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Research Article

Screening and Identification of Normal Flora Isolated from the Ocular Surface of Normal and Dry Eyes Subjects

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Abstract

Aim: To screen and identify the ocular normal flora in Saudi subjects with normal and dry eyes.

Methods: The right eyes of 40 Saudi male adults aged 19 - 35 years (mean ± standard deviation = 24.7 ± 4.4) were examined for eye dryness and screened for the presence of microorganisms. Patients with a history of ocular surgery, eye infections, use of medication, or use of contact lens were excluded from the study. All patients completed the ocular surface disease index. Also, the subjects were sub-divided into smokers and non-smokers based on the smoking habit, followed by the phenol red thread test. After 10 minutes, the non-invasive tear break-up time test was performed followed by the osmolarity test, with a 10-minute gap in between the two tests. Tear specimens were collected from the ocular surface of each subject and cultured over chocolate and blood agars supplemented with horse blood. The bacterial cultures were identified using Gram staining and a Staphylococcal identification system.

Results: Different gram-positive bacterial species were identified in 36 subjects (90%). The identified bacteria were *Staphylococcus epidermidis* (N = 29; 72.5%), *Staphylococcus saprophyticus* (N = 4; 10%), *Staphylococcus aureus* (N = 2; 5%), and *Streptococcus pneumoniae* (N = 1; 2.5%). The number of bacterial isolated found in younger subjects (19 - 25 years) was 28 compared with 6 in subjects aged 25 - 30 years, and 2 in subjects older than 30 years. Bacterial isolates were more commonly found in non-smoking subjects (52.5%) than in smoking subjects (37.5%).

Conclusion: Various types of ocular bacterial species were identified in both normal and dry eye subjects. It would appear that the diversity of the ocular normal flora between smoking and non-smoking subjects, and between younger and older subjects is significant ($p < 0.05$).

Keywords: *Ocular Surface; Normal Flora; Dry Eye; Bacteria; Osmolarity Test; Tear Film*

Introduction

The ocular surface consists of the tissues of the cornea, conjunctiva, and eyelid glands which are responsible for the secretion of the tear film and good vision [1,2]. The structure of the tear film is complicated since tear composition depends on the state of the eye (open or closed) and the type of tears (stimulated or non-stimulated) [3,4]. The stability of tear film is vital for the maintenance of the cornea and conjunctival epithelium [5-7]. Therefore, any instability or irregularity within the tear film could lead to eye diseases such as dry eye [8-10]. Dry eye is a common ocular disorder that leads to a disturbance in vision, inflammation, sandy sensation, and discomfort and in severe cases can also lead to corneal damage [11]. It also results in an increase in tear osmolarity [12]. Dry eye can be caused by excessive

tear evaporation or low aqueous content secretion. Various diagnostics tests can be used to test the stability and regularity of the tear film [13]. Such tests should be non-invasive, reliable, repeatable and have a short turnaround time [1]. Also, the use of mobile equipment is always preferable.

The most common dry eye tests are tear break-up time (TBUT) [14], osmolarity [15,16], tear evaporation rate [17], tear ferning [18], phenol red thread (PRT) [19], Schirmer [19] and tear meniscus height [20]. However, there is a poor correlation among these tests since each test is used to assess a different parameter and therefore a combination of several tests is recommended to evaluate the severity of eye dryness.

The ocular surface provides a suitable environment for the growth of various types of normal flora [21]. Such microorganisms play an important role in maintaining a healthy ocular surface and inhibiting the growth of pathogens *via* the secretion of antibodies and competing for nutrition [22,23]. The presence of normal flora usually prevents ocular infections from occurring although opportunistic infections may occur in some cases. Therefore, scanning for ocular normal flora is essential, especially in patients undergoing ocular surgery. Normal ocular flora varies and depends on various parameters such as nutrition, sex, hormonal state, age, degree of eye dryness, use of contact lens, use of antibiotics, and climate [24]. Ocular surface inflammation can be reduced by the use of oral antibiotics leading to a reduction in the number of bacteria and their enzymes [25]. In addition, the number of ocular surface microorganisms can be further reduced by frequent eye blinks that wash away foreign objects. The most commonly isolated microorganisms from the ocular surface are gram-positive bacteria such as *Staphylococci* and *Streptococcus* species [26]. Gram-negative bacteria and fungi are less commonly isolated from the ocular surface [26]. The current study sought to isolate and identify the ocular surface normal flora in Saudi males with normal and dry eyes. Also, to test the association between types of ocular microorganisms and smoking habit and age among Saudis. As far as we are aware, no similar studies were carried out in Saudi Arabia.

