ORIGINAL ARTICLE

Bacterial Flora of Conjunctiva in Medical Students of Mohtarma Benazir Bhutto Shaheed Medical College Mirpur Azad Kashmir

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Abstract:

Objectives: To determine the bacterial conjunctival flora present among medical students of Mohtarma Benazir Bhutto Shaheed Medical College, Mirpur, Azad Kashmir

Methodology: A microbiological analysis on 196 conjunctival swabs from 196 students with healthy eyes was carried out in this prospective, cross-sectional study conducted at the Divisional Headquarters Hospital, Mirpur, Azad Kashmir. These swabs were used to inoculate blood agar and chocolate agar plates for culture.

Results: The study included a total of 196 students. The mean age was 21.26±1.555 years. Of the 196 participants, 131 (66.8%) were males and 65 (33.2%) were females. The number of right and left eyes were 100 and 96, respectively. Only 86 (43.88%) specimens showed growth while the remaining 110 (56.12%) showed no growth. 76 (88%) of the 86 positive isolates were Gram Positive Bacteria. The most common bacteria were Staphylococcus epidermidis (67%), followed by Staphylococcus aureus (6%). There were only 10 (12%) gram-negative bacteria found in the positive isolates. With 1% of the total, the three most often isolated species were Citrobacter freundii, Burkholderia cepacia, and Pseudomonas aeruginosa.

Conclusion: The most common isolated bacteria from the conjunctiva were game positive bacteria, with Staphylococcus epidermidis being the most common. *Al-Shifa Journal of Ophthalmology 2024; 20(1): 9-14. © Al-Shifa Trust Eye Hospital, Rawalpindi, Pakistan.*

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Originally Received: 05 February 2024
Revised: 2 April 2024
Accepted: 9 April 2024

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Introduction:

The mucous membrane that lines the inside of the eyelids and continues to the orbital globe is known as the conjunctiva[1], and the area between the palpebral and bulbar conjunctiva is known as the conjunctival sac. The conjunctival sac is continuously exposed to the external environment, allowing for the detection of both possible pathogens and normal commensal flora[2]. By taking up potential pathogen colonization sites, generating antimicrobial compounds, or inducing an immune response that shields the host from infection, the typical commensal flora can defend the host[3]. On the other hand, endophthalmitis may result from accidental or surgical penetrating injuries that encourage the migration of local bacterial flora into the eye[4].

Although a very tiny percentage of people have sterile conjunctival sacs, they are present in the conjunctival sac from birth and throughout life[5]. Numerous organisms, both pathogens and non-pathogens, have been cultured from the normal conjunctiva, but their numbers are typically modest[6]. This results from frequent blinking, which cleanses the conjunctiva every few seconds and mechanically wash away foreign objects, including bacteria[7]. Bacteriostatic agents such as lysozyme, IgA, and IgG, as well as exposure, a moderate blood supply, and a dropped conjunctival temperature brought on by tears evaporating all work to prevent the growth of germs[8]. Excessive usage of antibiotics has been linked to alterations in both pathogenic and normal flora types[9].

However, due to differences in genetic makeup, age, gender, race, and geography, the range of these microorganisms varies in different individuals[10]. There are various studies which were carried out in different regions to evaluate the normal flora of human conjunctiva[11, 12].

This research aims to characterize the bacterial flora of the conjunctiva in medical students at Mohtarma Benazir Bhutto Shaheed Medical College in Mirpur, Azad Kashmir, as such data is lacking according to the best of our knowledge.

Materials and Methods:

The study aimed to investigate different bacteria identified as typical conjunctival flora by analyzing 196 conjunctival swabs from 196 students with healthy eyes. The research was conducted as a prospective cross-sectional study at Divisional Headquarters Hospital, Mirpur Azad Kashmir after taking ethical approval, from 1st January 2022 to 30th June 2022. Participant consent was taken before inclusion in the study. Inclusion criteria comprised all registered medical students who provided consent and were free from clinical ocular infections, while individuals with existing eye infections or those undergoing topical antibiotic treatment were excluded from the study. The conjunctival swabs were collected by the same ophthalmologist to maintain consistency. The specimens were taken from the lower conjunctival sac without a topical anesthetic. Using a cotton swab, the lower lid was pushed to reveal the lower conjunctival fornix. A sterile swab dampened with normal saline was used to gently wipe the lower fornix conjunctiva from the medial to the lateral side after requesting each individual to look upward. To prevent contacting the lid margins, the participants were instructed not to blink during the process. The specimens were streaked onto blood and chocolate agar plates right away, and they were then incubated for a period 24 to 48 hours at 37 °C. Using particular biochemical assays, the species of bacteria were identified. Results were documented by a microbiologist on a pre-designed proforma. Data were analyzed using SPSS version 21.0. Numerical variables like age were expressed as mean and standard deviation. Categorical variables like gender, eye involved, class & isolated bacteria were expressed as frequency and percentages.

