

Aerobic bacterial flora of the normal conjunctiva at high altitude area of Shimla Hills in India: a hospital based study

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Abstract

- **AIM:** To study the normal aerobic conjunctival flora in lower to mid Himalayan region of Shimla Hills.
- **METHODS:** Samples from normal conjunctiva of 200 individuals above the age of 13 years who visited Indira Gandhi Medical College Hospital Shimla for refraction or cataract surgery were taken by anaesthetising the conjunctival sac with sterile 4% Xylocaine solution. Samples were obtained by gently rubbing lower fornix with a sterile cotton wool swab moistened with normal saline and keeping the eye lids wide apart to avoid contamination from lid margins. It was immediately inoculated in Brain Heart infusion and subjected to standard aerobic culture and identification techniques.
- **RESULTS:** Totally, 72 eyes (36%) conjunctival sacs were sterile. Predominant aerobes isolated were *Staphylococcus* spp. in 120 (60%) followed by *Haemophilus* in 16 (8%), diphtheroids in 10 (5%), and *Escherichia coli* (*E. coli*) in 4 (2%). A single aerobe was isolated from 98 eyes (49%) while 30 eyes (15%) yielded more than 1 aerobe. *Staphylococcus epidermidis* (*S. epidermidis*) was the most common bacterium, found alone in 58 eyes (29%) and in combination with another aerobe in 30 eyes (15%), followed by *Staphylococcus aureus* (*S. aureus*) in 18 (9%) and 10 eyes (5%) respectively.
- **CONCLUSION:** *S. epidermidis* is the most common commensal organism followed by *Haemophilus* species. diphtheroids occupied the third position which otherwise are found more abundantly in literature. Pathogens like *S. aureus*, *Staphylococcus citreus* and *E. coli* were also found. Therefore, preoperative administration of topical broad spectrum antibiotics is extremely important in prophylaxis against ocular infection. The variation in

microflora of normal conjunctiva in this part of world can be attributed to geographical, climatic and ethnic characteristics of the population under study.

• **KEYWORDS:** normal conjunctival flora; high altitude; geographic and climatic conditions

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INTRODUCTION

Conjunctival sac is relatively free from organisms, thanks to antibacterial action of immunoglobulins and components of the complement pathways namely lactoferrin, lysozymes and B-lysin in tears that help kill microorganisms and decrease their adherence to the ocular surface and their flushing action^[1]. However, some microfloras still flourish in normal conjunctiva and have the potential to cause postoperative intraocular infection. Speaker *et al*^[2] demonstrated that an organism isolated from the vitreous was genetically indistinguishable from an isolate recovered from the patient's eyelid, conjunctiva, or nose in 82% cases of endophthalmitis. Therefore, aerobic bacterial cultures of normal conjunctiva and identification of ocular microflora are generally ordered preoperatively to avoid postoperative endophthalmitis by prophylactic administration of appropriate antibiotics.

Wide variations in composition of normal conjunctival flora are reported from different parts of world but no cause could be attributed. A recent report by Rubio^[3] has blamed climate as the causative factor for this variation. Shimla Hills in India are situated at an approximate range of 1 800m to 2 500m from mean sea level in mid Himalayan region where relatively cold climatic conditions prevail for most part of the year and the population is predominantly rural, engaged in agricultural activities. Therefore, it interested us to study the normal aerobic bacterial conjunctival flora of the local inhabitants visiting this institute.

SUBJECTS AND METHODS

Subjects Two hundred normal healthy individuals of age 13 years or above hailing from rural areas of Shimla Hills

Bacterial flora of normal conjunctiva at high altitude

Table 1 Age and sex distribution of healthy individuals

Groups	Age(a)	M		F		Total	
		n(%)	Mean age	n(%)	Mean age	n(%)	Mean age
Group 1	13-25	58(29)	21.2	32(16)	21.2	90(45)	21.2
Group 2	26-50	62(31)	35.6	22(11)	38.7	84(42)	36.4
Group 3	≥51	20(10)	62.6	6(3)	59.7	26(13)	61.8
Total		140(70)	33.5	60(30)	31.4	200(100)	32.9

visiting Indira Gandhi Medical College Hospital Shimla for refraction or cataract surgery were selected at random after obtaining consent and clearance from ethics committee for research. The patients were divided into 3 age groups of young (13-25 years), middle aged (26-50 years) and elderly (≥51 years).

