ORIGINAL ARTICLE

Normal Conjunctival Flora in Healthy Population of Quetta Balochistan: A Cross-Sectional Study

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ABSTRACT

Objective: To investigate the normal conjunctival flora in a healthy population of Quetta, Balochistan.

Study Design: A cross-sectional observational study.

Place and Duration of Study: This study was conducted in the Department of Microbiology and Ophthalmology at the Combined Military Hospital (CMH), Quetta, Pakistan, from July 2023 to December 2023.

Methods: This study was performed on 379 healthy patients, divided into three age groups ranging from 20 to 70 years. Sterile Stuart's swab samples were collected from the conjunctiva of individuals, avoiding contact with the eyelashes or lid margins. The swabs were gently applied to the conjunctiva of both eyes and then placed in Stuart's transparent media for transport to the microbiological laboratory.

Results: Out of 379 individuals, 239 were male (with a mean age of 41.92 ± 16.91 years), and 140 were female (with a mean age of 38.21 ± 11.61 years). Growths were obtained from 231 (60.9%) eyes, and the remaining 148 (39.1%) did not yield any organism. Predominantly isolated microorganisms were Staphylococcus epidermidis in 120 (51.95%) followed by Staphylococcus aureus in 42 (18.18%), Haemophilus influenzae in 28 (12.12%), Diphtheroids in 23 (9.96%), Streptococcus viridans in 10 (4.33%), Staphylococcus citreus in 6 (2.60%), and Escherichia coli in 2 (0.87%) of culture-positive specimens. The *P*-value (0.962) was not statistically significant, indicating no association between age groups and swab test results for conjunctival flora in the population under study.

Conclusion: Normal conjunctival flora in the present study exhibited a wide range of compositions. While S. epidermidis is the most common microbe, E. coli is considerably less prevalent. Identification of the initial pathogen is crucial in determining the appropriate treatment for ocular infections.

Keywords: *Conjunctival Sac, Culture Media, Escherichia, Staphylococcus*.

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Introduction

The conjunctiva refers to the mucous membrane that lines the inside of the eyelids and extends to the orbital globe. The conjunctival sac denotes the space between the palpebral and bulbar conjunctiva.[1] The normal flora is the collective term for the range of bacteria and fungi that thrive on the surfaces of the body.[2] Additionally, the outer layer of the eye is home to a unique microbiome that controls the development of pathogens and offers protection without needlessly causing inflammation. Ocular barriers, including the lids, tear film, and antimicrobial substances, physically shield the eye under normal physiological conditions. The innate and adaptive immune mechanisms, in conjunction with the microbiome, also inhibit the proliferation of pathogenic organisms.[3]

The rationale for this study was to establish a regional microbial baseline for Quetta. Given the unique environmental conditions and pollution levels in Balochistan, understanding the typical, non-

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pathogenic microorganisms present on the healthy eye surface is vital. This baseline allows clinicians to distinguish normal flora from opportunistic pathogens, facilitating early detection of infections and the selection of appropriate empirical therapies.

The conjunctival sac is continuously exposed to the external environment, allowing detection of potential infections and typical commensal flora.[4] However, components of the complement pathway, including lactoferrin, lysozymes, and B-lysin, as well as antibacterial compounds such as immunoglobulins, are also present in the lacrimal fluid in the sac of the conjunctiva and exhibit antimicrobial action.[5] It helps in killing microorganisms and reducing their adherence to the ocular surface, and also helps in flushing them away.[6] However, trauma, surgeries, or compromised immune systems can result in eye infections such as endophthalmitis, keratitis, and dacryocystitis.[7] When an eye infection does arise, it must be treated quickly and effectively to avoid harm from the infection and the immune system. The preliminary identification of a pathogen provides the foundation for clinical decisions that impact the management of eye infections. Thus, to effectively manage patients before, during, and after surgery, it is critical to understand and monitor the distribution of conjunctival bacteria.[8]

The typical conjunctival flora was defined in the 19th century.[9] The normal bacterial flora of the conjunctiva is composed of both anaerobic and aerobic bacteria that support immunoregulation.[10] Although Corynebacterium, Propionibacterium, and Klebsiella species are also found, Staphylococcus epidermidis is the most frequently grown bacterium from the ocular surface.[10] On the conjunctiva, corynebacteria often predominate, whereas gram-negative bacteria and streptococcal species are less common. While normal conjunctiva revealed the presence of non-hemolytic streptococcus, Staphylococcus aureus, and Staphylococcus epidermidis, the children were frequently infected with Haemophilus influenzae.[10] It's critical to understand the microbial flora to develop effective health and preventive strategies. While several studies have shown the presence of bacteria in the conjunctival sac, these investigations were conducted in individuals with various ocular diseases. Bacteria present in the conjunctival sac can be affected by surgical procedures, antibiotic usage, and seasonal variations, which may alter the microbial flora.[7,11]

