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BACTERIAL FLORA OF CONJUNCTIVAS OF NORMAL AND DISEASED INDIVIDUALS

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ABSTRACT

A variety of organisms were isolated from conjunctivas of 32 subjects, to evaluate the occurrence of different gram positive and gram negative bacteria in normal and diseased subjects. The specimens were obtained from individuals belonging to 2-61 years of age which were divided into 6 groups with respect to age. One hundred and six isolates were obtained from 64 specimens. Thirty eight (36%) specimens revealed single isolates whereas double, triple and quadruple isolates were obtained from 30%, 13% and 8% specimens, respectively. Culture results were negative for 13% specimens. Characterization of ocular flora isolates to genus level revealed that *Staphylococcus* (64%) was the most frequent genus isolated from conjunctivas. *Micrococcus* (10%)turned out to be the second most prevalent genus followed by *Bacillus* (7%), *Streptococcus* (5%), *Corynebacterium* (5%), *Pseudomonas* (3%), *Moraxella* (2%), *Neisseria* (1%), *Branhamella* (1%), *Acinetobacter* (1%), *Hemophilus* (1%) and *Klebsiella* (1%). The incidence rate of overall number of isolates with respect to sex was noted as 34/106 in males and 72/106 in females. The number of isolates obtained in different age groups was 5, 10, 43, 8, 26 and 14 from age groups of 2-11, 12-21, 22-31, 32-41, 42-51 and 52-61 years, respectively.

Key words: Conjunctiva, ocular flora, *Staphylococci*.

INTRODUCTION

The term normal microbial ocular flora can be defined as the population of microorganisms that are the residents of the eye of healthy individuals. The diversity and prevalence of these commensals varies from person to person on the basis of their dietary habits, their age and environmental and geographical condition (Brooks *et al.*, 2002). Normal commensal bacteria play very significant role in defense mechanism of eyes by competing for nutrition and space with pathogenic bacteria. They also produce certain substances, which make the conditions unfavorable for the growth of other bacteria that may be pathogenic (Khan *et al*, 2004).

Any alterations or imbalance of these organisms may lead to opportunistic infection, which is caused by this normal micro biota. Eyes are also constantly exposed to the environment therefore it is the first source of acquiring infection after that contaminated water used for washing and bathing may also contribute (Pelczar *et al.*, 1993). There are many risk factors that facilitate pathogenicity and opportunistic conversion of the normal flora and make the conditions favorable for other pathogenic bacterial growth. These predisposing factors include chemical burn, glaucoma i.e. increased pressure of interlobular fluid, corneal transplantation, trauma, implantation of intraocular lens, diabetes, haematogenous spread from distant infection site, obstruction in tears and mucous secretions, immunocompromized state of a person, use of contact lenses, twig of trees, flying insects and swimming in stagnant or contaminated swimming pools (Sechi *et al*., 1999; Sankaridurg *et al.*, 2000; Lakkis and Fleiszig, 2001; Badenoch *et al.*, 2002; Namdari *et al*., 2003; Basak *et al.*, 2005). It is an established fact that gram positive organisms are mostly found as commensals as compared to gram negative organisms. *Staphylococcus* particularly *S. epidermidis* is the most frequent which is followed by *Corynebacterium*. Beside these, streptococcal species, gram negative bacilli, *Neisseria*, anaerobic *Propionibacterium*, *Hemophilus influenzae* and diphtheroids are also reported as normal flora in 26% cases. *S.aureus* is also some times considered as normal flora of eye because of the close contact with the skin of lid margin (Khan *et al.*, 2004). There are different types of eye infections, which are caused by multiple, or a particular type of bacterial species. Studies on bacterial etiological agent of ocular infection indicates that gram positive cocci constituted 55.6%, which is followed by gram positive bacilli 13.1%, gram negative cocci accounted for 2.8% and gram negative bacilli or rods 28.5%. Prevalence of gram positive coagulase negative *Staphylococcus* (CoNS) mainly S. *epidermidis* have been reported as 25.3% then *Pseudomonas* 18.8% followed by *Micrococcus* 11.7%, *Corynebacterium* 10.1% and *Staphylococus aureus* 8.2% (Tritz, 1999; Sun *et al*., 2002). Involvement of other causative agents is also reported like *Chlamydia trachomatis* causes trachoma (Stevens *et al*,2004), *Neisseria gonorrhoea* causes gonococcal conjunctivitis, *H.influenzae* causes conjunctivitis in children as well as in contact lens users (Tritz, 1999), *Serratia ficaria* (Badenoch et al, 2002), *Bacillus cereus* (Callegan *et al*., 1999), and *Proteus* species (Tritz, 1999) cause endophthalmitis, *Rhizobium radiobacter* causes chronic endophthaomitis (Namdari *et al*., 2003), *Streptococus pneumoniae* and other alpha and beta haemolytic streptococci and *Serratia marcescens* are involved in corneal infilterative events (Tritz, 1999; Sankaridurg *et al*., 2000).

