Bacteriological Findings and Antimicrobial Susceptibility Pattern of Isolated Pathogens from Visual Threatening Ocular Infections

Usanee Reinprayoon MD*, Supapan Sitthanon MD**,
Ngamjit Kasetsuwan MD***, Anan Chongthaleong MD****

** Department of Ophthalmology, King Chulalongkorn Memorial Hospital, Bangkok, Thailand*
*** Department of Ophthalmology, Prapokklao Hospital, Bangkok, Thailand*
**** Department of Ophthalmology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*
***** Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*

Objective: *To study the profiles and antimicrobial susceptibility pattern of isolated bacteria from visual threatening ocular infection.*

Material and Method: *This is a retrospective review of all microbiological culture reports from visual threatening ocular infection patients admitted at King Chulalongkorn Memorial Hospital from 2005 to 2009. The culture reports with antimicrobial susceptibility pattern were analyzed and correlated with clinical presentation.*

Results: *There were 682 specimens from 282 patients (286 eyes). Three most common diagnoses were corneal ulcer, endophthalmitis and scleritis, which yielded positive culture of 31.1% (38/122 eyes), 24.2% (25/103 eyes), and 55.6% (5/9 eyes), respectively. Overall, positive cultures were demonstrated from 77 eyes (27%). Pseudomonas aeruginosa was the most common causative pathogens of corneal ulcer and scleritis, while Enterococcus faecalis was the most common pathogens of endophthalmitis. All isolated Pseudomonas spp. were susceptible to ciprofloxacin with 4% resistance to both gentamicin and amikacin. Enterococcus faecalis demonstrated 50% of intermediate resistance to ciprofloxacin. There was no methicillin-resistant Staphylococcus aureus identified.*

Conclusion: *Overall positive cultures obtained from visual threatening ocular infection from a 5 year-review were 27%. Microbial profile remained stable from 2005 to 2009, and antimicrobial resistance was not obviously observed in our study. The findings may be used as guidelines for the prompt management of antimicrobial agents used in presumed severe bacterial ocular infection, in order to prevent devastating ocular tissue damage.*

Keywords: *Antimicrobial susceptibility, Visual threatening ocular infection, Bacteria*

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Serious ocular infection either from anterior or posterior segments of the eye such as keratitis, scleritis, endophthalmitis or orbital infection, are major causes of ocular morbidity and visual impairment[1,2]. Adequate and proper treatments of these serious conditions were guided with update information of causative microorganisms regarding spectrum of pathogens in geographic area, chances of positive culture in each center and emerging of antimicrobial resistance strains[1-5]. The present retrospective study reviewed the microbiological culture reports from visual threatening, ocular infection patients in a tertiary referral center in Bangkok, Thailand from 2005 through 2009. The purpose of our study was to determine the current microorganisms that caused severe ocular infection and the sensitivity pattern of these isolated pathogens.

Correspondence to:
Reinprayoon U, Department of Ophthalmology, King Chulalongkorn Memorial Hospital, 1873, Rama IV Road, Pathumwan, Bangkok 10330, Thailand.
Phone: 0-2256-4420-4
E-mail: usaneer@gmail.com

Material and Method

A database search was obtained for all ocular specimens collected from patients diagnosed with visual threatening ocular infection. These patients were admitted at King Chulalongkorn Memorial Hospital from 2005 through 2009. The present study was approved by the institutional review board of the Faculty of Medicine, Chulalongkorn University. The authors reviewed demographic data, previous antibiotic treatment, clinical diagnosis, isolated bacteria and

susceptibility of antimicrobial agents. Visual threatening ocular infection was defined as severe ocular infection that may affect vision due to destruction of any part of the ocular tissues. Exclusion criteria were non-ocular specimens, positive isolated pathogens besides bacteria, and incomplete or loss of data. Antibiotic sensitivity testing of isolated bacteria was performed according to King Chulalongkorn Memorial Hospital protocol. Susceptibility of antimicrobial agents was done by antimicrobial disk and dilution susceptibility testing[6,7]. Specimen collection with standard techniques and direct smears on blood, chocolate, Sabouraud's agar and thioglycolate broth were performed by residents in training or staff of the Department. Any of isolated bacteria was considered pathogens; however, it did not exclude contamination.