Methods

The cross-sectional study was carried out at the Department of Microbiology and Ophthalmology, Combined Military Hospital (CMH), Quetta, Pakistan, from July 2023 to December 2023, after obtaining approval from the Institutional Review Board (IRB) of the hospital, vide reference number: 1ERB/17/2023, dated: 17th March 2023. After a comprehensive review of the literature, the WHO calculator determined a sample size of 380, maintaining a 5% margin of error at a 95% confidence level, with a 44% prevalence.[5] A non-probability consecutive sampling method was used to select 380 healthy individuals coming for routine eye check-ups. The Participants were divided into three age groups: young (20-40 years), middle-aged (41-50 years), and elderly (51-70 years). After obtaining consent, a thorough examination was performed to eliminate the possibility of ocular disease or surface infection, with particular attention to the exclusion criteria, including signs of redness, swelling, inflammation, and discharge from the eyes over the past two weeks. Other exclusion criteria included known immunodeficiency disease or systemic illness, and recent topical antibiotic treatment. Using the patients' conjunctiva, sterile Stuart's swab samples were taken from the lower fornix conjunctiva after administering 4% Xylocaine anesthesia. All samples were subjected to Gram staining for preliminary morphological identification. Samples were transferred to Stuart's clear medium before use in the microbiological laboratory. The swabs were used to smear the entire medium.

To culture the samples, the following culture media were utilized: blood agar, Sabouraud agar, MacConkey agar, and chocolate agar. In accordance with Clinical Laboratory Sensitivity Institute (CLSI) guidelines, the culture media were incubated at 37°C for 24-48 hours to ensure optimal growth of aerobic isolates.[5,8] Data was analyzed on SPSS version 26 using frequency, percentage, mean, standard deviation, and the Chi-Square test was applied to check the association between age group and swab test results.

Results

This study involved 379 participants. Out of the total, 239 were male (mean age 41.92 ± 16.91 years), and 140 were female (mean age 38.21 ± 11.61 years). Growths were obtained from 231 (60.9%) eyes, and the remaining 148 (39.1%) yielded no organism, as shown in Table 1.

Predominantly isolated microorganisms were S. epidermidis in 120 (51.95%), followed by S. aureus in 42 (18.18%), Haemophilus influenzae in 28 (12.12%), Diphtheroids in 23 (9.96%), S. viridans in 10 (4.33%), S. citreus in 6 (2.60%), and E. coli in 2 (0.87%) of culture-positive eyes as shown in Table 2.

Table 3 illustrates the association between age groups and swab test results among individuals in the study of normal conjunctival flora. The non-significant *P*-value (0.962) indicated no statistically significant association between age groups and swab test results for conjunctival flora in the study population.

To confirm these findings, a Pearson Chi-Square test of independence was performed (2\times 3 contingency matrix). The calculated Chi-Square value was $X^2 = 0.078$ with 2 degrees of freedom (df = 2), yielding a non-significant *P*-value of 0.962. The observed distribution of positive conjunctival cultures closely mirrored the mathematically expected frequencies across all three age cohorts (20–40, 41–50, and 51–70 years), verifying that age is not a significant determinant of conjunctival flora culture positivity in this sample population.

Table-1: Swab test evaluation (N=379)

	N (%)
Growth	231 (60.9%)
No Growth	148 (39.1%)
Total	379 (100%)

Table-2: Occurrence of bacteria in sample (N=231)

Micro-Organisms	N (%)
S.epidermidis	120 (51.95%)
S.aureus	42 (18.18%)
S.citreus	6 (2.60%)
Haemophilus spp.	28 (12.12%)
Diphtheroids	23 (9.96%)
S.viridans	10 (4.33%)
E.coli	2 (0.87%)
Total	231

Table-3: Association between age group and swab test evaluation (N=379)