Staphylococus aureus is the most important pathogen on the basis of its occurrence and incidence rate. *Staphylococus aureus* is gram positive and coagulase positive organism. Infections of cornea by *S. aureus* often involve the spread of bacteria from the skin or lid margin to the tear film and then to cornea. It is the most common cause of bacterial eye infection i.e. 97% of all bacterial eye infections however it is also found as normal flora in about 6.7% individuals (Modarres *et al*., 1998; Khan *et al*., 2004). It is involved in conjunctivitis (Modarres *et al*., 1998), suppurative keratitis (Tritz, 1999; Basak *et al*., 2005), periorbital cellulites (Tritz, 1999), acute dacryocystitis, endophthalmitis, and chalazia (Tritz, 1999). The second important etiological agent is *Staphylococcus epidermidis*, which is a gram-positive coagulase negative organism and also the inhabitant of eye as normal resident flora in 51.7% individuals (Khan *et al*., 2004) but it is also responsible for 77% ocular infections (Modarrses *et al*., 1998). It is an ubiquitous organism and possess relatively low virulence as compared to *Staphylococus aureus* and commonly cultured from the conjunctiva and lid margins of normal subjects. Its significant role has been reported in several ocular external diseases such as corneal infection, blepharitis and suppurative keratitis i.e. eye lid conjunctival or corneal infection. It is also a major cause of infections in contact lens users (Callaghan *et al*., 1997).

Pseudomonas aeruginosa is an opportunistic pathogen and most common cause of eye infections. Different infections like suppurative keratits, chalazia, hordeola, periorbital cellulitis and bacterial conjunctivitis are etiologically associated with *Pseudomonas aeruginosa* (Tritz, 1999; Basak *et al*., 2005). It is also a leading cause of lens related infections i.e. 70% of microbial keratitis associated with contact lens wearers involve *Pseudomonas aeruginosa*. They can gain access to the eye via contaminated lens, lens case and lens care solutions and could stimulate corneal epithelial cells to release chemotactic factors, especially recruite polymorphonuclear cells, indicates a mechanism of producing corneal infiltration (Modarres *et al*., 1998). Tear fluid shows no antibacterial activity after prolonged contact, suggesting ability of bacteria to degrade tear film components thus may result in corneal infiltrative events and contact lens acute red eye (Sankaridurg *et al*., 2000) which in complication may result in blindness.

Haemophilus influenzae also have importance in ocular infections. It is responsible for chronic dacryocystitis, keratitis (Tritz, 1999) and contact lens associated ocular inflammation. It is responsible for 43% of infection and contributes to 80% of conjunctivitis in children (Sankaridurg *et al*., 1996). *Corynebacterium diphtheriae* and *Corynebacterium xerosis* are morphologically identical and encountered as resident flora of conjunctiva very frequently (Miller, 1978). It is responsible of causing membranous conjunctivitis, which is termed as diphtheritic conjunctivitis. In conjunctivitis patients it is found in 13.4% of cases (Miller, 1978). *Streptococcus pneumoniae* is also major cause of eye infections like conjunctivitis (Baron *et al.*, 1994; Tritz, 1999).

