

Research Article

Conjunctival Flora of Normal Human Eye

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Abstract

Background: The normal flora of the eye plays an important role in maintaining ocular homeostasis by various mechanisms. They comprise of mainly bacteria which do not cause infection in normal conditions but can be a main source of infection after ocular surgery, trauma or in immune compromised. The ranges of these microorganisms vary with age, sex and geographical distribution. Therefore it is very important for the ophthalmologist to know the ocular normal flora before giving prophylactic antibiotics and treating infections.

Objectives: To describe the conjunctival flora of normal human eye which vary with age, sex, geographical distribution, right and left eye.

Methodology: A total of 200 conjunctival swabs from 100 patients with healthy eyes were sent for microbiological evaluation to describe the various microorganisms isolated as normal flora of conjunctiva.

Result: The growth of bacteria was seen in 78.5% of patients, the commonest flora isolated was Coagulase negative *Staphylococcus* in 51%. Greater number of male patients had sterile conjunctiva than females and conjunctiva of old people were found to be increasingly more colonised than young. However there was no variation in normal ocular flora in different geographical conditions.

Conclusion: The diversity of normal ocular flora in different individual in relation to age, sex and geography makes it important to have a through knowledge about them in order to prescribe appropriate antibiotics in ocular infections and trauma.

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Keywords

- Coagulase positive *Staphylococcus*
- Normal flora
- Ocular infection
- Ocular trauma

ABBREVIATIONS

MCI: Mild Cognitive Impairment; AD: Alzheimer's Disease; SSRIs: Selective Serotonin Reuptake Inhibitors

INTRODUCTION

Eyelids and conjunctiva harbors a significant number of bacteria and occasionally fungi from the external environment and are called normal flora. They play an important role in normal body functions and health by secreting antibiotics and chemical mediators to maintain surface homeostasis and immunoregulation. They also out compete pathogenic bacteria for nutrition thereby inhibiting their growth [1].

These do not cause infections in normal condition but sometimes it is a source of organisms for ocular infections. Therefore knowledge about the normal flora of conjunctiva will be of particular concern to the ophthalmologist in that the infection in the wake of surgery, ocular injection, or minor trauma is likely linked to infectious agents resident on the normal ocular surface. One study mentioned that 50 to 85% of the vitreous aspirates culture was positive for coagulase-negative *Staphylococcus* (CONS) followed by *S. aureus* and *Streptococcus* species [2]. These

organisms responsible for postoperative endophthalmitis are part of the normal microbial flora of the conjunctiva and eyelids of the patient.

They are present in the conjunctival sac from birth and are present throughout the life though a very small percent of population have sterile conjunctival sac [1]. A variety of organisms have been cultivated from the normal conjunctiva both non pathogens and pathogens, but their number is usually small. This is due to frequent blinking that wipes the conjunctiva every few seconds, mechanically washing away foreign bodies including bacteria by the tears. Presence of bacteriostatic substances like lysozyme, IgA, and IgG, decreased temperature of conjunctiva due to evaporation of tears, exposure and moderate blood supply also inhibits the bacterial growth. Rampant use of antibiotics has been associated with changes in the type of normal flora as well as pathogenic bacteria [3].

The range of these microorganisms however vary in different individuals due to variation in genetic makeup, nutrition, age, sex, race, immunosuppression, ocular inflammation, dry eye, contact lens wear, antibiotic use, surgery, external exposure, climate and geography [4].

The objective of this study is to describe the normal flora of conjunctiva and determine their variation in right/left eye, gender, age and geographical distribution.

MATERIALS AND METHODS

This is cross sectional descriptive study conducted in the Ophthalmology Department and Microbiology Department of Dhulikhel Hospital, Kathmandu University Hospital, from March to July 2011. The report is based on bacteriological culture results of healthy conjunctiva of patients visiting the Eye Outpatient Department. The study populations were 200 normal eyes (right & left eyes individually) of 100 healthy subjects of variable age groups, sex, geographical distribution.

