Isolation and Diagnosis of the Conjunctival Normal Flora before and After Cataract Extraction Surgery

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Abstract

Background: The conjunctival flora is opportunistic microorganisms because under certain circumstances they can cause endogenous infections.

Objective: This study aimed to diagnose conjunctival flora before and after cataract surgery and their role in post-cataract surgical infections.

Method: Specimens from ninety-one patients were collected from the conjunctivas and eyelid margins of ninety-one eyes of ninety-one patients both immediately before and one day after experiencing cataract surgery. These specimens were subjected to microbiological and biochemical tests. Susceptibility of ninety isolates obtained preoperatively was performed toward fifteen antibiotics.

Results: *Staphylococcus epidermidis* followed by *Staphylococcus aureus* were the predominant bacteria isolated from the conjunctiva and eyelid margin of the eyes before and after cataract extraction surgery. Vancomycin followed by ciprofloxacin and amikacin were significantly responsive against conjunctival isolates. In this study two patients suffered from postoperative endophthalmitis with the predominant of *Staphylococcus epidermidis* and *Staphylococcus aureus.*

Conclusion: It was predicated that the most causative microbes of post cataract surgical infections were the normal conjunctival flora.

Keywords: conjunctival Normal Flora, Endophthalmitis, Ciprofloxacin, Vancomycin, Amikacin.

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Introduction

The conjunctiva is a thin mucous membrane, which lines the inner surface of the eyelids, as well as the ocular surface of the eye ball. The normal flora is a mixture of organisms regularly found at any anatomical site. The dominant conjunctival normal flora involves mostly ***Staphylococcus epidermidis*** and certain coryneforms.

Organisms from the patient's conjunctival normal flora may gain entry into the eye at the time of cataract surgery.

These germs are the principal causative agents of postoperative endophthalmitis, which is a serious complication that threatens the visual outcome of cataract surgery. The treatment of postoperative bacterial ocular infections requires coverage for possible pathogens where this could be attained by using a combination of vancomycin, amikacin and ciprofloxacin.

Materials and Methods

Specimens from Ninety – one patients with cataracts (Forty – three males and forty – eight females) resident in Ibn – Al – Haetham Eye Hospital in Baghdad were collected during the period of November 2001 to August 2002. Their ages ranged from 9 to 92 years. All specimens were obtained by sterile – swabs under supervising the consultant physician in the operating theater. Cultures were

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obtained from the conjunctivas and eyelid margins of ninety – one eyes (Forty – three left eyes and forty – eight right eyes) of ninety – one patients immediately before experiencing cataract surgery and again one day following the surgery. Swabs were soon brought to the laboratory. Blood agar, chocolate agar and MacConky's agar media were used for culturing bacteria, while sabouraud dextrose agar medium was used for cultivating fungi. Conjunctival and eyelid margin swabs were cultured by streaking each of the above media. The inoculated blood agar and MacConky agar plates were incubated aerobically at 37C^o for 24 – 48 hours. The inoculated chocolate agar plates were placed in a candle jar to offer 5 – 10 % CO_2 atmosphere with a candle flame and then incubated at 37C^o for 24 – 48 hours. The inoculated sabouraud dextrose agar plates were incubated aerobically at 25C^o for two weeks. For primary bacterial diagnosis the following morphological characteristics of colonies were recognized on blood and chocolate agar for their shape, size, color, odor [(1)]. Gram stain test was performed as mentioned in Jawetz [(2)]. Cells' shape, gram reaction and grouping were recognized. The following biochemical tests were performed: [(1)] catalase test [(2)], oxidase test [(3)], coagulase test which involved slide coagulase test and tubal coagulase test [(4)], Optochin susceptibility test to differentiate between ***Streptococcus pneumoniae*** and ***Streptococcus*** spp. [(5)] and Api system; Colonies of catalase – positive Gram – positive cocci were subjected to the identification in the Api Staph system; Colonies of catalase – negative Gram – positive cocci were identified in the Api 20 Strep system; Colonies of catalase – positive Gram – positive rods were subjected to the identification in the Api Coryne system; and colonies of catalase – positive Gram – negative rods were identified in the Api 20 E system. To determine antibiotic susceptibility, the disk diffusion test method was employed [(6)]. Mueller – Hinton agar was used [for ***Strep***. spp. Blood (5%) was added to the medium].