Statistical analysis

Statistical analysis was performed using percentage.

Results

The authors reviewed 682 specimens of 282 patients (286 eyes). Demographic data and present history are summarized in Table 1. In all, the cultures were positive in 77 eyes (27%) or 122 specimens (17.9%) (Table 2). Among these patients, three most common clinical diagnoses were corneal ulcer, endophthalmitis and scleritis, which yielded positive culture in 31.1% (38/122 eyes), 24.2% (25/103 eyes), and 55.6% (5/9 eyes), respectively (Table 3). Regarding the isolated pathogens, gram positive to gram negative bacteria ratio was 2:3. There were polymicrobial growth in 10 eyes (8 eyes from corneal ulcer, 1 eye from scleritis, and 1 eye from orbital cellulitis).

Among 38 eyes with corneal ulcer, 31.1% and 68.9% were gram positive and gram negative bacteria, respectively. *Pseudomonas aeruginosa* and *Streptococcus pneumoniae* were the two most common isolated bacteria (Fig. 1). From 25 eyes of endophthalmitis, 52% were gram positive and 48% gram

Table 1. Demographic data of patients with corneal ulcer, endophthalmitis and scleritis

	Corneal ulcer	Endophthalmitis	Scleritis
Total number	38	25	5
Gender (M:F)	24:14	17:8	3:2
Mean age (year)	43.8	49.4	52.5
Underlying diseases			
Diabetes Mellitus	8	5	-
HT	4	8	-
Chronic kidney disease	3	2	-
Pregnancy	2	-	-
Thyroid diseases	1	-	-
Malignancy	1	1	-
HIV infection	1	1	-
Glaucoma	1	2	-
Risk factors			
Undetermined post ocular surgery	3	-	-
Post cataract surgery	-	7	-
Post glaucoma surgery	-	1	-
Post pterygium surgery	-	-	4
Contact lens wearer	10	-	-
Post Trauma/Intraocular foreign body	4	9	1
Extraocular foreign body	8	-	-
Mean onset of symptoms (range, days)	18.8 (1-270)	8.7 (1-90)	54.8 (10-180)
Previous antibiotic treatment	21	11	4
Intervention			
Intravitreal tapping/injection	1	22	-
Anterior chamber tapping	6	7	-
Par plana vitrectomy	-	11	-
Debridement	-	-	2
Therapeutic penetrating keratoplasty	4	-	-
Evisceration/enucleation	3	4	-

Table 2. Number of isolated specimens

Type of specimen	Number of specimens	Number of isolated specimens	Percent
Cornea	260	43	(16.5)
Vitreous	157	30	(19.1)
Aqueous	148	7	(4.7)
Unidentified	64	20	(31.3)
Conjunctival discharge	49	20	(40.8)
Biopsy	4	2	(50)
Total	682	122	(17.9)

Table 3. Clinical diagnosis and percentage of positive culture

Diagnosis	Number of cases (%)	Positive culture (%)
Anterior segment diseases		
Corneal ulcer	38 (49.4)	31.1
Scleritis	5 (6.5)	55.6
Conjunctivitis	2 (2.6)	-
Trauma of anterior segment	2 (2.6)	16.7
Posterior Segment Diseases		
Endophthalmitis	25 (32.5)	24.2
Post-operative	8	-
Trauma/intraocular foreign body	9	-
Endogenous	4	-
Unknown	4	-
Orbital infection		
Orbital cellulitis	2 (2.6)	-
Others	3 (3.9)	12
Total	77 (100)	

