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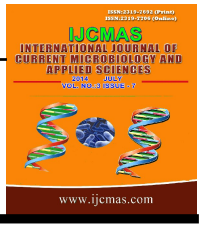

Original Research Article

Conjunctival microflora and their antibiotic susceptibility in north Indians prior to cataract surgery

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A B S T R A C T

This study determined the most common conjunctival microflora, antibiotic sensitivity and resistance pattern in North Indian patients scheduled for routine cataract surgery at Hind Institute of Medical Sciences, Safedabad, Barabanki, India. Conjunctival swab specimens were collected from patients scheduled to undergo routine cataract surgery and processed in Hind Institute of Medical Sciences. The specimens were cultured using standard techniques and those with bacterial growth were tested for antibiotic sensitivity and resistance pattern by using the Kirby Bauer disc diffusion technique in accordance with the guidelines of National Committee for Clinical Laboratory Standards (NCCLS). A total of 500 Samples were processed. Out of 500 patients 272 (54.4 %) were Males and 228 (45.6 %) were Females, 238 showed growth (47.6 %). The most common organism identified was *Coagulase Negative Staphylococci* (CONS 83.1 %). *Staphylococcus aureus* was identified in 12 patients (5.0 %). In gram negative organisms, *Pseudomonas aeruginosa* was identified as the most common isolates (2.5 %). *Candida* spp was also identified in 4 patients (1.7 %). Two cases showed growth of *Bacillus* spp which was ignored as non pathogenic organisms. Result of antibiotic sensitivity testing revealed that *Coagulase Negative Staphylococcus* isolates were showed 100% sensitivity against Vancomycin, Amikacin and Imipenam while higher resistance showed towards azithromycin, penicillin, cotrimoxazole and cefixime. *Coagulase Negative Staphylococcus* was the most frequently isolated organism in the conjunctiva of patients undergoing routine cataract surgery. The isolated bacteria showed higher resistance against penicillin, cefixime, cotrimoxazol and azithromycin, while maximum sensitivity was found with fluroquinolones like ciprofloxacin and ofloxacin, aminoglycosides such as amikacin, vancomycin and Imipenam.

Keywords

Cataract Surgery, Conjunctiva, Microflora, Antibiotic Sensitivity, Resistance

Introduction

Endophthalmitis is an inflammatory condition of the eye and a possible complication of all intraocular surgeries, particularly cataract surgery with possible

loss of vision. Various bacteria and fungi have been isolated as a cause of endophthalmitis. Cataract surgery is one of the most frequently performed operations in the world. The most common source of this complication is patients own conjunctival flora. The existence of bacterial flora in apparently healthy conjunctiva has been reported in several studies suggested that it is the commonest source of post operative infection *Staphylococcus aureus* and *Coagulase Negative Staphylococcus* including *Staphylococcus epidermidis* are important bacteria on the ocular surface (Fukuda *et al.,* 2002). *Staphylococcus aureus* is one of the most significant pathogen known for causing sporadic infection and epidemics. *Staphylococcus aureus* corneal infection results in extensive inflammation and tissue damage. *Coagulase Negative Staphylococci* (CONS) are the normal ocular surface bacterial flora isolated from patients undergoing anterior segment intraocular surgeries (Ta *et al.,* 2009). *Staphylococcus epidermidis* is the most frequent bacterium in normal conjunctival flora (Fukuda *et al.,* 2002).

The ophthalmologist use many pre and post operative method to prevent endophthalmitis, the most common of which is the use of topical antibiotic drops at regular intervals (Ciulla, 2002). Possible source of postoperative ocular infection are the ocular tear film, ocular adnexa, irrigation solutions, surgical instruments, respiratory and skin flora of the surgeon and assistants and operative room air (Höfling-Lima *et al.,* 2002). Once superficial flora enters the eye during the cataract surgery, several prophylactic measures are applied to suppress or limit the growth of these microorganisms, which could lead to endophthalmitis. Thus the use of topical antibiotics before and after cataract surgery is justified as they are efficient in reducing and, sometimes, temporarily eliminating the conjunctival microbiota (Ciulla *et al.,* 2002). Topical antibiotics are commonly prescribed in the preoperative period to possibly reduce the risk of infections following intraocular surgery. Development of drug resistance among ocular pathogens seems to be increasing due to many factors. It includes over use of antibiotics for eye, improper dosing regimen, misuse of antibiotics for viral infections, extended duration of therapy thus ophthalmologists must carefully choose the antibiotics that are most effective in minimizing ocular colonization. Knowing the organism found most frequently in the ocular flora and their antibiotic sensitivity may provide a better guide in choosing an antibiotic for prophylaxis of postoperative endophthalmitis.