Methods Detailed ocular and systemic examination was conducted to rule out presence of surface infection or an ocular disease with special emphasis on erythema, oedema and discharge from the eyes at least for a fortnight before. Other exclusion criteria were topical antibiotic use in the recent past and known patients of a systemic illness or an immunodeficiency disease. Only one eye of a patient was randomly selected for the study. Conjunctival anesthesia was done by instilling 1-2 drops of 4% Xylocaine and a sample was collected by gently rubbing the conjunctiva of lower fornix from medial to lateral side with a sterilized cotton-wool swab stick moistened with a drop of sterile normal saline, taking care not to touch the lid margins and ensuring that individuals should not blink during the procedure. The sample was put in Brain-Heart Infusion and inoculated to laboratory where standard aerobic culture and identification methods were carried out. Statistical analysis was carried out on API software.

RESULTS

There were 140 males (70%) and 60 females (30%) in the three age groups (Table 1). Growths were obtained from 128 eyes (64%) and remaining 72 (36%) did not yield any organism (Table 2). Predominantly isolated aerobes were *Staphylococcus* spp. in 120(60%) followed by *Haemophilus* in 16 (8%), diphtheroids in 10 (5%), *Streptococcus viridans* (*S. viridans*) in 8 (4%) and *Escherichia coli* (*E. coli*) in 4 (2%) of culture positive eyes. Age group wise isolation rate of aerobes is shown in Table 3. A single aerobe was isolated from 98 eyes (49%) while 30 eyes (15%) yielded more than 1 aerobe (Table 4). *Staphylococcus epidermidis* (*S. epidermidis*) was the most common microorganism, found alone in 58 eyes (29%) and in combination with another aerobe in 30 eyes (15%), followed by *Staphylococcus aureus* (*S. aureus*) in 18 (9%) and 10 eyes (5%) respectively (Table 5).

DISCUSSION

The microflora of conjunctival sac has been investigated all over the world. In India, authors have studied conjunctival microflora mostly from hot planes of the country. But no

Table 2 Isolation rate of aerobes n(%)

Groups	n=200	Growth present	No growth
Group 1	90	58(64.4)	32(35.6)
Group 2	84	52(61.9)	32(38.1)
Group 3	26	18(69.2)	8(30.8)
Total	200	128(64)	72(36)

Table 3 Pattern of isolated aerobes n(%)

Micro-organism	Group 1, n=90	Group 2, n=84	Group 3, n=26	Total, n=200
<i>Staphylococcus</i> spp.				
<i>S. epidermidis</i>	42(46.7)	38(45.2)	8.0(30.8)	88(44)
<i>S. aureus</i>	8(8.9)	14(16.7)	6(23.1)	28(14)
<i>S. citreus</i>	2(2.2)	0(0.0)	2(7.7)	4(2)
Total <i>Staph.</i> spp	52(57.8)	52(61.9)	16(61.5)	120(60)
Others				
<i>Haemophilus</i> spp.	10(11.1)	6(7.1)	0(0.0)	16(8.0)
<i>Diphtheroids</i>	0(0.0)	2(2.4)	8(30.8)	10(5.0)
<i>S. viridans</i>	6(6.7)	2(2.4)	0(0.0)	8(4.0)
<i>E. coli</i>	2(2.2)	0(0.0)	2(7.7)	4(2.0)
Total aerobes	70(77.8)	62(73.8)	26(100.0)	158(79)

Table 4 Incidence of isolation of one or more aerobes n(%)

Group	Examined(n)	1 aerobe	>1 aerobe
Group 1	90	44(48.9)	14(15.6)
Group 2	84	42(50.0)	15(17.9)
Group 3	26	12(46.2)	6(23.1)
Total	200	98(49.0)	35(17.5)