Results

Specimens were collected from 91 patients immediately before and one day after cataract extraction surgery. These specimens were subjected to vigorous microbiological identification and diagnosis. No fungal growth was recorded in this study. Thirteen (14.28%) out of ninty one patients showed mixed growth immediately prior to operation. Of these thirteen patients, nine patients (69.23%) exerted no growth one day after performing the surgery, one patient (7.69%) exhibited mixed growth one day following surgery, and three patients (23.07%) showed single bacterial growth one day postoperatively. Eleven out of 91 patients (12%) showed negative cultures immediately before and one day after the operation. Forty-one out of 91 patients (51.25%) revealed pre and one-day postoperative positive cultures. Thirty-six out of 91 patients (45%) exerted growth prior to surgery and no growth one-day post operation. Only 3 out of 91 patients (3.75%) showed negative cultures immediately prior to operation and positive cultures one day after surgery. This evidence suggested that these 3 patients were exposed to contamination either during the operation or after performing the surgery

Table 1 shows numbers and percentages of bacterial isolates detected immediately before experiencing cataract surgery.

Table 2 shows numbers and percentages of bacterial isolates detected one day following cataract extraction operation.

Susceptibility of the conjunctival preoperative bacterial isolates obtained from 77 out of 91 patients was performed against 15 different antibiotics. The following antibiotics were used: vancomycin, ciprofloxacin, cephalexin, erythromycin, chloramphenicol, cefotaxime, tobramycin, amikacin, gentamicin, rifampicin, tetracycline, amoxicillin, ampicillin, penicillinG, and streptomycin. The total number of each species and the number and percentages of the sensitive isolates of each species to the antibiotics used in the study is shown in table 3.

Table 1: Numbers and percentages of bacterial isolates detected immediately before experiencing cataract surgery.

Bacterial species	**Isolates**	
	Number	**Percentage%**
Staphylococcus aureus	14	15.55
Staphylococcus epidermidis	51	56.66
Staphylococcus xylosus	1	1.11
Staphylococcus hominis	1	1.11
Staphylococcus sciuri	1	1.11
Staphylococcus haemolyticus	2	2.22
Streptococcus mitis 2	1	1.11
Proteus mirabilis	1	1.11
Corynebacterium xerosis	10	11.11
Corynebacterium striatum	7	7.77
Rhodococcus equi	1	1.11
Total	90	

Table 2: Numbers and percentages of bacterial isolates detected one day following cataract extraction surgery.

Bacterial species	**Isolates**	
	Number	**Percentages %**
Staphylococcus aureus	7	15.55
Staphylococcus epidermidis	33	73.33
Staphylococcus haemolyticus	2	4.44
Staphylococcus hominis	1	2.22
Staphylococcus sciuri	1	2.22
Proteus mirabilis	1	2.22
Total	45	

Table 3: Numbers and percentages of pre-operative bacterial isolates susceptible to antibiotics used in the study