Previous studies have shown that use of preoperative prophylactic antibiotic significantly reduces the number of conjunctival bacteria at the time of surgery. Specific therapy should be based on laboratory data which identify the causative agents and provide antimicrobial susceptibility result (Reza *et al.,* 2008). Knowledge of the microbiological pattern of bacterial keratitis will be helpful for effective management of keratitis in situations where resources are limited.

Materials and Methods

Specimen: A total of 500 preoperative conjunctival swabs were obtained from eyes of patient scheduled for routine cataract surgery from North India to be processed in Department of Microbiology Hind Institute of Medical Sciences, India.

Sampling: Sterile disposable dry cotton swabs were used to swab the entire surface of the conjunctival cul-de-sac from outer to the inner canthus with special care to avoid lid margins, angels of eye and eye lashes before starting topical antibiotic for at least prior to 72 hours and immediately

transported to the laboratory for processing (Haggag *et al.,* 2011). These swabs were inoculated into liquid Thioglycolate media and subculture after 4–6 hrs on blood Agar, chocolate Agar, MacConkey Agar and Sabouraud's Dextrose Agar plates and were incubated aerobically at 37ºC for 24 hrs for bacterial and fungal growth. As the most ocular fungal growth observed in some cases were appeared on Sabourud's Dextrose Agar within three days of incubation thus the Sabouraud's Dextrose Agar plates were kept to be incubated for one week prior declaring to be negative for fungal growth.

Identification of the isolated strains: The positive culture was identified systematically (Cruickshank *et al.,* 1975). The obtained organisms were identified by the direct smears films using gram stain by their cultural characters and by using specific Biochemical test for each organism using the standard manual technique (by Chees brough 2000).

Antibiotic sensitivity test of the isolated bacteria: For performing antibiotic susceptibility testing of positive cultures, a 0.5 MacFarland solution of growth was prepared and performed by using Kirby - Bauer disc diffusion technique. Muller Hinton Agar media was used for antibiotic susceptibility testing procedure. The discs of 11 different antibiotics were obtained from Himedia: penicillin, cefixime, ceftriaxone, gentamycin, amikacin, ciprofloxacin, ofloxaacin, vancomycin, chloramphenicol and imipenam and were used in accordance with the guidelines of National Committee for Clinically Laboratory Standards (NCCLS).

Results and Discussion

A total of 500 patients from whom the conjunctival swabs were taken analysed. In this study 272 (54.4%) were males and 228 (45.6%) were females. No bacterial growth was seen in 262 (52.4%) eyes while 238 (47.6%) showed growth (Table 1). Out of 238 positive cultures showing growth, the most common organism identified was CONS (*Coagulase Negative Staphylococci*), which was found in 198 patients from a total of 238 (83.1%). *Staphylococcus aureus* was identified in 12 patients (5.0%). *Pseudomonas aeruginosa* was identified in 6 patients (2.5%). *Streptococcus viridans, Candida* spp, *Enterocouus faecalis* was identified in 4 patients each (1.7%). *Corynebacterium* spp was identified in 3 patients (1.3%). *Escherschia coli* and *Bacillus* spp in 2 patients each (0.9%). *Klebsiela* spp, *Morexella catarhalis* and *Proteus mirabilis* was identified in 1 patient each (0.4%) (Table 2).

This study showed CONS (*Coagulase Negative Staphylococci*), as the most commonly isolated conjunctival flora. This study was in confirmation with many of the studies. Results of sensitivity testing for CONS as a whole showed lower susceptibility towards penicillin, cefixime and azithromycin while higher susceptibility towards vancomycin, imipenam, amikacin, followed by gentamycin and chloramphenicol (Table 3). Antibiotic sensitivity for all other bacterial isolates showed low susceptibility to penicillin, cefixime and azithromycin where as high susceptibility was observed in vancomycin, imipenam, gentamycin and amikacin. Antibiotic susceptibility testing for other bacterial isolates did not include 2 strains of *Bacillus* spp (0.9%) being considered as non pathogenic organism. The major route of postoperative endophthalmitis is lid and conjunctival bacterial flora enter at the time of operation.