The aim of this study was to compare the bacterial distribution in conjunctivas of normal and diseased individuals.

MATERIALS AND METHODS

SUBJECTS

Eye swabs were collected from both the eyes of 32 (21 female and 11 male) individuals aged between 2-61 years from Fatima Eye Hospital Karachi. The subjects were categorized in two groups as normal and diseased individuals.

COLLECTION OF SPECIMEN

Eye swabs were collected in duplicates from the left and right eyes of the individuals from Fatima Eye Hospital.

CULTURE FOR PRIMARY ISOLATION

Blood agar, Chocolate agar and MacConkey agar media were used for the primary isolation of the organisms. The swabs with specimen were inoculated by rolling the swabs over the area of primary inoculation and then streaking was performed with the wire loop for isolation purpose by clock streak method (Sonnenwirth and Jarett, 1980). The inoculated chocolate agar plates were incubated at 35-37°C for 24-48 hours in the presence of CO_2 in the candle jar. Blood agar and MacConkey agar plates were incubated at 35-37°C for 24-48 hours in aired atmosphere. After incubation the isolated colonies were sub cultured on nutrient agar medium or blood agar medium to obtain pure culture for characterization.

CHARACTERIZATION OF THE ORGANISMS

All the pure cultures were characterized to species level by using different tests conforming with required standard diagnostic criteria as described by Sonnenwirth and Jarett, (1980); Baron *et al.* (1994) and Cheesbrough (2000).

RESULTS AND DISCUSSION

Normal flora is a collective term used for all microorganisms residing normally in or on the healthy human body as a permanent resident (Brooks *et al.*, 2002). Human eye is bacteriologically not a sterile part of the body and harbors a variety of organisms normally. It has been reported that coagulase negative staphylococci are the main residents of the eye and other predominant organisms encountered are *Staphylococcus aureus, Corynebacterium* (diphtheroids), *Streptococcus* (non-hemolytic), *Neisseria, Moraxella* and *Hemophilus* species (Brooks *et al*., 2002; Khan *et al.*, 2004). Other organisms may be present occasionally (Pelczar *et al*., 1993). It has been reported that the normal flora which acts as opportunists can cause different ocular infections like conjunctivitis, cellulitis, keratitis, blephritis, dacryocystitis, endopthalmitis etc. (Tritz, 1999; Khan *et al.*, 2004; Basak *et al*., 2005).

Sixty four (64) specimens from 32 subjects were collected from conjunctivas of individuals aged between 02-61 years. Out of 64, male subjects were 11 from which 22 (34.4%) specimens were collected and 42 were females from which 84(65.6%) specimens were collected (Table I). The specimens were categorized into two groups. Seventeen (53%) specimens were collected from normal individuals and 15 (47%) specimens were representative of diseased individuals (Table II).

In this study it was found that coagulase negative staphylococci had highest incidence rate (39%) in normal individuals followed by *Bacillus firmus* (11%). *Kocuria varians* and *K. kristinae* formerly known as *Micrococcus varians* and *M. kristinae*, respectively; *Corynebacterium* species, *Neisseria* species, *Hemophilus* species and *Branhamella* species were also recovered. *Pseudomonas aeruginosa* was recovered from 7% specimens of normal individuals. Bacillus species were also recovered in this study, although they have not been reported as the normal flora of the eye but since eye is the most important environmentally exposed part of the body, the environmental flora can be recovered from the eye. In the present study, coagulase negative staphylococci were isolated in the highest numbers (58%) from diseased subjects, followed by *Staphylococcus intermedius* (10%), *Micrococcus* (10%), *Staphylococcus aureus* (2.4%), *Streptococcus pyogenes* (2.4%), *Moraxella osleonsis* (2.4%) and *Corynebacterium* (2.4%) . These results are in fair correlation with the study carried out by Sun *et al*., (2002) who reported 55.6% of gram positive cocci, 13.1% of gram positive bacilli, 2.8% of gram negative cocci and 28.5% of gram negative bacilli in bacterial ocular diseases. Among these, coagulase negative staphylococci were the most common (25.3%) followed by *Pseudomonas* (18.8%), *Micrococcus* (11.7%), *Corynebacterium* (10.1%) and *Staphylococcus aureus* (8.2%).

