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Original Article

Aerobic bacterial flora of normal human conjunctiva

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Abstract:

Background and purpose: **The conjunctiva as a mucus membrane sustains permanent flora of indigenous bacteria which plays an important role to maintain normal conjunctival function. This study was carried out to see the pattern of conjunctival bacterial flora in healthy individuals. Methods: This prospective cross sectional study was carried out in Bangabandhu Sheikh Mujib Medical University during the period of January 2011 to December 2011. Total 400 conjunctival swabs were collected from both eyes of 200 healthy individuals those attending the Eye Out patient department (OPD) of BSMMU with complaints other than eye infections and their attendants. Results:** Out of 400 swabs, 95 (23.75%) **culture was positive in healthy individuals. *Staphylococcus epidermidis* (65.26%) was the predominant organism followed by *Moraxella sp.* and *Pseudomonas sp.* (7.37%). Unilateral culture positivity was higher (60%) than bilateral culture positivity (40%). Conclusion: The absence of growth in majority of the samples is because of the sterile nature of the normal conjunctiva.**

Key word: Conjunctival flora.

Introduction:

The surfaces of the body like the skin and mucous membranes are in contact with the environment. So they rapidly become colonized by organisms present in the environment which is termed to as normal flora[1]. The conjunctival sac is parasitized with microflora that changes dynamically through out lifetime because of its long-term exposure to the environment. These flora are part of the defense mechanism of the eye and prevent colonization by more pathogenic microorganisms[2]. Bacteria and fungi are considered as normal flora of conjunctiva. Viruses and parasites are not considered as the members of the normal flora[1].

The gram positive organisms; particularly *Staphylococcus epidermidis* (30-80%), *Micrococcus sp.* (1-28%), and *Diphtheroids* (5-83%) are the predominant flora of healthy conjunctiva. The less frequent microbial flora of conjunctiva includes *Staphylococcus aureus* (3-25%), *Streptococcus pyogenes* (0-3%), *Streptococcus pneumoniae* (0-3%), *Streptococcus viridans* (0-1%), *Moraxella catarrhalis* (5-83%), *Haemophilus influenzae* (0-1%), *Klebsiella sp.* (0-0.5%), *Pseudomonas aeruginosa* (0-2%) and *Escherichia coli* (0-1%). Anaerobic bacteria (0.33%) are occasionally present and 3 -15% population have fungal flora[3].

Bacterial infections are responsible for ocular disease. The source of bacteria can be from conjunctival sac. Thus it is vital to understand the role of commensals in these diseases. The present study was designed to see the pattern of conjunctival bacterial flora and to isolate their types in healthy individuals.

Materials and Methods:

It was a cross sectional observational type study carried out in BSMMU during the period of January 2011 to December 2011.

Study population: Healthy individuals were patients attending Eye OPD of BSMMU with complaints other than eye infections mostly refractive error and their

attendants who were non diabetic were enrolled in the study.

Sample collection: The patient was asked to look up; the inferior conjunctival fornix was swabbed, without touching eyelid or lashes. Two conjunctival swabs were collected from both eyes of the patient separately, one for culture and other for microscopy. The collected material was inoculated. When there was bacterial growth, cultures for isolation and identification of the bacteria were made[4].

Microbiological methods: Smears were prepared with one swab from each sample and Gram staining was performed to detect pus cells to exclude infection. Second swab was inoculated onto blood agar, chocolate agar, MacConkey agar, blood tellurite agar and Haemophilus selective agar media. Inoculated plates were incubated at 37ºC aerobically for 48 hours except Chocolate agar and Haemophilus selective agar plates which were incubated at 37ºC in candle extinction jar for 48 hours. After 48 hours colony morphology was observed and processed for further identification.

The isolated organisms were identified by standard microbiological procedure-colony morphology, Gram staining, pigment production and relevant biochemical tests (catalase, coagulase, novobiosin sensitivity, oxidase, MIU, mannitol fermentation, bile solubility, bile esculin test, rapid carbohydrate utilization test, growth factor requirement test, Haemophilus satellitism and butyrate esterase test)[5, 6, 7, 8, 9,1 0, 11].

Statistical analysis: SPSS (Statistical Package for Social Sciences) was used for data analysis. Chi-square test was applied to compare the study parameters of culture results. P-value ≤ 0.05 was considered statistically significant difference. P value < 0.05 was taken as minimum level of significance.

Results:

A total of 400 conjunctival swabs were collected from both eyes of 200 healthy individuals. Out of 400 swabs, 95 (23.75%) culture was positive in healthy individuals (23.75%). Unilateral culture positivity was higher (60%) than bilateral culture positivity (40%). (Table 1).