Isolates Bacterial species	Total no. of Isolates	No. and (%) of isolates susceptible to the antibiotics used														
		VA	CF	AN	S	KF	RA	GM	CE	C	TM	TE	AMX	E	AM	PG
Staphylococcus aureus	14	13 93%	12 86%	12 86%	11 78%	9 64%	11 78%	7 50%	6 42%	5 36%	7 50%	4 28%	0 0%	1 7%	1 7%	0 0%
coagulase-negative Staphylococci																
Staphylococcus epidermidis	51	50 98%	48 94%	45 88%	39 76%	35 68%	33 65%	25 49%	26 51%	28 55%	21 41%	24 47%	12 23%	8 16%	1 2%	1 2%
Staphylococcus haemolyticus	2	2 100%	2 100%	2 100%	2 100%	1 50%	1 50%	1 50%	0 0%	2 100%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
Staphylococcus xylosus	1	1 100%	1 100%	1 100%	1 100%	0 0%	0 0%	1 100%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
Staphylococcus hominis	1	1 100%	1 100%	1 100%	1 100%	1 100%	1 100%	1 100%	1 100%	1 100%	1 100%	0 0%	0 0%	0 0%	0 0%	0 0%
Staphylococcus sciuri	1	1 100%	1 100%	1 100%	1 100%	1 100%	1 100%	1 100%	1 100%	1 100%	1 100%	1 100%	0 0%	0 0%	0 0%	0 0%
Total no. and % of sensitive coagulase-negative Staphylococci	56	55 98%	53 94%	50 89%	44 78%	38 68%	36 64%	29 52%	28 50%	32 57%	23 41%	25 44%	12 21%	8 14%	1 2%	1 2%
Proteus mirabilis	1	0 0%	1 100%	0 0%	1 100%	1 100%	0 0%	1 100%	1 100%	0 0%	1 100%	0 0%	1 100%	0 0%	1 100%	0 0%
Streptococcus mitis 2	1	1 100%	1 100%	0 0%	1 100%	0 0%	1 100%	1 100%	1 100%	1 100%	0 0%	0 0%	1 100%	0 0%	1 100%	1 100%
Rhodococcus equi	1	1 100%	1 100%	1 100%	1 100%	1 100%	1 100%	1 100%	1 100%	1 100%	0 0%	0 0%	0 0%	0 0%	1 100%	0 0%
Corynebacterium xerosis	10	10 100%	9 90%	8 80%	8 80%	10 100%	8 80%	8 80%	8 80%	4 40%	9 90%	7 70%	7 70%	5 50%	6 60%	6 60%
Corynebacterium striatum	7	7 100%	4 57%	7 100%	5 71%	7 100%	2 28%	5 71%	5 71%	4 57%	6 86%	2 28%	3 43%	1 14%	1 14%	1 14%
Total and %	90	87 96%	81 90%	78 86%	71 79%	66 73%	59 65%	52 58%	50 55%	47 52%	46 51%	38 42%	24 27%	15 16%	12 13%	9 10%

VA = vancomycin; CF = ciprofloxacin; KF = cephalexin; E = Erythromycin
C = chloramphenicol; CE = cefotaxime; TM = tobramyin; AN = amikacin
GM = gentamicin; RA = rifampicin; TE = tetracycline; AMX = amoxicillin
AM = ampicillin; PG = penicillin G; S = streptomycin

Discussion

Information were obtained from 91 patients [43 males (47.25%) and 48 females (52.75%)] underwent cataract extraction surgery. Sixteen out of the 91 patients (17.6%) were diabetics. This indicates that there is a considerable correlation between development of cataracts and diabetes mellitus. Such interpretation agreed with that indicated by Cullom and Chang [(7)], who stated "Diabetics are at an increased risk of cataract.

Sixty five patients (71.4%) aged between 60 and 92 years. This indicates that there is an important relation between advanced ages and development of cataract. A plausible explanation is that old patients are usually suffering from senile degenerations. This quite agreed with that mentioned by Dreyer *et al*. [(8)] who demonstrated that senile degenerations might yield the degenerative type of cataracts.

Only one child (1.1%), who was suffering from congenital cataract, underwent cataract extraction surgery. Twenty four out of ninety one patients (26.37%) were from villages, while the remaining sixty seven (73.63%) were civilians. This reveals a significant decrease in the number of villagers in comparison with the number of civilians intending ophthalmic hospitals. It is illustrated that those rural patients had non-acceptable beliefs and worse habits concerning health care. Researches concerning the correlation between cataract patients resident in villages and cities and intending ophthalmic hospitals were not available.

Specimens were collected from 91 patients immediately before experiencing cataract extraction surgery and again one day after surgery.

Coagulase-negative staphylococci were the predominant isolates prior to surgery. This finding was similar with that found by Bialasiewicz and Welt [(9)]. The following coagulase-negative staphylococci were detected before surgery:
Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus sciuri, Staphylococcus xylosus.

