·Clinical Research·

Bacterial flora of conjunctiva after death

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Abstract

• AIM: To evaluate the frequency of bacterial flora of conjunctiva after death (cadaver eyes) which will give information about the bacterial contamination of donor eyes, and the *in-vitro* sensitivity of isolated bacteria to the commonly used antibiotics in ophthalmic practice.

• METHODS: Conjunctival swabs were taken from the cadavers (motor vehicle accident deaths and patients who died in the hospital), within 6h after death, and sent for culture and sensitivity test. Conjunctival swabs, taken from the healthy conjunctiva of patients admitted for cataract surgery, were sent for culture and sensitivity as controls (eyes in those of living status). The bacterial isolates were tested against the commonly used antibiotics (chloramphenicol, gentamicin, ciprofloxacin) in ophthalmology practice.

• RESULTS: Bacteria were isolated in 41 out of 100 conjunctival swabs (41%), taken from 50 cadavers (study group). Coagulase negative *staphylococcus* was the most common bacteria isolated (15%), followed by *pseudomonas aeruginosa* (5%). Gentamicin was effective against majority of the bacterial isolates (82%). Bacteria were isolated from 7 out of 100 conjunctival swabs taken as control group (eyes in living state). Coagulase negative *staphylococcus* was the most common organism (5%) isolated in control group; the others were *staphylococcus aureus* (1%) and beta hemolyticus *streptococci* (1%).

• CONCLUSION: Bacteria were isolated from 41% of the cadaver eyes. High percentage sensitivity of the bacterial isolates to gentamicin (82%) supports the practice of thorough irrigation of the eyes with gentamicin solution before starting the procedure of enucleation followed by immersion of the enucleated eyeballs in gentamycin solution, to prevent the bacterial contamination.

• **KEYWORDS:** bacterial flora; conjunctiva; cadaver eyes; cataract eyes

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INTRODUCTION

Vision can be improved by corneal transplantation in patients with corneal blindness, due to infections, injuries, corneal dystrophies and bullous keratopathy. The incidence of penetrating keratoplasty has increased worldwide, especially in the developing countries. Donor cornea (harvested after death from the cadaver eyes) is essential for corneal transplantation or keratoplasty. Donor cornea from local donations is not adequate in Malaysia and it is to be imported from abroad, which is evidenced in the fifth report of national transplant registry Malaysia. Out of 993 donor corneas utilized for transplantation from 2004 to 2008, the source of donor cornea was 16% from local donors, 64% from USA and 20% from Sri Lanka[1].

Variations in retrieval for cadaver donor eyes cannot be avoided because of social, emotional and ethical factors of organ donation. The practice of enucleation and preserving the whole globe by moist chamber method is still carried out in many developing countries, and in small eye hospitals, where proper sophisticated eye bank is not available. It is easy to perform enucleation procedure when compared to the removal of corneoscleral rim; and there is no need of preservation media to store the whole globe. Hence, it is much less costly than the donor cornea preserved in McCarey and Kaufman (MK) medium or Optisol medium.

Since there is a possibility of bacterial contamination of the eyes after death, a conjunctival swab is taken routinely before cleaning the donor eyeball with normal saline containing antibiotic. The donor cornea from the eyeball preserved by moist chamber method is usually utilized for corneal grafting within 24h or 48h (before the availability of culture and

sensitivity test). Hence, it is essential to take all measures to prevent bacterial contamination.

There are many reports available from different countries on the incidence of positive bacterial cultures from the conjunctival swabs taken from the donor eyeballs, prior to washing with saline containing antibiotic. Positive bacterial culture from the cadaveric eyes has been reported to be varying between 12.4% and 100%[2-7]. The literature search did not show any report on this subject from Malaysia. Therefore, the present study was undertaken to evaluate 1) the frequency of bacteria isolated from the conjunctival sac in the eyes after death and 2) their *in-vitro* antibiotic sensitivity to the commonly used antibiotics in ophthalmology practice.

SUBJECTS AND METHODS

Subjects The conjunctival swabs were taken from right eye and left eye of 50 cadavers (motor vehicle accident deaths and patients who died in University Hospital, Kuala Lumpur and kept in the mortuary) within 6h after death. The specimens were not taken from patients who died of septicaemia. All the bodies were in the cold storage room in the mortuary before the collection of the conjunctival swabs. In the control group, depending on the eye to be operated for cataract, the conjunctival swab was taken from the right eye or left eye in the outpatient department on the day of admission (before the instillation of any preoperative antibiotic eye drops).