All the participants were examined by an ophthalmologist in a slitlamp and patients with ocular infection and those on antibiotics were excluded from the study. Then ophthalmologist collected conjunctival samples from both eyes separately with a sterile cotton tipped swab moistened in brain heart infusion broth by rubbing them over the lower conjunctival sac from medial to lateral canthus and back again to the medial canthus very carefully without touching the cornea. The swabs were then inserted into separate sterile labeled test tubes and transported immediately to microbiology laboratory. Swabs were directly inoculated onto blood agar, chocolate agar, and Brucella Blood Agar and were examined after 24, 48 and 72hrs. The colonies were then extracted for Gram staining and categorized into cocci, rods, pair, tetrad and chain. Catalase test was done for cocci to distinguish from *Staphylococcus* (catalase +ve) from *Streptococcus* (catalase -ve). Coagulase tests were done for identification of different species of *Staphylococcus* – coagulase positive *Staphylococcus aureus* (the pathogenic strains) and the coagulase negative *Staphylococcus species* (the nonpathogenic strains). Antibiotic Sensitivity Tests were carried out by disc diffusion technique (Cruickshank) containing Optochin, Bacitracin and

Clotrimoxazole for distinguishing different *Streptococcus species*. Thereby bacteria were identified to the nearest Genus and species. The laboratory data were directly entered in SPSS 11.5 software and different statistical parameters were then calculated including frequencies, percentages, standard deviation and chi-square test. In this study, Conjunctival flora is dependent variable. Independent variable includes Left/right eye, gender, age and geographical distribution.

RESULTS

Growth of some bacteria was seen in 78% in Right Eye (RE) and 79% in Left Eye (LE) in total of 200 samples. The commonest flora isolated was Coagulase negative *Staphylococcus* (CONS), 50% in right eye & 52% in left eye. Others are tabulated in Figure 1. On statistical analysis, chi square test ($df=1, p>0.05$) showed no significant difference in microbial flora in right and left eye. Occurrence of CONS in both eyes of male and female is statistically not significant ($p>0.05$).

Among 100 subjects, 55 were male and 45 were female. 27.3% of male subjects had sterile conjunctiva compared to 15.6% in the females as shown in Figure 2. The swab culture of rest of the subjects showed that, CONS were the predominant normal flora in both, 47.3% and 53.3% in male and female respectively. However *Streptococcus species* were found significantly high in females ($df=1, p=0.08$).

The maximum number of patients belonged to age group 14-45 years (78 out of 100). The mean age was 30.21 as shown in Figure 3. Here also CONS followed by Coagulase Positive *Staphylococcus* (COPS) are the common organisms isolated among all age groups. The greater percent of sterile conjunctiva was seen in younger populations while with the increasing age (>60 years old) conjunctivae were found to be increasingly colonized. *Streptococcus species* were remarkably more in early adults (15-45 years).