Table 5 Isolation pattern of aerobic bacteria

Parameters	n(%)
<i>S. epidermidis</i>	58(29.0)
<i>S. aureus</i>	18(9.0)
<i>S. epidermidis</i> + <i>S. aureus</i>	10(5.0)
<i>S. viridans</i>	4(2.0)
<i>Haemophilus</i>	8(4.0)
<i>S. citreus</i>	4(2.0)
<i>E. coli</i>	2(1.0)
<i>Diphtheroids</i>	2(1.0)
Total <i>S. epidermidis</i>	88(44.0)
Total <i>S. aureus</i>	28(14.0)

such studies are available from climatically cold and geographically different hilly areas. Predictably all normal healthy conjunctival sacs do not yield a growth. However, coagulase negative staphylococci and diphtheroids constitute most of the isolates when a growth is obtained [4]. *Streptococcus pneumoniae*, *Haemophilus influenzae* and

other potential pathogens may also be recovered from otherwise an uninfected eye^[5]. Type of organisms present in normal conjunctiva differ widely for which climatic conditions are considered as one of the factors^[3].

The isolation rate of a growth varies widely. Smith^[6] in London found 47% conjunctival sacs sterile while McNatt *et al*^[7] found as low as 9%. In Indian subcontinent, Saleem *et al*^[8] and Puttana *et al*^[9] found as high as 85% and 72.9% sacs sterile respectively while Saxena and Goswami^[10] found none in their series. Liu *et al*^[11] found 62.23% of conjunctival sacs yielding a growth and rest 37.77 sacs sterile. In another study, Sinha and Das^[12] could find bacteria in 56.7% of 67 healthy eyes. In the present study, nearly 2/3rd of eyes yielded an aerobic growth and remaining were sterile. Our figure of 36% of sterile conjunctival sacs thus compares very well with the findings of Liu *et al*^[11].

The incidence of sterile sacs was nearly same in young and adult age groups (35.6% and 38.1%) respectively but decreased in elderly (30.8%), meaning thereby that latter have more tendency to harbor micro-organisms. However, the inter-age group variation in the three age groups was statistically not significant. Liu *et al*^[11] found that rate of positivity of growth increased significantly in elderly patients as compared to young and children. Khorazo and Thompson^[13] have also observed that conjunctival sacs in elderly are less often sterile than the young.

The most commonly isolated micro-organism was *Staphylococcus* spp. (60%) out of which *S. epidermidis* alone constituted 44% followed by *S. aureus* (14%). Among the other aerobes, *Haemophilus* spp. was the commonest aerobe (8%) followed by diphtheroids. Other aerobes were found in small numbers only. *Staphylococcus* spp. has been described in a vast range from different places-91% from Algeria by Orifila and Courden^[14] to as low as 6% from Manipal (India) by Srinivasa and Rao^[15]. In the present study also, Staphylococci were the most common isolated organism in 60% cultures which is same as found by Saleem *et al*^[8] from Pakistan.

Staphylococcus epidermidis is the commonest bacterium in normal conjunctival sacs reported universally. In the present study also, this was the commonest organism found in 44% eye followed by *S. aureus* in 14%. *Staphylococcus citrius* (*S. citrius*) was also found occasionally (Table 3). Rajvanshi *et al*^[16] have reported somewhat higher (53%) incidence for *S. epidermidis* but the same (14.7%) for *S. aureus*. Our figures also compare well with those of Khorqazo and Thompson^[13]. But Park *et al*^[17] found *S. epidermidis* accounting for 60.6% of isolates and Liu *et al*^[11] reported much higher incidence (76.07%) of *S. epidermidis* as well as of *S. aureus* at 20.51%. However, *S. aureus* was reported much lower-only 3.4% by Saxena and Goswami^[10] and 2.9%

by Sinha and Das^[12] respectively.

We found the incidence of *S. epidermidis* declined with increasing age. The incidence was minimum (30.8%) in elderly and nearly same (45.2%-46.7%) in middle and young age groups but that of *Staphylococcus aureus* was found to increase significantly from 8.9% in young to 16.7% in middle aged and further to 23.1% in elderly subjects. The variation in isolation rate of different species of Staphylococcus in the three age groups (Table 3) was significant ($P \leq 0.01$ ANOVA). It implied that with increasing age, the conjunctiva became somewhat hostile to *S. epidermidis* but hospitable to *S. aureus*. However the opposite has been reported by Liu *et al*^[11] -a high incidence of 94.64% of *S. epidermidis* in elderly, 83.87% in middle aged and considerably low-33.33% in children whereas for Puttana ST, D'Souza C, Bhargava MK *S. aureus*, incidence was same for children and elderly (26.67%-26.79%) but drastically low (3.23%) for middle aged.

