictly dependent on the oxygen concentration of the medium was only recovered in conventional culture media in the present study, could be associated with the lower oxygen content in FAN bottles. Similarly, it was also possible that more nonfermentative Gram-negative bacilli were recovered in conventional culture media because of higher oxygen tension.

Controversy in prevailing pathogens existed in microbial keratitis following PK in Taiwan.[2,3] Chen *et al.* have demonstrated the most frequently isolated microbes were Gram-negative microbes (50%), fungus (26%), and Gram-positive microbes (24%), which is contrasted by Sun *et al.* as Gram-positive microbes (58%), Gram-negative microbes (22%), and fungus (20%).[2,3] In Germany, the contamination rate and spectrum of microbes in 3306 organ-cultured donor corneas were shown that the most frequently isolated microbes were *Enterococci* (19%), *Staphylococci* (10.8%), and *Candida* (37.4%).[24] While in the National Eye Bank of Taiwan,[25] 39 out of 232 collected donor corneas isolated microbes (16.8%), with *Staphylococcus* species (57%) predominant. Because of the changing microbial environment in different region across time, the methods to recover the truly existed microbes seemed important. In the current study, we have shown that combination of blood culture media (FAN) and conventional culture media after aerobic/anaerobic cotton swabs could recover the wide spectrums of microbes existing in the corneoscleral rim.

Even though several recent studies challenged the cost-effectiveness of corneoscleral rim culture, it was evident that a positive corneoscleral culture

was associated with 5 times more frequent among recipients developing endophthalmitis than among negative culture.[15,26] Postoperative infection is the most disastrous complication of PK.[27-29] Several studies emphasized on early diagnosis and treatment based on culturing of the preservation medium.[5,6,30] If local medical therapy for infection succeeded, early removal of the infected corneal graft may be prevented.[31] Therefore, the corneoscleral rim culture at transplantation did provide important clinical information for the ophthalmologist.

The current study was limited by small number of patients. However, it revealed different patterns of bacterial isolate from the two culture system. Longer term follow-up is suggested.

Conclusion

The aerobic/anaerobic cotton swabs system with convention media and blood culture media (FAN) did not yield identical isolates of bacteria. The blood culture media (FAN) bottle could further recover Gram-positive bacteria in addition to those recovered from convention media. It seemed adding gentamycin and streptomycin could achieve bacteriostatic effect instead of bactericidal effect. The administration of postoperative antibiotics in the recipient was suggested.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

The authors declare that there are no conflicts of interests of this paper.

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