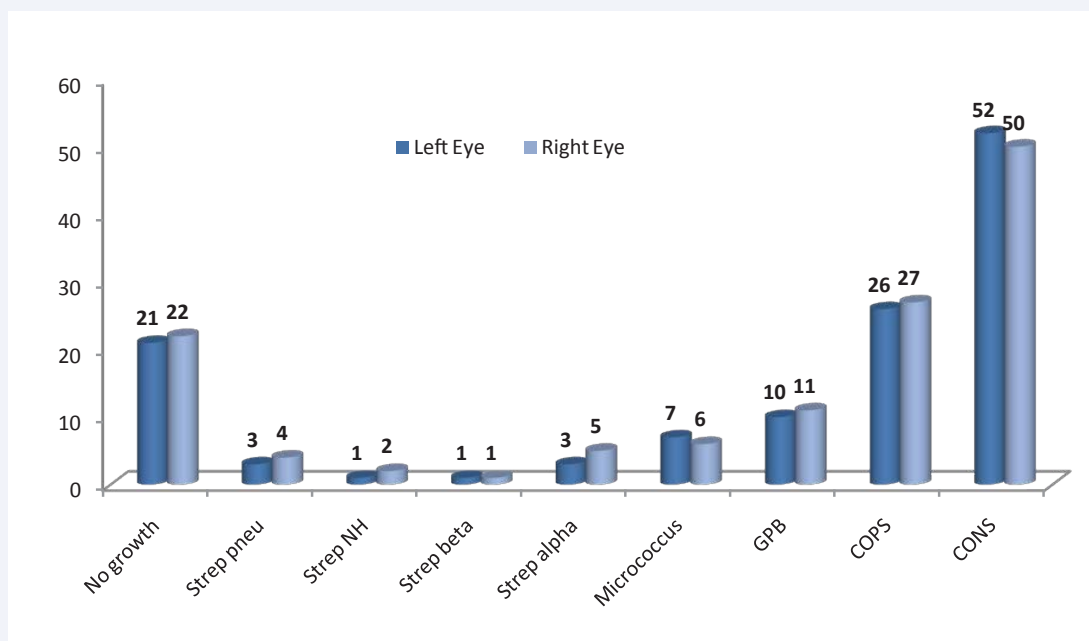


Figure 1 Percent of Conjunctival Normal Flora of Right Eye and Left Eye.

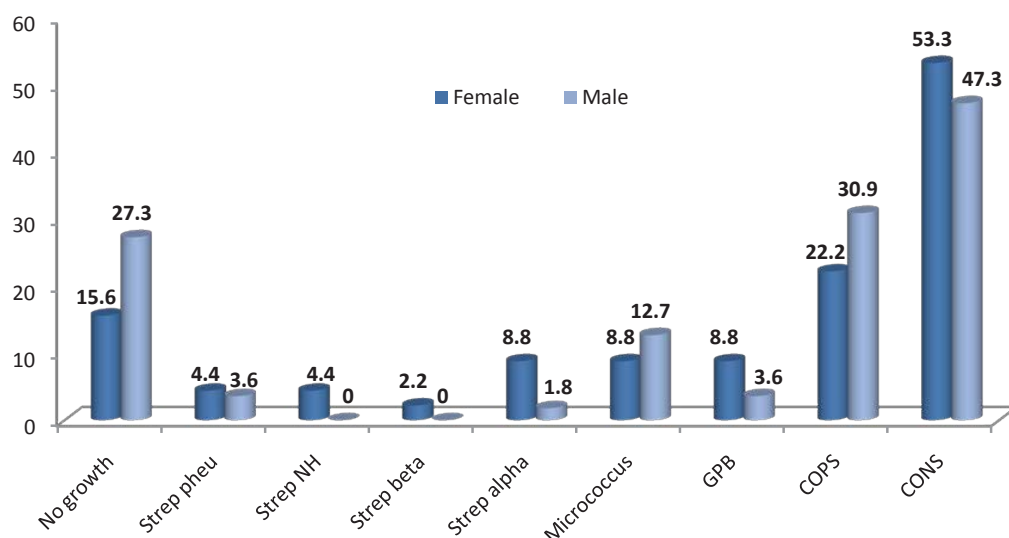


Figure 2 Gender wise Percent of Conjunctival Normal Flora.