S. epidermidis and *S. citreus* are commensal organisms in conjunctival sac but *S. aureus* is potentially more pathogenic. However, *S. aureus* and other pathogens may sometimes grow in the mucus film without causing eye disease^[16]. Relatively harmless members of *S. epidermidis* are commonly present on the skin, but the more dangerous *aureus* species is found on the hands of only a small proportion of healthy persons who on investigation are usually shown to be heavy nasal carriers^[18].

Corynebacterium xerosis, a diphtheroid, is considered a commensal organism of the normal conjunctiva. Its isolation rate has been reported from 31% eyes by Smith^[6], 32% by Orifila and Courden^[14] to 35.1% by Saxena and Goswami^[10]. However, we isolated diphtheroids only in 10 (5%) eyes, most of them from elderly age group individuals and none from young (Table 3).

A variety of other gram negative and gram positive micro-organisms are capable of a saprophytic existence in the conjunctiva. The most frequent offenders are pneumococci, diplobacilli of Mo-rax-Axenfeld, influenza bacilli, Koch-Weeks bacilli, streptococci and gonococci. Streptococci, diplobacilli and coliform organisms may occasionally grow in a similar fashion as normal flora^[19]. We found *Haemophilus* spp. in 8%, *Streptococcus viridans* in 4% and *E. coli* in 2% of culture positive eyes. We did not encounter *Pseudomonas* spp. in the present series although this organism has also been reported from normal eyes^[11,16,17].

We also observed that *Haemophilus* spp. and *S. viridans* were more prevalent in younger than middle aged individuals and were conspicuous by their absence in elderly. Conversely, diphtheroids were more prevalent in latter age group, with tapering incidence to zero towards younger age group. *E. coli* was not found in middle aged and incidence

was equal in other two age groups (Table 3). In their study of preoperative conjunctival sac flora on subjects undergoing cataract surgery, Suto *et al*^[20] also found these microflora in few sacs only, but the number of diphtheroids was more in comparison to *Haemophilus*, *E.coli* and other gram-negative bacilli.

Microorganisms are known to live in symbiosis with other types. We found more than one species of aerobes in many eyes. *S. epidermidis* and *S. aureus* were present as a single aerobe in 29% and 9% of culture positive eyes. In addition, *S. epidermidis* was found in combination with another aerobe in 15% eyes (Tables 4, 5). Saleem *et al*^[8] reported this combination as low as 4.5%. However, variation in isolation of more than 1 aerobe in 3 age groups was not significant. Rubio^[3] also isolated more than one bacterium in some patients' conjunctiva.

The variation of normal conjunctival flora as found in the present study can be attributed to age, geographical (high altitude), climatic conditions (relatively cold) and professional back ground of host population in this part of India. Since potential pathogenicity of ocular commensal organisms can occasionally result in severe postoperative morbidity including blindness, routine preoperative topical broad spectrum antibiotics administration is an appropriate prophylactic measure against it.

The limitations of this study are-a hospital based survey which does not truly represent a cross section of the population, and possibility of inclusion of an asymptomatic patient of diabetes mellitus, AIDS or any other disease leading to lowering of immunity, though apparently only healthy individuals were enrolled.

In conclusion, normal conjunctival flora in Shimla Hills shows considerable variation in its composition as compared to hot climatic conditions of the rest of country. Although *S. epidermidis* is the predominant aerobe, occurrence of diphtheroids is much less. *Pseudomonas* spp. was not found in any of the conjunctival sacs in this study. The characteristic variations in normal conjunctival flora can be attributed to geo-climatic conditions of the place and agriculture related profession. Common commensals of conjunctiva are also known to cause ocular infections after intraocular surgery, hence it is important to administer broad spectrum antibiotics for a few days prior to surgery as a preventive measure.

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