Table-1: Unilateral and bilateral culture positivity of conjunctival swabs in healthy individuals

Number of positive cultures	Unilateral culture positivity	Bilateral culture positivity
95 (23.75)	57 (60)	38 (40)

S. epidermidis (65.26%) was the predominant organism followed by *Moraxella sp.* and *Pseudomonas sp.* (7.37%). The less frequently isolated bacteria were *S. viridans* (6.32%), *S. aureus* (4.21%), *S. saprophyticus* (4.21%) and *Diphtheroids* (2.11%). (Table 2).

Table-2: Different bacterial species isolated from conjunctival swabs in healthy individuals.

Bacterial species	Healthy individuals (n=95)
S. epidermidis	62 (65.26)
S. aureus	4 (4.21)
S. saprophyticus	4 (4.21)
S. viridans	6 (6.32)
Diphtheroids	2 (2.11)
Moraxella sp.	7 (7.37)
Pseudomonas sp.	7 (7.37)
H. influenzae	3 (3.16)

Among healthy individuals no significant difference of culture positivity of conjunctival swab was observed in different age groups (Table 3).

Table-3: Culture positivity of conjunctival swab of healthy individuals in different age group.

Culture positivity of conjunctival swab in		
5-20 years	21-40 years	41-60 years
21 (21)	56 (26.19)	18 (20.93)

Among healthy individuals no significant difference of culture positivity of conjunctival swab was observed between male and females (Table 4).

Table 4: Culture positivity of conjunctival swab of healthy individuals according to sex.

Culture positivity of conjunctival swab in		P-value
Male	Female	
42 (23.6)	53 (23.87)	0.743

Figures in the parenthesis indicate percentage.

Discussion:

This study was conducted on both eyes of 200 healthy individual for isolation of conjunctival bacterial flora. Conjunctival bacterial flora isolated in healthy individual was 23.75%. In healthy person most microorganisms are usually removed by lacrimation, with only a relatively low-density microbiota being left behind, consisting of a reduced number of species[13].

In the present study, *S. epidermidis* (65.26%) was the predominant organism in healthy individuals. Similar findings were also observed by Javed *et al*[14] and Quadeer *et al*[15] 53.85% and 54.5% respectively. This frequent isolation might be due to environmental exposure, physical contact or unhygienic habits of the people allowing them to flourish on the skin, eye lids and mucous membrane etc[14].

Diphtheroids (5-83%)[3] is considered as the second most normal flora of conjunctiva. Quadeer *et al*[14] (23.6%) and Javed *et al*[15] (28.21%) observed similar higher isolation rate of *Diphtheroids* in healthy individuals which was not consistent with our study. The lower isolation rate of *Diphtheroids* (2.11%) in our study may be due to faster replicating bacteria such as *S. aureus* which causes the inhibition of slowly growing bacteria like *Diphtheroids* on the basis of depleted nutrition and living space[16].

The other predominantly isolated gram positive cocci were *S. viridans* (6.32%), *S. aureus* (4.21%), *S. saprophyticus* (4.21%), *Diphtheroid* (2.11%). The result showed similarity with a study in 2006 which showed an isolation frequency of *S. aureus* (8%), *S. saprophyticus* and *Diphtheroid* (2%) except *S. viridians*[17]. In present study higher isolation rate (7.37%) of *Pseudomonas sp.* than that of normal (0-2%) meant transient nature of normal flora & *S. saprophyticus* (4.21%) in conjunctiva might be due to contamination from environment & eyelid.

Unilateral culture positivity (60%) was higher than that of bilateral culture positivity (40%) of healthy individual. In China (2011), the isolation frequency from bilateral eyes was predominately (86.67%) higher than that of unilateral in elder groups [18]. But in our study bacterial isolation rate was less frequent in bilateral eye (40%) which is due to the intact local defense of the eyes in that age group and thereby preventing the fixation of invading microorganism.

In this study age does not influence the conjunctival bacteria significantly. This is because of small sample size & short duration of sample collection time (shorter than a complete year) which could not have the whole possible bacteria. A sample containing less than a few consecutive, is also not suitable for studying the influence of age in the conjunctival bacteria[21].

Study in China (2011), the elder people (68-78 yrs) showed a higher isolation rate (93.33%) than that of children and young (9-22 yrs) (45 - 45%). This differences in the conjunctival flora between adults and children is explained by several potential mechanisms include age-related changes in conjunctival normal flora, tear deficiency with age, goblet cell changes, and lipid dysregulatory states of eye[19, 20,21].

Among healthy individuals, no significant difference of culture positivity of conjunctival swab was observed between male and females in this study. A study in Pakistan (2008) showed higher percentage (68.15%) of normal conjunctival flora in female patients than the male patients (31.91%) which was not consistent with this study [15].

Conclusion:

This study highlights distribution of the normal flora of conjunctiva. A relationship may exist between resident normal flora and the etiology of ocular infections. These indigenous bacteria may become opportunistic and pathogenic in any immunosuppressed condition. A thorough knowledge about the conjunctival flora of healthy eyes is essential for ophthalmologists. It can help in interpretation of clinical culture results and in management of potential pathogens colonizing the ocular surface.

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