Materials and Methods

Subjects

The right eyes of 40 Saudi male subjects aged 19 - 35 years [mean ± standard deviation (SD) = 24.7 ± 4.4] were examined and screened for the presence of normal flora. Subjects with a history of ocular surgery, use of medication, eye infections, or contact lens wear were excluded from the study. All study participants provided informed written consent, and the study design was approved by the Ethics Committee of the College of Applied Medical Sciences, King Saud University. The measurements were carried out by the same examiner under normal conditions.

Dry eye diagnostic tests

All subjects completed the ocular surface disease index (OSDI), followed by the PRT test. After 10 minutes, the non-invasive tear break-up time (NITBUT) test was performed followed by the osmolarity test, with a 10-minute gap between the two tests. Tear specimens were collected from the subjects and cultured and the bacterial cultures were identified using Gram staining and a Staphylococcal identification system.

OSDI

An OSDI score of 12 or less indicates normal eye, 13 - 22 indicates mild dry eye, 23 - 32 indicates moderate dye eye, and a score of > 33 indicates severe eye dryness [27].

PRT test

A strip containing phenol red indicator was inserted in the lower lid of the eye of each subject and removed after 15s. The wetted portion of thread was measured in which the cut-off value for dry eye was less than 10 mm [19].

NITBUT test

The NITBUT test was performed on the Keratograph 4 (OCULUS Optikgeräte GmbH, Wetzlar, Germany). Dry eye was defined as a tear break-up time of less than 10s [28].

Tear osmolarity test

Tear osmolarity was measured on TearLab™ Osmolarity System (TearLab™ Corp., San Diego, California). The cut-off for normal eyes was 298 - 302 mOsm/L [15].

Tear culturing

Specimens were collected from the ocular surface of each subject and transferred to 2 mL of a brain-heart infusion broth (Thermo Fisher Scientific Oxoid Ltd., Basingstoke, UK). The broth was mixed and 100 µL transferred to plates containing chocolate and blood agars supplemented with horse blood (Oxoid Ltd.) within 2h of sampling. The chocolate agar plates were incubated for 48h at 35°C in 5% by volume carbon dioxide and the blood agar plates were incubated at 37°C under aerobic and anaerobic conditions. Positive bacterial cultures were identified using Gram staining and a Staphylococcal identification system (API; bioMérieux, Marcy l'Etoile, France) [29].

Statistical analysis

The data were recorded onto Excel (Microsoft™ Office 2010, Microsoft Corp., Redmond, USA) and analyzed using the Statistical Package for the Social Sciences software, version 22.0 (SPSS Inc., Chicago, IL, USA). To test the association between the type of identified bacteria and tear film status, the subjects were divided into two categories as normal and dry eye groups based on the scores obtained from the OSDI, and PRT, NITBUT, and osmolarity tests. Also, the subjects were sub-divided into smokers and non-smokers based on the smoking habit.

Results

The scores obtained from the OSDI sheet, and PRT, NITBUT, and osmolarity tests were not normally distributed (Kolmogorov-Smirnov test, $p < 0.05$) and the medians (interquartile ranges; IQR) were used to describe the averages (Table 1). The scores obtained from the OSDI ranged from 0 to 80. The scores obtained from the PRT, NITBUT, and osmolarity testes ranged from 3 to 38 mm, from 2 to 19s, and from 275 to 336 mOsm/L, respectively.