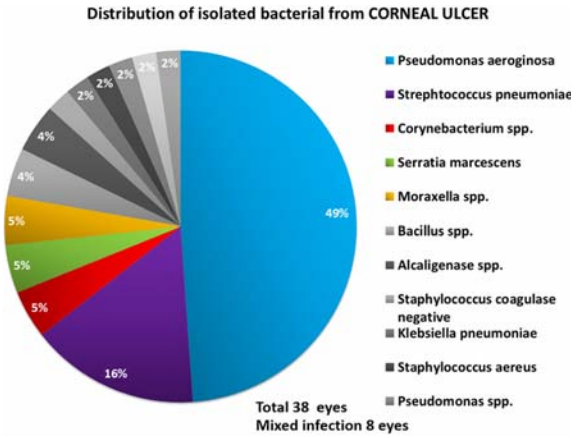

Fig. 1 Isolated bacterial from patients with corneal ulcer.

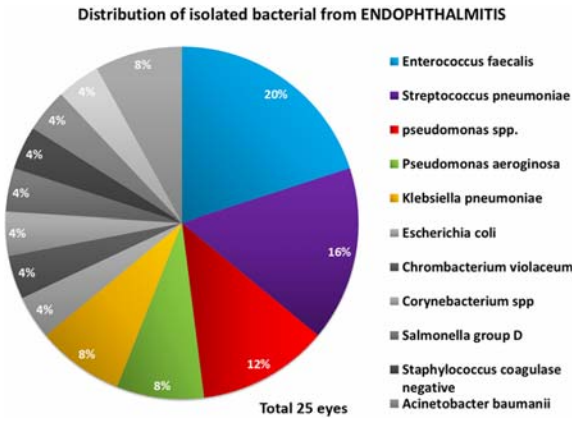

Fig. 2 Isolated bacterial from patients with endophthalmitis.

negative bacteria. Three common pathogens were identified namely: *Enterococcus faecalis*, *Pseudomonas spp*. and *Streptococcus pneumoniae* (Fig. 2). Regarding the types of endophthalmitis, the isolated bacteria are shown in Table 4. The distributions of isolated pathogens in 5 years were stable. *Pseudomonas aeruginosa* was also the most common causative pathogens of scleritis (60%).

Regarding the susceptibility pattern, *Pseudomonas spp*. was sensitive to gentamicin, amikacin, ciprofloxacin and ceftafzidime. *Staphylococcus aureus* showed very good susceptibility. Neither resistant bacterial strains nor more the serious methicillin-resistant *Staphylococcus aureus* (MRSA) were identified. Isolated *Streptococcus pneumoniae* had high rate of sensitivity to test antimicrobial agents and less than 5% of resistance. The authors found 50% of intermediate resistance of *Enterococcus faecalis* (Table 5, 6).

Discussion

This is the first study in a tertiary eye center that reviewed the isolated bacterial pathogens from ocular specimens. Cultures were overall positive in 77 out of 286 eyes (27%), which were rather low. Rate of culture positive varies from 50-70% among studies in bacterial keratitis and endophthalmitis[8-12]. The authors, hereby, propose some factors that might affect this result. Limited amount of specimens is still the main problem. Specimens obtained from infected eye tissues have to be processed immediately with special techniques and training medical personnel had to be trained. Secondly, our center is the tertiary/referral center in which approximately 60% of the referred patients had prior treatment with either topical and/or systemic antimicrobial drugs, which may directly affect cultivation. Van der Meulen showed that untreated patients had significant higher culture positive rates

Table 4. The distribution of isolated bacteria from endophthalmitis/panophthalmitis

Endophthalmitis/panophthalmitis	Number of eyes	Isolated bacteria	
Endogenous	4	*Klebseilla pneumoniae*	2
		Pseudomonas aeruginosa	1
		Acinetobacter baumanii	1
Post-operative	8	*Enterococcus faecalis*	4
		Streptococcus pneumoniae	2
		Pseudomonas aeruginosa	1
		Staphylococcus coagulase negative	1
Trauma/Intraocular foreign body	9	*Pseudomonas spp.*	3
		Corynebacterium spp.	1
		Streptococcus pneumoniae	1
		Staphylococcus aureus	1
		Escherichia coli	1
		Chrombacterium violaceum	1
		Enterobacter spp.	1
Unknown	4	*Bacillus spp.*	1
		Salmonella group D	1
		Streptococcus pneumoniae	1
		Enterococcus faecalis	1
Total	25		25

