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Original Article

Conjunctival bacterial flora in healthy individuals and health care workers (HCWs)

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Abstract

This study was designed to see the pattern of conjunctival bacterial flora in healthy individuals and health care workers. This prospective study was conducted during the period of January 2011 to December 2011 in BSMMU. A total of 480 conjunctival swabs were collected from both eyes of 200 healthy individuals and 40 HCWs of BSMMU. Significant number of culture was positive in HCWs (52.5%) compared to healthy individuals (23.75%). Staphylococcus epidermidis was predominant in healthy individuals (65.26%) and HCWs (66.66%). Isolation of Enterocooci (11.9%) was significantly higher in HCWs. Bilateral culture positivity was significantly higher in HCWs (71.43%) than that from healthy individual (40%) and in healthy individuals, unilateral culture positivity was 60%, which was higher than that of HCWs (28.57%).

Key word: Conjunctival flora, HCWs.

Introduction

The conjunctiva is a thin semitransparent mucous membrane that covers the posterior surface of the eyelids and is then reflected onto the eyeball[1]. An intact conjunctival epithelium helps in preventing most microbes from invading the conjunctiva. Blinking sweeps debris and organism from conjunctiva[2]. Tear contains lysozyme, IgA, IgG, ß-lysine, lactoferrin and complement which serves as a defense against microorganisms[3].

The predominant microbial organisms retrieved from normal uninfected conjunctiva are *Staphylococcus epidermidis, Corynebacterium sp., Staphylococcus aureus* and *Micrococcus sp. Streptococcus sp., Moraxella catarrhalis, Haemophilus influenzae, Klebsiella sp., E. coli* and *Pseudomonas aeruginosa* are occasionally found[4].

Factors such as age, dry eye, contact lens use, immunocompromising disease, medications and environmental factors may affect the normal flora of conjunctiva[5]. Alteration of conjunctival flora is noticed in persons who work in hospital environments and the constant exposure to this environment may increase the possibility of developing diseases[6].

Patients, hospital staff and visitors represent the primary reservoir of normal human microbiota in the hospital. The secondary reservoirs include hydrotherapy and dialysis equipments, equipment fitted with air humidifiers, nebulizers. Intensive use of chemotherapeutic antibiotics also allows transmission of infections[6].

Cross-infection may occur through contaminated instruments, hands, contaminated irrigation solutions and disinfectants[7]. Other risk factors are low immunity, malnutrition, general disease and extremes of age[8]. Eye infection from the patients can be spread by HCW through simple social greeting of patients such as shaking of hands, during physical examination or patient care and or through contaminated instruments. These HCWs may act as a reservoir and transmit the infection to other patient. HCWs who carry the modified conjunctival flora may act as a source of hospital acquired and laboratory acquired infections[9]. By maintaining the personal hygiene, hospital and laboratory staff can minimize the risk associated with infectious microorganisms [10].

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Therefore, the present study was designed to see the pattern of conjunctival bacterial flora in healthy individuals and HCWs and alteration of the pattern of conjunctival bacterial flora in HCWs.

Material and methods

Ethical aspects: Ethical clearance was taken from Institutional Review Board of BSMMU and was approved on 17/10/11.

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

Study population: Patients attending Eye OPD with complaints other than eye infections and their attendant who were non diabetics were selected as healthy individuals. HCWs were selected from the department of Ophthalmology, Microbiology and Immunology and Surgery, BSMMU (physician, nurse, ward boy, laboratory technician) with no clinical evidence of occular infections in previous 3 months and without history of diabetes mellitus.

Sample collection: A prior consent was obtained before taking eye culture swabs from each subject. With two moistened sterile cotton tipped applicators without touching the eyelid margins or lashes conjunctival swabs were collected from both eyes of the patient separately. Two swabs were collected from each eye, one for culture and other for microscopy[11].

The collected swabs were placed in sterile test tube and immediately transported to Microbiology and Immunology Department, BSMMU for further processing and identification.

Microbiological methods:

Culture: 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. Chocolate agar and Haemophilus selective agar plate were incubated at 37ºC in candle extinction jar for 48 hours. After 48 hours colony morphology was observed and processed for further identification[12].

All the 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[12-16, 17, 18].

Statistical analysis: Statistical analysis was performed using chi-square test. The data obtained from healthy individuals and HCWs were compared by Chi-square (x^2) test. P value < 0.05 was taken as minimum level of significance.