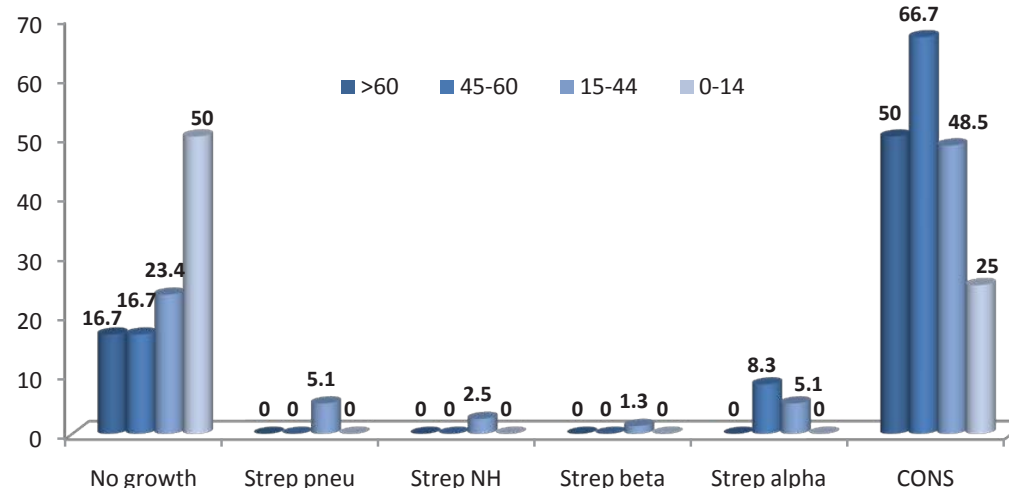


Figure 3 Age wise Percent of Conjunctival Normal Flora.

Table 4 indicates the district wise conjunctival swab results. Of the total 100 subjects maximum numbers of the subjects were from Kavrepalanchowk district (64) while other subjects were reported from Kathmandu (17), Bhaktapur (16) and Lalitpur (3). among the four districts under study, lalitpur showed the highest percent of no growth(33.3%) while Katmandu showed the least(17.6%).CONS showed the highest percent followed by COPS, Gram Positive Bacilli and *Micrococcus*.

DISCUSSION

Many researchers and scientists have worked on identification of normal flora of conjunctiva but none has been published so far from Nepal.

A study by Rajvanshi VS on conjunctival swabs of 102 normal patients found 53% *Staphylococcus albus*, 14.7% *Staphylococcus*

aureus, 11.7% Diphtheroids and 6.8% *Streptococcus* [5]. Another study by Khoraza and Thompson carried out in normal conjunctiva of 1122 patients found 64% staphylococci, 36% Diphtheroids, 3-4% α H *Streptococci* and 17% no growth [6].

Also found higher prevalence of CONS in 88.3%, *Diphtheroids* in 58.1%, *Propionibacteria* in 31%, *Streptococci* in 23.1%, *Staphylococcus aureus* 10.2%, *Haemophilus* plus Gram-negative diplococci 7.5% and other Gram-negative rods 4.5%, *Enterococci* 2% [7].

Another study by Ansari MR, Madani H and Ghaderi E, 52.4% had positive cultures in the eyes out of which 79 eyes (88.8%) had coagulase-negative *Staphylococcus* (CONS) and eighty two cases (95.3%) had *Staphylococcus* [2].