Among the staphylococcal species, *S. warneri* was obtained in the highest number (12.3%) in the present study and *S. epidermidis* (9.4%) was observed as second highest in occurrence. *S. lugdunensis* and *S. intermedius* were also found in similar numbers as *S. epidermidis* (9.4% each). These were followed by *S. simulans*, reported as 6.6%. It is occasionally found as normal flora on the skin, so it may be introduced in the eye tnrough skin. *S. auricularis* had an incidence rate of 5.7%. The next species in occurrence was *S. schleiferi* (3.7%). In the present study, incidence of *S. aureus* was 2% and was isolated from diseased cases only. It is considered as nosocomial as well as opportunistic pathogen (Ieven *et al*., 1995). *S. hemolyticus, S. capitis, S. saprophyticus* and *S. saccharolyticus* were also isolated. *S. hemolyticus* has been reported in a variety of human infections such as septicemia, conjunctivitis, urinary tract infections and wound infections.

Gram positive cocci other than Staphylococci which were obtained in this study were Micrococcus species and Streptococcus species. *Streptococcus pyogenes* was recovered in 3% infected individuals. It is considered as normal flora. The incidence of Micrococcus species i.e., *M. nishinomyaensis, K. varians, K. kristinae* and *M. sedentarius* was observed as 4%, 4%, 2% and 1% respectively. Micrococcus and Kocuria species have been recovered from various clinical specimens e.g., bacteremia, meningitis. Although they have been isolated frequently but their clinical relevance is questionable and mostly considered as contaminants (Basaglia *et al*., 2002).

Corynebacterium species have been reported in many studies as an ocular pathogen like conjunctivitis (Baron *et* al., 1994; Sun *et* al., 2002; Basak *et al*., 2005). They have been reported as normal flora in 26.6% individuals (Khan *et al.*, 2004). In the present study it constituted about 7% of isolates from normal specimens and 5% from diseased individuals.

Pseudomonas aeruginosa, Neisseria subflava, Morexalla osloensis, Hemophilus aprophilus, Klebsiella rhinoscleromatis, Branhamella catarrhalis and *Acinetobacter calcaoceticus* were the gram negative organisms isolated in the present study (Table III). *Ps. aeruginosa* was isolated from 3% of all the specimens. Although *Ps. aeruginosa* is a common habitant of soil and water, it is associated with a variety of human infections ranging from superficial skin infections to acute infections of damaged sites such as eyes and invasion of tissues through severe burns and wounds and it is also an important nosocomial pathogen (Liang *et al.*, 2001). It has also been reported as a common pathogen in ocular infections like karatitis, corneal ulcer suppurative keratitis, corneal infilterative events (Modarres *et al*., 1998; Liang *et al*., 2001; Sun *et al*., 2002; Bonjar *et al*., 2004; Basak *et al*., 2005) and most

commonly in contact lensassociated infections because it has been found to be resistant to most of the lens care solutions (Sankaridurg *et al*., 2000; Lakkis *et al*., 2001). In the present study, *Ps. aeruginosa* was isolated from normal and post operative specimen indicating that the individual is at high risk of establishing infection. Other than *Ps. aeruginosa*, *Moraxella osleonsis*, *Branhamella catarrhalis*, *Haemophilus aphrophilus*, *Klebsiella rhinoscleromatis* and *Acinetobacter calcoaceticus* have been recovered in the present study. Moraxella species and *Branhamella catarrhalis* are considered as normal flora of the eye as well as frequently isolated pathogen of the eye (Buchman *et* al., 1993; Khan *et al*., 2004). Berrocal *et al.* (2001) recovered *Moraxella* species from 1.3% cases of bacterial endopthalmitis including *M. catarrhalis* and *M. osloensis* and Buchman *et al.* (1993) reported the first case of central venous cathetar infection caused by *M. osloensis*. However, bacterial flora tends to vary due to different geographical and cultural conditions.