Test	Median (IQR)
OSDI	17.7 (15.5)
PRT (mm)	25.6 (5.6)
NITBUT (s)	8.5 (4.0)
Osmolarity (mOsm/L)	297.6 (14.4)

Table 1: *The average scores for the OSDI sheet, and PRT, NITBUT and tear osmolarity tests.*

The ocular bacteria isolated from the subjects (N = 40) were identified and are represented in figure 1. Four different types of bacteria were identified in 36 subjects (90%). The bacteria isolated were *Staphylococcus epidermidis* (*S. epidermidis*; N = 29), *Staphylococcus saprophyticus* (*S. saprophyticus*; N = 4), *Staphylococcus aureus* (*S. aureus*; N = 2) and *Streptococcus pneumoniae* (*S. pneumoniae*; N = 1), which all are gram-positive bacteria.

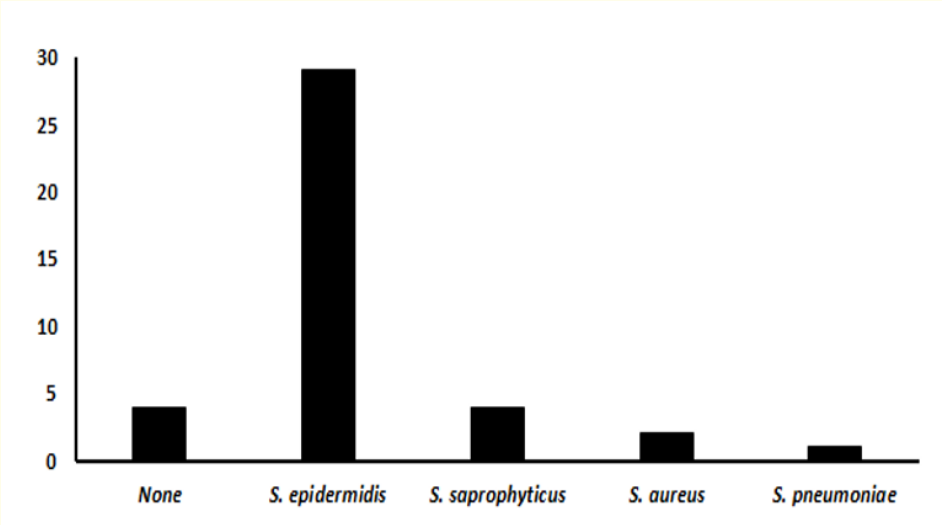

Figure 1: *Ocular bacteria isolated from study subjects (N = 95).*

The number of bacteria isolated from younger subjects (19 - 25 years) was 28 (70%) compared with 6 (15%) from subjects aged 25 - 30 years, and 2 (5%) from subjects older than 30 years. In addition, bacterial isolates were found to be more common in non-smoking subjects (N = 21; 52.5%) compared with smokers (N = 15; 37.5%). Table 2 shows the number of bacteria isolated from study subjects based on their age and smoking status.

Characteristic	Subjects		Ocular bacteria (N; %)	
	N	%	Yes	No
Age (year)				
19 - 25	29	72.5	28 (70)	1 (2.5)
26 - 30	7	17.5	6 (15)	1 (2.5)
> 30	4	10	2 (5)	2 (5)
Smoking habit				
Smoking	17	42.5	15 (37.5)	2 (5)
Non-smoking	23	57.5	21 (52.5)	2 (5)

Table 2: *Ocular bacteria isolated from study subjects (N = 40) based on their age and smoking status.*

The number of dry eye subjects based on the diagnostic tests varied from 18 to 21 (Table 3). For example, the number of dry eye subjects based on PRT and osmolarity tests was 18 and 21, respectively. These discrepant results clearly highlight the importance of using a combination of different diagnostic tests to assess eye dryness since no single test can give a clear picture of tear film stability.

The ocular bacteria identified in both normal and dry eye subjects based on different diagnostic tests are shown in Table 3. *S. epidermidis* was common to all subjects regardless of the eye dryness. Based on the OSDI, PRT and NITBUT scores, four types of bacteria were identified in normal eye subjects, while, only two bacterial species were identified in dry eye subjects (Table 3). In contrast, based on the osmolarity test, three types of ocular bacteria were identified in normal eye subjects and four types were isolated from in dry eye subjects (Table 3).