In our study as well, commonest flora isolated was Coagulase

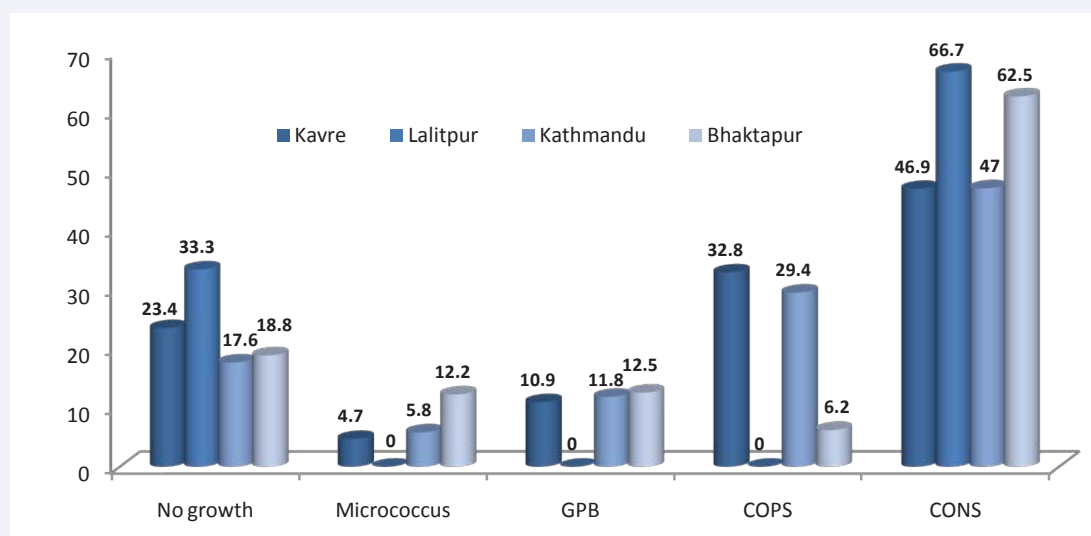


Figure 4 District wise Percent of Conjunctival Normal Flora.

Negative *Staphylococcus* (CONS), 50% in right eye & 52% in left eye followed by Coagulase Positive *Staphylococcus* (COPS) in 53%, *Micrococcus* in 13%, Gram positive bacilli in 21% and *Streptococcus* species in 20%. All the above studies shows that occurrence of CONS and COPS is the predominant flora of conjunctiva. The fact that they are the common resident flora of skin and mucus membrane and are acquired in conjunctiva from the adjacent eyelid or from hand also explains their predominant presence.

Studies on frequency of sterile conjunctival sac has shown marked disparity among different studies. Chang [8], Matuura [9] and Locatcher-Khorazo and Seegal [10] reported 9%, 2.5% and 0% respectively of sterile conjunctiva. Whereas other studies reported higher percentage of sterile conjunctiva like Lally [11], Bachrach et al [12], Debnath [13], Smith [14] reported 24%, 33%, 30% and 47% respectively. While we found only 21.5% of patients with sterile conjunctiva. Agrawal and Khosla in their series found variation in urban and rural population with 23% and 30% of sterile conjunctiva respectively. He explains this difference as due to the indiscriminate use of corticosteroid and antibiotic eye, ointment by the urban people [15].

Though the geographical areas we covered in our study are both rural as well as urban areas, our study does not agree with above result by Agrawal and Khosla. In our study, lesser patients (17.6%) from Kathmandu had sterile conjunctiva but Lalitpur also being urban area, 33.3% of patients had sterile conjunctiva. Whereas Kavre being mostly rural area had 23.4% of sterile conjunctiva. So there was no co-relation in isolation of flora in relation to geographical distribution and urbanization.

Sterile conjunctiva was more in younger age group while conjunctiva of more than 60 years old were found to be increasingly colonised in our study. Singer TR et al. in their study of conjunctival cultures from 229 eyes of 144 unaffected individuals showed adults with a greater number of species isolated per eye than did younger subjects (1.47 versus 1.13; $p < 0.05$) [3]. They also found that more *Streptococcus* species were cultured from 14.9% of the children's eyes as opposed to only

2.2% from adults ($p < 0.005$). This could be because colonization of skin and upper respiratory tract are found to be more frequent in children than in adults, which are believed to be an important source of conjunctival flora [6]. So one might expect more streptococci in the conjunctivae of children.

Our findings also showed consistent increase in percent of CONS with the increase in age and increase in number of various *Streptococcus* species in younger age group. The shift in floral composition may be related to aqueous tear deficiency with age, goblet cell changes, and lipid deregulatory states that accumulate over time.

A study by Srinivasa Rao PN, showed that conjunctival bacterial flora is more or less the same in either sex, 22.5% males 18.2% females but there is predominance of fungal isolates in the males (28.2% males 18.2% females) [16]. A study by Ejaz Ahmad Javed et al [17] with 500 swabs showed that male patients had higher percent of growth of normal flora (68.1%) than the female (31.9%). They suggested that such result may be attributed to the outdoor work in the agricultural area in which male takes part more than females. Our study found no statistically significant difference (male 93% and female 85% with $p = 0.317$) in the prevalence of normal flora. This may be attributable to the fact that, in Nepal, both sexes are equally involved in agricultural work.