Table.1 The number of patients with Growth & No Growth

Growth Status	Number of Patients n= 500	Percentage %
A) Sterile(No bacterial & fugal growth)	262	52.4%
B) Microbial growth	238	47.6%

Table.2 Identification of Conjunctival isolates in patients undergoing cataract surgery

S.No.	Microbial Flora	No of Strains N=238	%
1	*Coagulase Negatives Staphylococcus*	198	83.1 %
2	*Staphylococcus aureus*	12	5.0 %
3	*Pseudomonas aeruginosa*	6	2.5 %
4	*Streptococuus viridians*	4	1.7%
5	*Candida spp*	4	1.7%
6	*Enterococuus faecalis*	4	1.7%
7	*Corynebacterium spp*	3	1.3%
8	*E.coli*	2	0.9%
9	*Klebsiella spp*	1	0.4%
10	*Bacillus*	2	0.9%
11	*Proteus mirabilis*	1	0.4%
12	*Morexella catarhalis*	1	0.4%

Table.3 Antibiotic susceptibility of *Coagulase Negative Staphyloccoccus* (n=198)

	Peni	Gent	Ak	Cipr	Oflox	Van	Chloram	Cfm	Ctr	Azit	Imp
S %	66.2	98.0	100	88.3	95.4	100	91.4	67	89	69	100
I %	2.6	0	0	1.7	1	0	0.6	4	2	3.5	0
R %	31.2	2	0	10	3.6	0	8	29	9	27.5	0

S=sensitive, I=Intermediate, R=Resistant, Peni=Penicillin, Gent=Gentamycin, Ak=Amikacin, Cipr=Ciprofloxacin, Oflox=Ofloxacin, Van=Vancomycin, Chloram=Chloramphenicol, Cfm=Cefixime, Ctr=Ceftriaxone, Azit=Azithromycin, Imp=Imipenam

Table.4 Antibiotic susceptibility of all bacterial isolates (n=34)

	Peni	Gent	Ak	Cipr	Oflox	Van	Chloram	Cfm	Ctr	Azit	Imp
S %	62.0	90.5	92.0	86.0	90.8	96.0	86.0	62	72	60	98.2
I %	8.0	2.0	0	8	2.0	0	6	6	2	7	0
R %	30	7.5	8	6	7.2	4	8	32	26	33	1.8

S= sensitive, I= Intermediate, R= Resistant, Peni=Penicillin, Gent=Gentamycin, Ak=Amikacin, Cipr=Ciprofloxacin, Oflox=Ofloxacin, Van=Vancomycin, Chloram=Chloramphenicol, Cfm=Cefixime, Ctr=Ceftriaxone, Azit=Azithromycin, Imp=Imipenam

So evaluation of the conjunctival bacterial flora and their sensitivity pattern is of at most important to prevent most dreaded complication of cataract surgery. Although there are many published paper from other countries on the normal flora of the eye and their sensitivity pattern to antibiotics these data cannot be applied wholesale to Indian as several factors affect the type and sensitivity and resistance pattern of bacterial in developing countries.

In the present study *Coagulase Negative Staphylococcus* (CONS) was identified as the most isolated bacteria among the conjunctival microbial flora 198 strains out of 238 growth representatively, (83.1%). These results agree with many studies that proved CONS are the most frequent organism identified by culture of eye flora with different percentages.

The result of our study was very similar to *Ta et al.,* (2009) showed 82% of the isolates were CONS. Haggag *et al.,* (2011) and *De*-Kaspar *et al.,* (2005) demonstrated that CONS was the most common bacterial flora reported 76% and 76.2% CONS respectively. *Barnabas Mshengila et al.,* from Uganda also reported the similar findings in 2013 but they find 62% positivity of CONS isolates. They also reported 2nd most common bacteria was *Staphylococcus aureus* (21.0%) and Haggag *et al.,* (2011) found 10.5% *Staphylococcus aureus* which agree with our present study in which we found *Staphylococcus aureus* as 2nd most frequently isolated bacteria (5.0%).

With regards to sensitivity patterns particularly in case of CONS vancomycin, imipenam, amikacin, gentamycin, ofloxacin and chloramphenicol were found to be most effective in a study by Keshav and Basu in 2012 almost similar sensitivity patterns were noticed.

In a study by Terence *et al.,* also reported high sensitivity with ciprofloxacin, gentamycin, vancomycin and chloramphenicol against CONS. In contrast Harper *et al.,* 2007 demonstrated high resistance to fluroquinolones among CNS isolated from patients with endopthalmitis.

In conclusion, we find (52%) of healthy north Indian eyes were culture positive. CONS isolated predominantly (83.1%), while some gram negative isolates also recovered. CONS showed high sensitivity with vancomycin, imipenam, fluroqunilones and aminoglycosides. Our study may help to eye surgeons in choosing pre and post operative treatment to avoid post operative complications. It is important not only for cataract surgeons but all ophthalmologists to know the most common ocular bacterial flora and the antibiotics most effective against them.

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