Rusults

A total of 480 conjunctival swabs were studied from 240 participants. Of the conjunctival swabs, 400 were collected from both eyes of 200 healthy individuals and 80 from 40 HCWs. Significant number of culture were positive in HCWs (52.5%) compared to healthy individuals (23.75%) Bilateral culture positivity was significantly higher in HCWs (71.43%) and was also significantly higher than that from healthy individual (40%). In the healthy individual, unilateral culture positivity (60%) was higher than that of HCWs (28.57%). (Table I)

Table I : Unilateral and bilateral culture positivity of conjunctival swabs in healthy individuals and HCWs

Study group	Number of positive cultures	Unilateral culture positivity	Bilateral culture positivity
Healthy individuals	95 (23.75)	57 (60)	38 (40)
HCWs	42 (52.5)	12 (28.57)	30 (71.43)

Significant proportion of bilateral culture positivity in HCWs. (P < 0.001)

S. epidermidis was most commonly isolated bacteria in healthy individuals (65.26%) and HCWs (66.66%). *S. aureus* (4.21%), *S. viridans* (6.32%), *S. saprophyticus* (4.21%) and Diphtheroids (2.11%) were isolated in healthy individuals only. *Enterocooci* (11.9%) was isolated only in HCWs. No significant difference was observed among isolation rate of *Pseudomonas* sp., *Moraxella* sp. and *H. influenzae* in between groups. (Table II)

Table II : Different bacterial species isolated from conjunctival swabs in healthy individuals, diabetic patients and HCWs

Bacterial species	Healthy individuals (n=95)	HCWs (n=42)
S. epidermidis	62(65.26)	28(66.66)
S. aureus	4 (4.21)	0 (0)
S. saprophyticus	4 (4.21)	0 (0)
S. viridans	6 (6.32)	0 (0)
Diphtheroids	2 (2.11)	0 (0)
Moraxella sp.	7 (7.37)	2 (4.76)
Pseudomonas sp.	7 (7.37)	5 (11.90)
H. influenzae	3 (3.16)	0 (0)
E. coli	0 (0)	1 (2.38)
Enterobacter	0 (0)	1 (2.38)

Figures in the parenthesis indicate percentage.

HCWs showed significant culture positivity (50%) than healthy individuals (26.19%) in between 21-40 years age group. In

between 41-60 years group HCWs (65%) showed significant culture positivity than healthy individuals (20.93%) (Table III)

Table III : Culture positivity of conjunctival swab of healthy individuals, diabetic patients and HCWs in different age group

Study population	Culture positivity of conjunctival swab in		
	5-20 years	21-40 years	41-60 years
Healthy individuals (n=95)	21 (21)	56 (26.19)	18 (20.93)
HCWs (n=42)	0 (0)	29 (50)	13 (65)

Figures in the parenthesis indicate percentage

The bacteria isolated from Department of Ophthalmology were *Pseudomonas* sp. (50%) followed by *S. epidermidis* (40%) and *Moraxella* sp. (10%). *Enterococci* (55.56%) was the most frequently isolated bacteria from Department of Surgery followed by *S. epidermidis* (22.22%), *E. coli* and Enterobacter (11.11%). In Microbiology and Immunology department, *S. epidermidis* (95.65%) and *Moraxella* sp. (4.35%) were isolated (Table IV).

Table IV : Bacterial species isolated from HCWs in different departments of BSMMU

Bacterial species	Ophthalmology department (n = 10)	Microbiology & Immunology department (n = 23)	Surgery department (n = 9)
S. epidermidis (n = 28)	4 (40)	22 (95.65)	2 (22.22)
Pseudomonas sp (n = 5)	5 (50)	0 (0)	0 (0)
Moraxella sp (n = 2)	1 (10)	1 (4.35)	0 (0)
Enterococci (n = 5)	0 (0)	0 (0)	5(55.56)
E. coli (n = 1)	0 (0)	0 (0)	1 (11.11)
Enterobacter (n = 1)	0 (0)	0 (0)	1 (11.11)

Figures in the parenthesis indicate percentage.

Of the 27 follow up samples collected after one month of first collection from the HCWs, 7 (25.93%) showed no growth, 6 (22.22%) showed bacteria of same genus and 14 (51.85%) showed alterations involving bacteria of different genera compared to those isolated in the first collection (Table V).

Table V : Pattern of conjunctival bacterial flora of 27 culture positive HCWs during follow up collection after one month of the first collection

Conjunctival bacterial flora in follow up HCWs	Number of HCWs (n=27)
Same organism	6 (22.22)
Alteration	14 (51.85)
No growth	7 (25.93)

Figures in the parenthesis indicate percentage.