Methods Under aseptic precautions (using sterile gloves and sterile cotton swab), the conjunctival swabs were taken from the lower fornix of the conjunctiva (without touching the eyelids) using Copan sterile transport swab, M40 Transystem and put into Amie's transport medium (Italy), Figure 1. This medium with charcoal is a non-nutritional, phosphate buffered type medium used to maintain the viability of microorganisms without a significant increase in growth. The swabs were sent immediately to the microbiology laboratory for processing the culture and sensitivity test. In the laboratory, swab with culture material was streaked across the media (blood agar, chocolate agar, and McConkey agar) in lines, after burning the wire loop in between the media plates. The blood agar and McConkey agar plates were incubated at 35℃, and chocolate agar plate was incubated in 5% CO_2 at 30℃. The plates were read after 48h for any growth of organisms. Any growth obtained was processed and the organism was identified using standard microbiology methods. Antibiotic sensitivity testing of the isolated bacteria was performed using NCCLS disc diffusion method against commonly used antibiotics (chloramphenicol, gentamicin, ciprofloxacin) in ophthalmology practice [8]. The swabs were taken during office hours so that they would be processed immediately in the laboratory.

Figure 1 Showing Copan swab with transport medium.

In the control group, one drop of 0.5% proparacaine was put in the conjunctival sac before taking the swab. The conjunctival swab was taken under aseptic precautions (using sterile gloves and sterile cotton swab) from the lower fornix, without touching the eyelids. The swabs were processed for culture and sensitivity test in microbiology department in the same way as described above. The results were entered on a data sheet and analysed using SPSS programme. This research project was approved by ethics committee of Faculty of Medicine, University of Malaya.

RESULTS

The conjunctival swabs were taken from 100 cadaver eyes and from 100 eyes with healthy conjunctiva of cataract patients, over a period of six months. Out of 100 conjunctival swabs taken from cadaver eyes, bacteria were isolated in 41 eyes (41%). Out of these 41 positive cultures, 34 had monomicrobial growth and 7 had polymicrobial growth (2 organisms in 5 cultures and 3 organisms in 2 cultures). Out of total 50 bacterial isolates from cadaver eyes, coagulase negative *staphylococcus* (15%) was the most common bacteria isolated followed by *pseudomonas aeruginosa* (5%), Table 1.

The isolation of bacteria was less in the specimens sent within 2h after death when compared to the specimens sent at later time (Table 2).

Gentamicin was effective against majority of the bacterial isolates (41/50, 82%), followed by ciprofloxacin (31/50, 62%) and chloramphenicol (28/50, 56%), Table 3.

Bacteria were isolated from 7 out of 100 conjunctival specimens taken from patients admitted for cataract surgery

Table 1 Showing various bacteria isolated from the conjunctival swabs in cadaveric eyes (*n*=100)

Bacteria	*n*	Percentage(%)
Coagulase negative *staphylococcus* [1]	15	15
Pseudomonas aeruginosa [2]	5	5
Staphylococcus aureus [3]	3	3
Streptococcus pneumonia	2	2
Streptococcus viridance	1	1
Beta haemolyticus *streptococci*	2	2
Diphtheroids	4	4
Haemophilus influenza	3	3
Acetobacter species	3	3
Klebsiella species [4]	3	3
Eschericia coli	2	2
Citrobacter species	2	2
Enterobacter species	1	1
Neisseria lactamica	1	1
Branhamella catarrhalis	1	1
Proteus species	1	1
Leuconostoc species	1	1

[1]Coagulase negative s*taphylococcus+Haemophylus influenza*; Coagulase negative s*taphylococcus+Streptococcus viridians*; Coagulase negative *staphylococcus+Acenetobacter* species
[2]*Pseudomonas aeruginosa+Klebsiella pneumonea; Pseudomonas aeruginosa+Acenetobacter* species
[3]*Staphylococcus aureus+Acenetobacter* species+*Enterobacter* species
[4]*Klebsiella* species+*Eschericia coli+Branhamella catarrhalis*.