Test/ocular flora	Normal eye subjects	Dry eye subjects
OSDI		
N	20	20
None	1	3
S. epidermidis	13	16
S. saprophyticus	3	1
S. aureus	2	-
*S. pneumoniae**	1	-
PRT test		
N	22	18
None	1	3
S. epidermidis	15	14
S. saprophyticus	4	-
S. aureus	1	1
*S. pneumoniae**	1	-
NITBUT test		
N	21	19
None	1	3
S. epidermidis	15	14
S. saprophyticus	2	2
S. aureus	2	-
*S. pneumoniae**	1	-
Osmolarity		
N	19	21
None	1	3
S. epidermidis	16	13
S. saprophyticus	1	3
S. aureus	1	1
*S. pneumoniae**	-	1

Table 3: *Ocular bacteria identified in both normal and dry eye subjects. OSDI: Ocular Surface Disease Index; PRT: Phenol Red Thread; NITBUT: Non-Invasive Tear Break-Up Time; S: Staphylococcus; *: Streptococcus pneumoniae.*

Discussion

The current study showed that ocular normal flora was identified in 36 subjects (90%), in which *S. epidermidis* contributed to 72.5% (N = 29) of cases. The number of ocular bacteria identified in non-smoking and younger subjects was higher than that identified in smoking and relatively older subjects, respectively. Dry eye subjects may be at a lower risk for bacterial infection possibly because their innate immunity protects the tear film and because of the aqueous layer deficiency [30]. It has been reported that dry eye symptoms are associated with various types of normal flora such as coagulase-negative *Staphylococcus*, *Bacillus subtilis*, *S. aureus*, *Streptococcus* species etc [31-33]. However, strong evidence is still needed to establish such an association. Since one report showed that no significant differences were found between the normal flora isolated from the ocular surface of dry and normal eye subjects [34]. In the current study, *S. aureus*, which is known as a major pathogen to infect the tear duct, eyelid, cornea, conjunctiva and the anterior, posterior and vitreous chambers [35] was identified in only two subjects (5%). Such infections are serious and can lead to a reduction in visual acuity and in severe cases may also cause blindness [35].

The relationship between ocular normal flora and gender, age, and geography has not been clearly established [21,36]. Previous studies showed a variation in the number of ocular isolates in rural and urban areas [37]. However, another study showed that there was no remarkable diversity in the ocular flora based on geographical differences [21]. Females tend to have less normal flora compared to males but the differences were not significant and could be attributed to the outdoor work that males tend to do more than females [21,38].

The ocular bacteria isolated from the subjects in the current study were mainly similar to those reported. Previous reports have shown huge variations in the number of ocular normal flora identified, from 2.5 to 87.5% in most cases [21,39-44]. The most commonly identified bacteria in healthy subjects are usually coagulase-negative *Staphylococcus*, coagulase-positive *Staphylococcus*, *S. epidermidis*, *S. aureus*, *Diphtheroids*, and *Streptococcus* species [21,39-44]. A study showed that in 170 subjects with ocular flora, 52.4% had coagulase-negative *Staphylococcus* as the main bacteria [32]. Another study showed that ocular flora were identified in 78.5% of the subjects (*N* = 100) of which half showed the presence of coagulase-negative *Staphylococcus* [21].

The current study has some limitations such as the use of a relatively lower number of male subjects (N = 40) that are placed in the city of Riyadh. Therefore, a more detailed study is still needed that involve a larger number of both male and female subjects based in different geographical locations in Saudi Arabia.

Conclusion

Colonies of ocular bacteria were identified in more than one-third of the subjects tested. The most common isolated normal flora were *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. The number of bacterial species isolated from younger and non-smoking subjects was relatively higher than that isolated in relatively older and smoking subjects, respectively.

Disclosure

The authors report no conflicts of interest in relation to this work.

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