The conjunctival flora isolated from right and left eye are not statistically significant 78% and 79% respectively. However unhygienic practices like rubbing the eye with contaminated hands in right or left handed patients can make the difference. Research on the conjunctival flora concerning left and right eye individually have not been reported so far for comparison.

This study has limitation in that this is a hospital based study and not a community based study, so the results may not apply to the general population as a whole.

CONCLUSION

Bacterial colonies were isolated from 78.5% of normal

conjunctiva. The commonest flora isolated was Coagulase negative *Staphylococcus* followed by Coagulase positive *Staphylococcus*, Gram positive bacilli and *Streptococcus pneumoniae*. There was no significant difference in microbial flora in right and left eye. Male has more sterile conjunctival sac compared to female.

Older patient's harbour more bacterial colonies than young among which CONS was more common in elderly while in young it was *Streptococcus* species. Diversity of normal conjunctival flora among patients of different geographical locations is not remarkable.

REFERENCES

1. Malathi J, Madhavan HN, Therese KL, Joseph PR. A hospital based study on the prevalence of conjunctivitis due to *Chlamydia trachomatis*. Indian J Med Res. 2003; 117: 71-75.
2. Ansari MR, Madani H, Ghaderi E. Conjunctival bacterial flora and antibiotic resistance pattern in patients undergoing cataract surgery. Pak J Med Sci. 2008; 24: 581-585.
3. Singer TR, Isenberg SJ, Apt L. Conjunctival anaerobic and aerobic bacterial flora in paediatric versus adult subjects. Br J Ophthalmol. 1988; 72: 448-451.
4. Jawetz Levinson. Medical Microbiology and Immunology. USA: Practice Hall International. 1989; 20-21.
5. Rajvanshi VS. Bacterial flora of the conjunctiva. J All India Ophthalmol Soc. 1968; 16: 24-28.
6. Khorazo D, Thompson R. The bacterial flora of the normal conjunctiva. Am J Ophthalmol. 1935; 18: 1114-1116.
7. Fernández-Rubio E, Urcelay JL, Cuesta-Rodríguez T. The antibiotic resistance pattern of conjunctival bacteria: a key for designing a cataract surgery prophylaxis. Eye (Lond). 2009; 23: 1321-1328.
8. Chang HL. Bacterial flora of the normal conjunctiva. Chin Med J. 1957; 75: 233-235.
9. Matuura H. Anaerobes in the bacterial flora of the conjunctival sac. Jpn J Ophthalmol. 1971; 15: 116-124.
10. Locatcher- Khoranzo D, Seegal BC. Microbiology of the eye. St. Louis: Mosby. 1972:13-18.
11. Starr MB, Lally JM. Antimicrobial prophylaxis for ophthalmic surgery. Surv Ophthalmol. 1995; 39: 485-501.
12. Bachrach U, Gurevitch J, Landau J, Birnbaum D. The flora of the normal conjunctiva of healthy people in Israel. Acta Med Orient. 1953; 12: 10-13.
13. Debnath Sc. Effect of preoperative antibiotics on bacterial colony counts of the conjunctival sac. J Ocul Ther Surg. 1985; 4: 122-124.
14. Smith CH. Bacteriology of the healthy conjunctiva. Br J Ophthalmol. 1954; 38: 719-726.
15. Agarwal LP, Khosla PK. Mycotic keratitis (experimental & clinical study). J All India Ophthalmol Soc. 1967; 15: 1-10.
16. Rao PN, Rao KN. Study of the normal conjunctival flora (bacterial and fungal) and its relations to external ocular infections. Indian J Ophthalmol. 1972; 20: 164-170.
17. Ejaz Ahmad Javed, et al. Normal Conjunctival flora (2006). APMC. 2008; 2: 17-20.

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