Table 2 Showing the positive cultures at different time intervals after death when the conjunctival swabs were sent (*n*=100)

Time interval after death when swab was taken	*n*	No. of +ve cultures	Percentage (%)
up to 60min (1h)	8	1	12.5
65-120min (2h)	14	3	21.4
125-180min (3h)	28	16	57.1
185-240min (4h)	18	10	55.5
245-300min (5h)	26	8	30.8
305-360min (6h)	6	2	33.3

(eyes in living state-control group). Coagulase negative *staphylococcus* was the most common organism (5%) isolated in the control group also; the others were *staphylococcus aureus* (1%) and beta hemolyticus *streptococcus* (1%). *In vitro*, all these isolates were sensitive to all three antibiotics tested.

DISCUSSION

The frequency of positive bacterial cultures in the eyes after death noted in our study (41%) is within the range of previously reported figures (Table 4). Coagulase negative *staphylococcus* was earlier known as *staphylococcus albus*, and isolation of this organism was reported in some of the earlier studies[4,9,10]. Isolation of *Pseudomonas aeruginosa* was reported in 5.8%, 17.5%, 18.5% of donor eyes in earlier studies[2,4,9].

Gentamicin sensitivity against all bacterial isolates (82%) in our study is slightly lower than 86.4% reported by Pardos and Gallagher [2], but much higher than 53.9% reported by Satpathy and Angra[4]. However, gentamicin resistance (18%) seen in our study is much lower than previous reports 63.4% and 69.8%[11,12]. Gentamicin has been reported to be the most effective antibiotic used for decontamination of donor eyes prior to enucleation procedure[13,14]. It is the most widely used antibiotic in all the commercially available corneal storage media.

Panda *et al* [15] have suggested that pretreatment of cadaveric eyes with 50mg/mL chloramphenicol and 0.5mg/mL gentamicin for 10min before preservation and 10min before keratoplasty, followed by usage of same drops post operatively until the culture report is available, to reduce the postoperative infections.

Thorough irrigation with 20mL of sterile saline prior to enucleation decreased the incidence of bacterial contamination by 12.4%[2]. Gopinathan *et al*[16] reported that a contact of 3min duration between povidone-iodine 5% and donor globe remains satisfactory decontamination procedure when compared to ciprofloxacin 0.3% or gentamicin 0.3%.

Panda *et al* [10] reported that a 20mL sterile saline wash of enucleated eyeball resulted in a 20% decrease in the amount of bacterial contamination. They suggested saline wash followed by treatment with 1% povidone-iodine for 3min is a more effective method of decontamination (64% decrease) of donor eyes than 0.3% ciprofloxacin for 10min (47.6% decrease) or 0.3% gentamicin for 10min (21.7% decrease).

Pels and Vrensen [17] studied the effectiveness of microbial decontamination and corneal toxicity of povidone-iodine by immersing the donor eyes in different concentrations (5-100mg/mL) for different times (2-30min). They concluded that immersion of human donor eyes in 5mg/mL of povidone-iodine solution for 2min reduces microbial contamination of donor corneas without relevant penetration of iodine into corneal layers. Higher concentrations do not further reduce contamination, whereas the iodine penetration into corneal layers is toxic for corneal fibroblasts.

In our study, the numbers of positive cultures was more from the swabs sent after 2h of death when compared to the swabs sent before 2h (Table 2). Panda *et al* [10] and Matsumoto *et al* [18] support this finding. They reported that longer intervals between death and enucleation (tissue harvesting) were associated with higher positive microbial growth rates. Therefore, it is recommended that the donor eyeballs or

Table 3 Showing sensitivity report of bacterial isolates against commonly used antibiotics in ophthalmology practice