In addition to that, the following species were isolated preoperatively:
Staphylococcus aureus, proteus mirabilis, Streptococcus mitis 2, Rhodococcus equi, Corynebacterium xerosis, **and** ***Corynebacterium striatum*.**

Staphylococcus epidermidis was the predominant preoperative microorganism isolated. These results were mostly accepted by Taylor et *al*. [(10)], who mentioned that ***Staphylococcus epidermidis*** was the commonest microorganism isolated among the normal preoperative lid and conjunctival microbial flora.

In this study, the dominant preoperative conjunctival microbes were ***Staphylococcus epidermidis*** and ***Corynebacterium spp***. This result agreed with that found by Mims *et al*. [(11)], who demonstrated that

Staphylococcus epidermidis and ***Corynebacterium spp***. were the principal microbial flora of the conjunctiva. Fourteen isolates of ***Staphylococcus aureus*** and only one isolate of ***proteus mirabilis*** were detected prior to surgery, which represented 15.6% and 1.1%, respectively. The rates above were approximately accepted by those indicated by Bialasiewicz and Welt [9], who stated that coagulase-positive staphylococci and Proteus spp. represented 13.5% and 3.0% out of the total preoperative conjunctival isolates; respectively the following one day postoperative species were isolated: ***Staphylococcus aureus,*** Staphylococcus ***epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus sciuri,* and *proteus mirabilis*.**

Ocular microbial infections following cataract surgery are related predominantly to the normal conjunctival flora and to a lesser degree from air borne microorganisms or certain endogenous sources such as the genitourinary tract [13]. Herde *et al.* [14] pointed out "The conjunctival flora is of great interest for each case of intraocular operation preventing postoperative infections." In the present study, two patients suffered from postoperative endophthalmitis. Of these two patients, the conjunctival swabs showed heavy growth of ***Staphylococcus epidermidis* or *Staphylococcus aureus***, which were detected from the preoperative and one day postoperative conjunctival smears. A plausible interpretation was that the conjunctival normal flora resulted in postoperative endophthalmitis in these two patients. This explanation agreed with that found by Binder *et al*. [15] who stated "Most germs causing postoperative endophthalmitis derive from the conjunctival bacterial normal flora." Bannerman *et al*. [16] mentioned that patient's conjunctival normal flora was a major source of postoperative endophthalmitis following cataract extraction surgery. The authors Ormerod *et al*. [17], Somani *et al*. [18], and Versteegh *et al*. [19] demonstrated that organisms mostly isolated in cases of postoperative endophthalmitis were coagulase-negative staphylococci. Han *et al*. [20] documented that coagulase-negative staphylococci followed by ***Staphylococcus aureus*** played a considerable role in the pathogenesis of bacterial endophthalmitis following cataract surgery. Mandle [21] indicated that *Staphylococcus aureus* was a significant causative agent of acute infections following cataract extraction surgery. Oguz *et al*. [22] stated "The organisms most commonly recovered in cases of post-surgical endophthalmitis include primarily ***Staphylococcus aureus*** and ***Staphylococcus epidermidis, Streptococcus spp., Proteus spp***., and less frequently ***Pseudomonas spp***.". Lam *et al*. [23] documented that a diabetic patient, who underwent cataract surgery, developed endophthalmitis caused by ***Proteus mirabilis***, while Joussen *et al*. [24] indicated that diphtheroid resident in the conjunctiva were recognized as potential causatives of serious ocular diseases. However, Watkins *et al*. [25] regarded that ***Corynebacterium striatum*** was a potent microbe causing conjunctivitis. Valenton [26] indicated that infections of the sclerocorneal incision following cataract surgery could be caused by ***Staphylococcus aureus, Staphylococcus epidermidis,*** and viridans streptococci group. These results predicate that the conjunctival normal flora is the principal causative of postoperative infections; therefore preoperative microbial diagnosis is of great importance in inhibiting postoperative infections by administering the effective prophylactic drugs and for the prescription of the best therapy in cases of postoperative infections. This suggestion agreed with that found by Herde *et al.* [14], who stated "The preoperative bacteriological diagnostic of the conjunctiva is important mainly for the prevention of postoperative endophthalmitis despite the transience and fluctuation of the conjunctival flora but also in case of endophthalmitis for rapid specific antibiotical therapy. ".