Discussion

Conjunctival flora develops soon after birth, and its composition depends on age, seasonal variation, environmental conditions, immune status, and general hygienic conditions[19]. Alteration of conjunctival flora is also observed in persons who work in hospital environments. The microbiota of the hospital environment is rich in pathogens and continuous exposure in health care setting may lead to infections[6].

This study was conducted on both eyes of 200 healthy indiduals and 40 HCWs for isolation of conjunctival bacterial flora. Conjunctival bacterial flora isolated in healthy individual was less (23.75%) than HCWs (52.5%). 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 species6. HCWs showed significant number of culture positivity (52.5%) compared to healthy individuals (23.75%). This result may be due to the continuous contamination of the conjunctival sac of the HCWs by the hospital environment. In hospital, infection may be transmitted from patient to staff, staff to patients, patient to patient and staff to staff by direct contact, aerosol formation or contamination of equipment or instruments in the practice[9]. Resident flora may temporarily become part of the transient flora as a consequence of any environmental alteration. However, when a there is breach in the equilibrium between the resident and transient flora, disease process may progress[6]. No study was found previously where culture positivity of HCWs was compared to healthy individuals.

In the present study, S. epidermidis was the most commonly isolated bacteria in HCWs (66.66%) and healthy individuals (65.26%). Similar findings were also observed by Javed et al (53.85%) 20and Quadeer et al [21](54.5%) where they isolated S. epidermidis in healthy individuals in their study. The possible explanation of 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 [20].

Bilateral culture positivity was significantly higher in HCWs (71.43%) than that from healthy individual (40%). Unilateral culture positivity was significantly higher in healthy individual (60%). The bilateral culture positivity in HCWs may result from frequent exposure to antibiotics or contact with health care settings.

The higher isolation rate of conjunctival bacteria in HCWs

(50%) in 21-40 years age and in (65%) 41-60 years age group in this study may be explained by several potential mechanisms such as weakened immune response and tear deficiency due to old ages, patterns of exposure to bacteria, contamination from environment or adjacent areas such as skin or upper respiratory tract, contact with health care setting [22, 23, 24]. The bacterial isolation rate was less frequent in healthy individual (20.93%) than HCWs (65%) which can be explained by the intact local defense of the eyes in that age group and thereby preventing the fixation of invading microorganism[6].

Pattern of bacterial species isolated from HCWs in different departments of BSMMU were studied. Isolation of *S. epidermidis* (66.66%) was highly significant in HCWs. Trindale and his associate6 reported *S. epidermidis* (45%) to be the most frequently isolated bacteria from all the sectors of hospital which was consistent with our study. Isolation of *Enterocooci* (11.9%) was highly significant in HCWs of Surgery Department. Other organisms isolated were *Pseudomonas* sp (11.9%), *Moraxella* sp. (4.76%), *E. coli* (2.38%) and *Enterobacter* (2.38%) though their isolation was not statistically significant. *E. coli, Enterobacter* and *Enterocooci* are the modified conjunctival flora because they are not found in normal healthy individual. In hospital settings, antimicrobial treatment may lead to a large modification in the endogenous flora[25]. Trindale *et al*.,[6] isolated modified conjunctival bacteria from all the sectors of hospital and found *Bacillus* sp. (29.0%), *Proteus* sp. (6.1%), *Enterobacter* sp. (4.1%), *Alcaligenes* sp. (4.1%), *Citrobacter* sp. (2.1%) and *P. mirabilis* (2.1%) which was not consistent with our study.

Pseudomonas sp. (50%) was the most frequently isolated bacteria from Ophthalmology Department followed by *S. epidermidis* (40%) and *Moraxella* sp. (10%). Though *Pseudomonas* sp. is considered as normal flora of conjunctiva but its higher isolation rate may be the source of nosocomial infection. Any mucosa and skin of hospitalized patients can be colonized with *P. aeruginosa*[26]. *Enterococci* (55.56%) was the significantly isolated bacteria from Surgery Department. *E. coli* and *Enterobacter* also isolated from Surgery Department though the number was insignificant. The modification of conjunctival flora may be due to repeated exposure of the organisms in the hospital settings[22]. In Microbiology and Immunology department, *S. epidermidis* (95.65%) was the most frequently isolated bacteria followed by *Moraxella* sp. (4.31%).

Conclusion:

HCWs have significant number of positive conjunctival cultures in comparison to healthy individual. *S. epidermidis* was the most common organism in both groups. HCWs are more exposed to hospital environment and also to microorganisms. They should be aware of basic infection control principles like hand washing, contact isolation and barrier nursing and should take measures for preventing or the emergence of resistance by the judicial use of antibiotics

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