Organism	No. of strains	Chloramphenicol No. sensitive (%)	Gentamicin No. sensitive (%)	Ciprofloxacin No. sensitive (%)
Coagulase negative *staphylococcus*	15	14 (93)	12 (80)	13 (87)
Pseudomonas aeruginosa	5	0 (0)	5 (100)	3 (60)
Staphlococcus aureus	3	2 (67)	2 (67)	3 (100)
Streptococcus pneumonia	2	1 (50)	1 (50)	2 (100)
Streptococcus viridance	1	0 (0)	1 (100)	1 (100)
Beta hemolyticus *streptococci*	2	2 (100)	1 (50)	2 (100)
Diphtheroids	4	3 (75)	4 (100)	3 (75)
Haemophilus influenza	3	2 (67)	3 (100)	2 (67)
Acetobacter species	3	1 (33)	2 (67)	2 (67)
Klebsiella species	3	0 (0)	2 (67)	2 (67)
Eschericia coli	2	0 (0)	2 (100)	1 (33)
Citrobacter species	2	1(50)	2 (100)	0 (0)
Enterobacter species	1	0 (0)	1 (100)	0 (0)
Neisseria lactamica	1	1 (100)	0 (0)	1 (100)
Branhamella catarrhalis	1	1 (100)	1 (100)	1 (100)
Proteus species	1	0 (0)	1 (100)	0 (0)
Leuconostoc species	1	0 (0)	1 (100)	0 (0)

Table 4 Showing the frequency of positive bacterial cultures from donor eyes reported in the literature

Author	No. of eyes studied	Positive bacterial cultures (%)	Most common organism isolated (%)
Bobergrans *et al*, 1962[6]	65	87.3	*Streptococcus pneumonia* (49)
Rollins & Stocker, 1965[5]	100	61	*Staphylococcus epidermidis*
Polack *et al*, 1967[7]	240	100	--
Pardos & Gallagher, 1982[2]	4167	12.4	*Staphylococcus epidermidis* (66.4)
Poole & Michael, 1984[3]	70	20	--
Satpathy & Angra, 1993[4]	1557	39.2	*Staphlococcus albus* (28.1)
Panda *et al*, 1997[9]	2150	41.1	*Staphlococcus albus (37)*
Panda *et al*, 2006[10]	200	75.5	Coagulase negative *staphylococcus* (29.1)
Present study	100	41	Coagulase negative *staphylococcus* (15)

corneoscleral buttons be removed as soon as possible after death.

In a large survey involving 10 271 individuals between 1952 and 1968, Locatcher-Khorazo and Gutierrez [19] found *staphyloccus epidermidis*, *staphyloccus aureus* and diphtheroids to be the most commonly isolated organisms from the normal conjunctival sac. In a study of 276 healthy eyes, Capriotti *et al* [20] reported that the commonly isolated organisms from healthy conjunctiva were coagulase negative *staphylococcus* (28.6%), *staphylococcus aureus* (19.9%), *pseudomonas/haemophilus* (9.8%), *nocardia actinomyces* (6.5%), and *pseudomonas aeruginosa*(6.2%). Singh and Lim [21] reported *staphyloccus epidermidis* (24.5%) as the most common organism isolated from 200 healthy individuals in Malaysia; the others being *corynebacterium* species (21.5%), *staphyloccus aureus* (3.5%), acetobacter species (2.5%), *streptococcus* species (1.5%) and *micrococcus* species (1%). In our study, bacterial growth was noted in 7% of control group (eyes in living status); the organisms were coagulase negative *staphylococcus* (5%), *staphylococcus aureus* (1%) and beta hemolyticus *streptococcus* (1%). The preservative present in the topical proparacaine may affect the culture positivity in the living eyes to certain extent.

The three fold increase in isolation of coagulase negative *staphylococcus*(15%) in the study group (after death) when compared to 5% in the control group (in living status) is suggestive of contamination after death. Positive bacterial cultures in 41% of the cadaver eyes and 82% sensitivity of all isolates to gentamicin noted in our study supports the clinical practice of irrigation of the cadaver donor eyes with normal saline containing gentamicin prior to enucleation, and immersion of the enucleated eyeballs in gentamicin solution, to prevent the chances of bacterial contamination.

The isolation of pathogens from the donor eyeballs and awareness of their direct contribution to post transplant infections have made it possible to take proper care by using

combination of broad spectrum topical antibiotics to cover the resistant organisms. From the reports available in the literature, it seems that a thorough saline wash containing gentamicin before and after enucleation of the donor eyeballs followed by treatment with 0.5% povidone-iodine for 2min is the most appropriate procedure to be practiced for effective decontamination of the donor eyes.