Table 1. Distribution of individuals with respect to age and sex.

S.NO	AGE (years)	MALE		FEMALE		TOTAL	
		NO.	%AGE	NO.	%AGE	NO.	%AGE
1.	02 – 11	1	3.2	2	6.2	3	9.4
2.	12 – 21	2	6.2	2	6.2	4	12.4
3.	22 – 31	3	9.4	10	31.2	13	40.6
4.	32 – 41	2	6.2	1	3.2	3	9.4
5.	42 – 51	2	6.2	3	9.4	5	15.6
6.	52 -61	1	3.2	3	9.4	4	12.6
	TOTAL	**11**	**34.4**	**21**	**65.6**	**32**	**100**

Table 2. Categorization of individuals on the basis of clinical findings.

S.NO.	CATEGORIES	NO. OF SUBJECTS					
		MALE		FEMALE		Total	
		NO.	%	NO.	%	No.	%
1.	Normal	5	15	12	38	17	53
2.	Diseased	6	19	9	28	15	47
	Total	**11**	**34**	**21**	**66**	**32**	**100**

Table 3. Comparison of incidence rate of organisms isolated from different groups of specimens.

S.No.	ORGANISM	NORMAL		DISEASED		TOTAL	
		No.	%AGE	No.	%AGE	No.	%AGE
1.	Coagulase Negative Staphylococci	11	10	45	42	56	52
2.	*Staphylococcus aureus*	-	-	2	2	2	2
3.	*Staphylococcus intermedius*	2	2	8	10	10	9
4.	*Micrococcus nishinomyaensis*	-	-	4	5	4	4
5.	*Micrococcus varians*	2	2	2	2	4	4
6.	*Micrococcus kristinae*	2	2	-	-	2	2
7.	*Micrococcus sedentarius*	-	-	1	1	1	0.9
8.	*Streptococcus morbillorium*	-	-	4	4	4	4
9.	*Streptococcus pyogenes*	-	-	1	1	1	0.9
10.	*Bacillus firmis*	3	3	1	1	4	4
11.	*Bacillus sphaericus*	-	-	2	2	2	2
12.	*Bacillus coagulans*	1	1	-	-	1	0.9
13.	*Corynebacterium pseudodiphtheriticum*	1	1	3	3	4	4
14.	*Corynebacterium mycetoides*	1	1	-	-	1	0.9
15.	*Pseudomonas aeruginosa*	2	2	1	1	3	2.8
16.	*Neisseria subflava*	1	1	-	-	1	0.9
17.	*Branhamella catarrhalis*	1	1	-	-	1	0.9
18.	*Moraxella osloensis*	-	-	2	2	2	2
19.	*Acinetobacter calcaoceticus*	-	-	1	1	1	0.9
20.	*Hemophilus aprophilus*	1	1	-	-	1	0.9
21.	*Klebsiella rhinoscleromatis*	-	-	1	1	1	0.9
	TOTAL	**28**	**-**	**77**	**-**	**106**	**100**

In the present study, the bacterial flora of the conjunctiva was compared among healthy and diseased individuals and isolation among male and female subjects. There was no significant difference in the bacterial flora among the male and female individuals, indicating that gender of the individual does not influence the rate of colonization. However, potential pathogens like Streptococcus species, *S. aureus* were isolated from diseased individuals and not from normal subjects, and a higher isolation rate of opportunistic organisms was observed in the diseased individuals. Coagulase negative *S taphylococci* were isolated from 58% of diseased cases and 39% of normal individuals.

Since the eyes are continuously exposed to the environment there are chances of acquiring infection from various sources, especially if there is any breach in the defense mechanisms due to trauma, chemical irritation or allergic reactions. As the bacterial flora tends to change with time it is important to develop a surveillance system in order to identify emerging pathogen. It is also very important for accurate diagnosis and treatment to examine bacterial culture particularly to prevent post operative infections. Some of the eye infections can result in serious complications and even loss of eye sight, therefore, awareness should be developed among the population to observe preventive measures and seek proper medical care in case of any trauma or allergy.

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