Age Group	Swab Test Evaluation		Total	*Chi-Square* (X^2)	*P*-value
	Growth	No Growth			
20 to 40 Years	136 (61.5%)	85 (38.5%)	221 (58.3%)	0.078	0.962
41 to 50 Years	27 (60%)	18 (40%)	45 (11.9%)		
51 to 70 years	68 (60.2%)	45 (39.8%)	113 (29.8%)		
Total	231 (60.9%)	148 (39.1%)	379 (100%)		

Discussion

The conjunctival sac flora is important in the treatment of eye illnesses following trauma or surgery. Previously, normal ocular flora was thought to be nonpathogenic; however, bacteria isolated from the conjunctiva frequently correspond with pathogens causing bacterial endophthalmitis, where commensals like S. epidermidis have emerged as major pathogens.[11,12]

In the current study, 60.9% of eyes produced growth. As noted in the recent report on the microbial composition of the conjunctiva across different regions, which indicated that environmental and demographic factors are associated with the composition of microorganisms on the conjunctiva, no statistically significant association between culture positivity and age group was found in this

study.[13] However, recent studies in Beijing found that age-related changes in the composition of microorganisms in the conjunctiva were observed and could suggest a difference in the susceptibility of the ocular surface.[14]

Staphylococci were the most common isolated organisms in our study. S. epidermidis (51.95%) was followed by S. aureus (18.18%).

There was no significant inter-age group variance for the three age groups in the present study. More recently, however, studies have shown that in older people, conjunctival microbial diversity changes and there is a greater abundance of aerobic bacteria.[15] This indicates that the microbial diversity of the ocular surface may be altered with age and that older people may be more susceptible to infection.[9,10]

Staphylococcus spp. accounted for 60% of the most frequently isolated microorganisms in the study by Shrestha LB et al., with S. epidermidis accounting for 44% and S. aureus for 14%. Haemophilus spp. was the most prevalent aerobe (8%) among the other aerobes, followed by diphtheroids. Only a few more aerobes were discovered.[16] In the present study, Staphylococci were the most common isolated organism.

According to worldwide studies, Staphylococcus epidermidis is the most prevalent bacterium in healthy conjunctival sacs. This was the most common organism in 51.95% of eyes in the current study, followed by S. aureus (18.18%). According to recent studies, Staphylococcus epidermidis remains the predominant commensal microorganism of the healthy ocular surface and plays an important role in maintaining ocular immune homeostasis.[17] Furthermore, prophylactic antibiotics have been shown to reduce the risk of postoperative ocular infections by limiting the pathogenic potential of conjunctival flora, highlighting the clinical importance of understanding baseline microbiota.[18] The latest studies have consistently shown Staphylococcus epidermidis as the most predominant bacterial species on the ocular surface, but the prevalence is different among populations and geographical regions. These variations might be due to differences in environmental exposure, age distribution, and methods used to identify microbes.[19] A diphtheroid is regarded as a commensal microbe of a healthy conjunctiva. Other types of microorganisms, both gram-positive and gram-negative, were observed as well. The research found Streptococcus viridans in 4% of cases, while Haemophilus spp. in 12.12% of cases. Culture-positive rarely showed the presence of E. coli. The presence of *Escherichia coli* was rare among culture-positive isolates.

These findings reflect the diversity and variability of the conjunctival microbiome reported in recent literature.[20]

This study was hospital-based; a community-based survey would better reflect the general population. The research focused primarily on aerobic bacteria; anaerobic flora and fungi were not extensively investigated. Environmental variables such as seasonal changes and humidity in Quetta were not controlled.

Conclusion

Normal conjunctival flora in the present study exhibited a wide range of compositions. While S. epidermidis is the most common microbe, E. coli is considerably less prevalent. The most prevalent kind of normal conjunctival flora was Staphylococcus epidermidis. Thus, it is the most common organism that causes endophthalmitis. In this study, no conjunctival sac contained Pseudomonas spp. The differences observed in the normal conjunctival flora can be attributed to various reasons. Identification of the initial pathogen is crucial in determining the appropriate treatment for ocular infections.

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Author Contributions

NA: Conception, design of the work, writing the original draft, proofreading, and approval for final submission

AH: Manuscript writing for methodology design, investigation, and approval for final submission

FH: Data acquisition, curation, statistical analysis, and approval for final submission

WY: Validation of data, interpretation, write-up of results, and approval for final submission

MA: Revising, editing, supervising for intellectual content, and approval for final submission

NA is the nominated guarantor and takes full responsibility for the overall content and integrity of the work