Table 5. Antibiotic sensitivity profiles of isolated *Pseudomonas aeruginosa*

Antimicrobial agents	Number of specimens		
	Sensitive (%)	Intermediate resistant (%)	Resistant (%)
Gentamicin	27 (96.4)	-	1 (3.6)
Amikacin	27 (96.4)	-	1 (3.6)
Ciprofloxacin	28 (100)	-	-
Ceftriazone	13 (48.1)	13 (48.1)	1 (3.8)
Ceftazidime	28 (100)	-	-
Trimetroprim-sulfamethoxazole	10 (62.5)	1 (6.3)	5 (31.2)

Table 6. Antibiotic sensitivity profiles of isolated Gram-positive

Antimicrobial agents	Number of specimens		
	Sensitive (%)	Intermediate resistant(%)	Resistant (%)
Staphylococcus aureus			
Oxacillin	6 (100)	-	-
Vancomycin	6 (100)	-	-
Cefoxitin	6 (100)	-	-
Ciprofloxacin	6 (100)	-	-
Moxifloxacin	6 (100)	-	-
Fucidic acid	6 (100)	-	-
Streptococcus pneumoniae			
Penicillin	6 (75)	1 (12.5)	1 (12.5)
Vancomycin	8 (100)	-	-
Ofloxacin	8 (100)	-	-
Ciprofloxacin	7 (87.5)	1 (12.5)	-
Levofloxacin	8 (100)	-	-
Clindamycin	6 (75)	-	2 (25)
Enterococcus faecalis			
Chloramphenicol	3 (75)	-	1 (25)
Ampicillin	4 (100)	-	-
Vancomycin	4 (100)	-	-
Ciprofloxacin	2 (50)	2 (50)	-
Tetracycline	1 (25)	-	3 (75)

than those treated with antimicrobial agents before culturing[10]. The present study did not exclude patients who were infected with unidentified pathogens besides bacteria, *i.e.* fungus, virus or protozoa. This may dilute and cause of lower positive culture yield.

All specimens were obtained from serious ocular infection patients. Corneal tissues, vitreous and aqueous humors were the three most common specimens. Comparing the percentage of positive yields among these specimens, the aqueous humor was the lowest. Reconsidering of aqueous tapping should be concerned or else new microbiological tools such as polymerase chain reaction (PCR) should be applied[13,14].

Surprisingly, the pattern of isolated pathogens regarding the diagnoses was stable compared to a previous study[2]. *Pseudomonas aeruginosa* and *Enterococcus faecalis* were the two most common pathogens from corneal ulcer and endophthalmitis, respectively. *Pseudomonas aeruginosa* still remained the most common isolated bacterial in keratitis patients as shown in a previous study at our center[2]. The spectrum of bacterial isolated in endophthalmitis patients was resembled to other reports, except *Pseudomonas spp*., which was found to be the most common organism in post-traumatic endophthalmitis[15-17]. Awareness of this difference may facilitate our practice. The small number of endophthalmitis patients was still our limitation, however.

Regarding antimicrobial susceptibility testing, many studies reported resistant strains of ocular pathogens to frequent ophthalmic-used antimicrobial agents, fluoroquinolone[18-21]. In the USA, the prevalence of methicillin-resistant *S. aureus* (MRSA) has increased from 29.5% to 41.6% within 5 years[19]. The authors fortunately found no emerging antibiotic resistance among isolated pathogens at our center. Hence, overall rate of antimicrobial resistance in the present study was low. However, treatment with fluoroquinolone alone in endophthalmitis caused by *Enterococcus faecalis* may be inappropriate due to its intermediate resistance.