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REFERENCES

1 Fifth report of National Transplant Registry Malaysia 2008-Corneal transplantation, p54. http://www.mst.org.my/ntrSite. Accessed on 9th April 2013

2 Pardos GJ, Gallagher MA. Microbial contamination of donor eyes. A retrospective study. *Arch Ophthalmol* 1982;100(10):1611-1613

3 Poole TG, Insler MS. Contamination of donor cornea by gentamicin-resistant organisms. *Am J Ophthalmol* 1984;97(5):560-564

4 Satpathy G, Angra SK. Changing patterns of microbial contamination and antimicrobial sensitivity in donor eyes. *Ann Ophthalmol* 1993;25 (12): 442-446

5 Rollins HJ Jr, Stocker FW. Bacterial flora and preoperative treatment of donor corneas. *Am J Ophthalmol* 1965;59:247-249

6 Boberg-Ans J, Badsberg E, Rasmussen J. Frequency of infection in donor eyes post-mortem: A method of obtaining sterile eyes for corneal grafting. *Br J Ophthalmol* 1962;46(6):365-368

7 Polack FM, Locatcher-Khorazo D, Gulterrez E. Bacteriological study of donor eyes: evaluation of antibacterial treatments prior to corneal grafting. *Arch Ophthalmol* 1967;78(2):219-225

8 National committee on clinical laboratory standards (NCCLS)-M2-A7 Performance standards for antimicrobial disc susceptibility tests: Approved standard, 7th ed, USA, 2011;121(1):M100-S11

9 Panda A, Sharma N, Angra SK, Kumbar A. Microbial contamination of donor eyes. In: Lass JH (ed), *Advances in corneal research*, New York, Springer US, Plenum press, 1997:549-555

10 Panda A, Saxena R, Vajpayee RB, Satpathy G, Angra SK, Sethi HS. The efficacy of postenucleation saline wash and the effect of different antimicrobial agents on microbial contamination of donor eyes. *Ophthalmic Res* 2006;38(5):287-293

11 Gopinathan U, Agarwal V, Sharma S, Rao GN. Donor corneoscleral rim contamination by gentamicin resistant organisms. *Indin J Ophthalmol* 1994;42(2):72-74

12 Farrel PL, Fan JT, Smith RE, Trousdale MD. Donor cornea bacterial contamination. *Cornea* 1991;10(5):381-386

13 Baum J, Barza M, Kane A. Efficacy of penicillin G, cefazoline, and gentamycin in M-K medium at 4°C. *Arch Ophthalmol* 1978;96 (7): 1262-1264

14 Liesegang TJ, Robinson N, Jones DB. Modified tissue culture medium for corneal storage 1: Investigation of effect of antibiotics on bacterial contamination. *Arch Ophthalmol* 1984;102(4):619-624

15 Panda A, Angra SK, Venkateswarulu K, Mahajan VM, Mohan M. Microbial contamination of donor eyes. *Jpn J Ophthalmol* 1988;32 (3): 264-267

16 Gopinathan U, Reddy MK, Nadkarni MS, Dasari S, Rao GN. Antimicrobial effect of ciprofloxacin, povidone-iodine, and gentamicin in the decontamination of human donor globes. *Cornea* 1998;17(1);57-61

17 Pels E, Vrensen GF. Microbial decontamination of human donor eyes with povidone-iodine: penetration, toxicity, and effectiveness. *Br J Ophthalmol* 1999;83(9):1019-1026

18 Matsumoto M, Suzuma K, Miyamura N, Imamura N, Kitaoka T. Conjunctival swabs and corneoscleral rim cultures from corneal transplantation donors as possible early indicators for posttransplant endophthalmitis. *Jpn J Ophthalmol*2011;55(4):321-326

19 Locatcher-Khorazo D, Gutierrez E. The bacterial flora in the normal eye. In Locatcher-Khorazo D. Segal BC (eds). *Microbiology of the eye*. St Louis, CV Mosby Co, 1972:13-23

20 Capriotti JA, Pelletier JS, Shah M, Caivano DM, Ritterband DC. Normal ocular flora in healthy eyes from a rural population in Sierra Leone. *Int Ophthalmol* 2009;29(2):81-84

21 Singh M, Lim VKE. Bacteriology of healthy conjunctivae in Malaysians. *Med J Malaysia* 1987;42(1):50-52