Antimicrobial resistance of bacteria has become a worldwide problem; the prevalence of resistant bacteria can lead to selection of either non-effective or expensive drugs, prolonged illness, or greater risk of death [27].

These isolated microbes exerted very high resistance to penicillin G, ampicillin, and amoxicillin. Akhter *et al.* [28] denoted that high rates of resistance were observed *among* Gram-negative and Gram-positive species to penicillin G, ampicillin, and amoxicillin.

The following antibiotics could be used as topical ophthalmic therapy: vancomycin, ciprofloxacin, tobramyin, amikacin, gentamicin, tetracycline, chloramphenicol, and rifampicin [29, 20, 8].

Gram-positive species exhibited higher sensitivity to vancomycin. All ***Corynebacterium spp.*** were responsive to this drug. Among coagulase-negative staphylococci (only one isolate) was resistant to vancomycin. Kunimoto *et al.* [30] reported similar results and indicated that all ***Corynebacterium spp.*** and high rates of coagulase-negative staphylococci were sensitive to vancomycin. In the present study, 13 out of 14 isolates of ***Staphylococcus aureus*** responded to vancomycin. A close finding was described by *Akhter et al.* [28], and Han *et al.* [20], who observed that all ***Staphylococcus aureus*** isolates complied with vancomycin.

Amikacin was effective against coagulase-negative Staphylococci and ***Corynebacterium spp***. that were detected in the study. Kunimoto *et al.* [30] pointed almost similar results when mentioned that 89.5% of coagulase-negative Staphylococci and all ***Corynebacterium spp.*** responded to amikacin. Han *et al.* [20] recognized that 81.3% of ***Staphylococcus aureus*** *microorganisms* were sensitive to amikacin. This rate was close to that observed in the study.

All ***Corynebacterium spp.*** responded to cephalexin, while the remaining isolates showed moderate sensitivity to it.

It was found that coagulase-negative Staphylococci and ***Staphylococcus aureus*** microbes obtained in the study, were highly resistant to tetracycline.

Knauf *et al.* [31] illustrated that the susceptibility of conjunctival isolates to ciprofloxacin was relatively high and represented 91.7%. This rate was almost similar with that found in this study.

The authors Kunimoto *et al.* [30] recorded that the sensitivity of ***Streptococcus spp***. to amikacin, chloramphenicol, ciprofloxacin, gentamicin, and vancomycin represented 81.8%, 92.3%, 76.9%, 53.8%, and 81.8% respectively.

The microorganism ***Rhodococcus equi*** was found to be resistant to tobramyin and tetracycline.

Gram-negative organisms do not comply to vancomycin; therefore ***Proteus mirabilis*** did not respond to it.

In this study, ***Proteus mirabilis*** exhibited intermediate resistance to amikacin. In addition, Akhter *et al.* [28] showed that ***Proteus mirabilis*** agents were highly responsive to ciprofloxacin and gentamicin and represented 95% and 91%, respectively.

Owing to the wide use of gentamicin as the preferred ophthalmic therapy in our country, a notable decrease in the susceptibility of ***Staphylococcus aureus*** and coagulase-negative staphylococci to this drug was observed. At present time, gentamicin is currently used as a prophylactic agent preventing ophthalmic postoperative infections in Ibn-Al-Haetham Eye Hospital. As it is mentioned previously, 51.25% of patients exhibited growth pre and one day after cataract surgery. This rate indicated that gentamicin was not active sufficiently in prophylaxis and that ocular infections could appear in the first few days following the surgery.

This study suggests that gentamicin can be substituted by ciprofloxacin as a prophylactic drug, which exhibited high efficacy against the obtained isolates in the study. In addition, vancomycin and amikacin can be used in the treatment of endophthalmitis.

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