Conclusion

Overall bacterial positive cultures obtained from visual threatening ocular infection in 5 years reviewed was 27%. The most common causative pathogens of bacterial keratitis were *Pseudomonas aeruginosa*; *Enterococcus faecalis* was found to be the most common pathogen in endophthalmitis patients. Bacteriological profile remained stable from 2005 through 2009, and antimicrobial resistance was not obviously observed in the present study.

Acknowledgement

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Potential conflicts of interest

None.

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เชื้อจุลชีพและการตอบสนองต่อยาปฏิชีวนะในโรคติดเชื้อทางตาที่ส่งผลต่อการมองเห็น

อุษณีย์ เหรียญประยูร, สุภาพรรณ ศิษย์ธานนท์, งามจิตต์ เกษตรสุวรรณ, อนันต์ จงเลิง

วัตถุประสงค์: เพื่อศึกษาเชื้อจุลชีพและการตอบสนองต่อยาปฏิชีวนะในการก่อโรคติดเชื้อทางตาที่ส่งผลต่อการมองเห็น

วัสดุและวิธีการ: การศึกษานี้เป็นการศึกษาแบบย้อนหลังโดยค้นข้อมูลจากประวัติผู้ป่วยที่เป็นโรคติดเชื้อทางตาที่อาจจะส่งผลต่อการมองเห็นผู้ป่วยเหล่านี้พักรักษาในโรงพยาบาลจุฬาลงกรณ์ระหว่างปี พ.ศ. 2548 ถึง พ.ศ. 2552 ผู้วิจัยได้ประเมินผลการเพาะเชื้อ และการตอบสนองต่อยาปฏิชีวนะของเชื้อกับการแสดงของโรค

ผลการศึกษา: ผู้วิจัยเก็บตัวอย่างทั้งหมด 682 จากผู้ป่วย 282 คน (286 ตา) โรคติดเชื้อ 3 ลำดับที่พบบ่อยคือ กระจกตาติดเชื้อ การติดเชื้อในลูกตา และการติดเชื้อที่ตาขาว ทั้งนี้ร้อยละของการเพาะเชื้อที่ให้ผลบวกตามรายโรคคือ 31.1 (38/122 ตา), 24.2 (25/103 ตา) และ 55.6 (5/9 ตา) ตามลำดับ โดยคิดผลรวมการเพาะเชื้อที่ให้ผลบวกเป็นร้อยละ 27 การติดเชื้อที่กระจกตาและตาขาวพบว่า Pseudomonas aeruginosa เป็นจุลชีพที่พบเป็นสาเหตุสูงสุดในขณะที่ Enterococcus faecalis เป็นจุลชีพที่พบเป็นสาเหตุสูงสุดในโรคติดเชื้อในลูกตาเชื้อ Pseudomonas aeruginosa ทุกตัวให้การตอบสนองของต่อยา ciprofloxacin โดยมีเพียงร้อยละ 4 ของเชื้อที่ดื้อต่อยา gentamicin และ amikacin เชื้อ Enterococcus faecalis แสดงผลดื้อระดับกลางต่อยา ciprofloxacin ร้อยละ 50 การศึกษานี้ผู้วิจัยไม่พบเชื้อ Methicillin-resistant staphylococcus aureus

สรุป: การเพาะเชื้อที่ให้ผลเป็นบวกจากโรคติดเชื้อทางตาที่อาจจะส่งผลต่อการมองเห็นในช่วงเวลา 5 ปีพบร้อยละ 27 ชนิดของเชื้อจุลชีพก่อโรคไม่เปลี่ยนแปลงในช่วงปี พ.ศ. 2548 ถึง 2552 ผู้วิจับพบเชื้อดื้อยาในสัดส่วนไม่มาก ผลการศึกษานี้อาจจะใช้เป็นแนวทางในการรักษาผู้ป่วยโรคติดเชื้อทางตาที่สงสัยว่าเกิดจากเชื้อแบคทีเรียในระยะแรก เพื่อป้องกันเนื้อเยื่อไม่ให้ถูกทำลาย