Results:

A total of 196 students were included in this study. 31 students from 1st year, 36 from 2nd year, 34 from 3rd year, 41 from 4th year, and 54 students participated from final year. The mean age of patients included in this study was 21.26±1.555 years (Table 1). There were more males as compared to females as shown in Figure 1. There were 100 right and 96 left eyes from which the sample was taken.

Out of 196 samples, only 86 (43.88%) showed growth while the remaining 110 (56.12%) showed no growth (Figure 2). Out of 86 positive isolates, 76 (88%) were gram-positive bacteria. Staphylococcus epidermidis (67%), followed by Staphylococcus aureus (6%), were the most prevalent bacteria. Only 10 (12%) of the positive isolates had gram-negative bacteria. Citrobacter freundii, Burkholderia

cepacia, and Pseudomonas aeruginosa were the three most frequently isolated species, accounting for 1% each of the total gram-negatives. Table 2 displays the list of microorganisms that were isolated from the conjunctival sac.

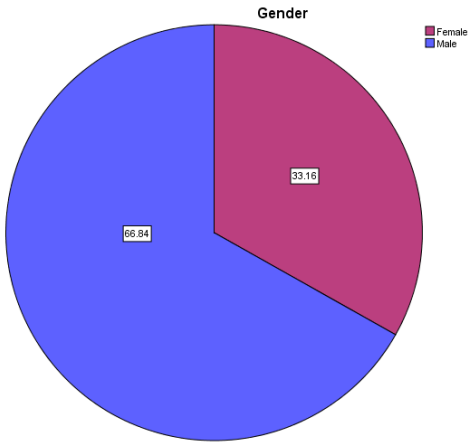
Table 1: Mean age in the study (n=196)

Mean Age in the Study (Years)			
Mean	Std. Deviation	Maximum	Minimum
21.26	1.555	25	17

Table 2: Bacterial Isolates from Conjunctiva of students (n=86)

Bacteria	No of Isolates	Percentage
Gram Positive	76	88%
Staphylococcus epidermidis	58	67%
Staphylococcus aureus	5	6%
Corynebacterium species	5	6%
Others	8	9%
Gram Negative	10	12%
Citrobacter freundii	1	1%
Burkholderia cepacia	1	1%
Pseudomonas aeruginosa	1	1%
Others	7	9%

Figure 1: Gender Distribution in this study (n=196)



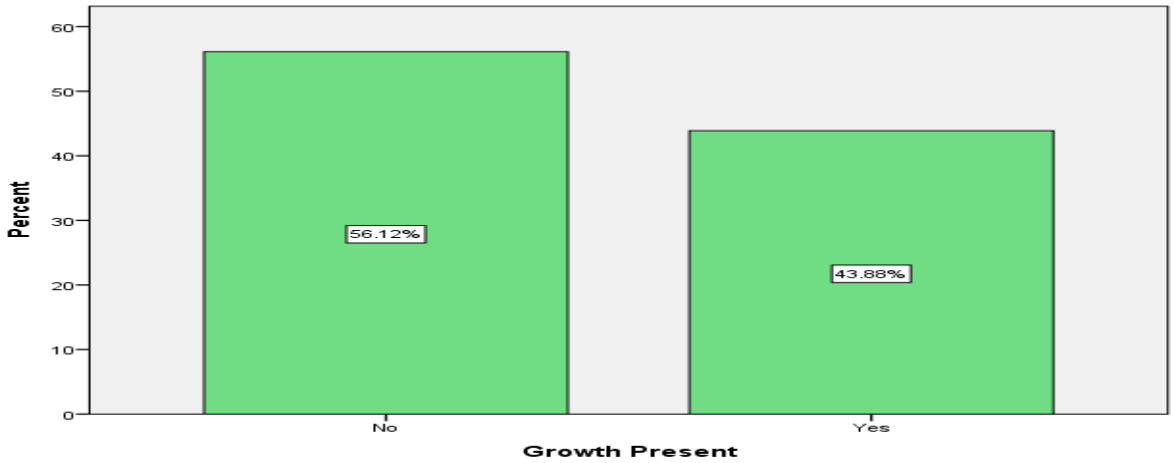


Figure 2: Growth Present in Samples after culture (n=196)

Discussion:

The normal flora of the conjunctiva refers to the diverse microorganisms that typically inhabit the surface of the eye[13], specifically the conjunctiva, which is the thin, transparent membrane covering the sclera and inner eyelids[14]. These microorganisms, primarily bacteria, play a crucial role in maintaining ocular health by forming a protective barrier against potential pathogens[15]. Common bacterial species found in the conjunctival flora include Staphylococcus epidermidis, Streptococcus pneumoniae, and Corynebacterium species[16].

Xu S et al[17] conducted a similar study to determine the bacteriological profile of conjunctiva bacterial Flora in Northeast China. The two studies diverge notably in participant demographics, with our study focusing on a younger cohort (mean age 21.26 years) and having more male participants, whereas this study involved older participants (mean age 60.73 years) with a majority of females. Both studies observed positive culture rates, with our study reporting a rate of 43.88% and this study reporting a higher rate of 48.20%. While both studies identified Staphylococcus epidermidis as the most prevalent bacterium, their percentages slightly differed. This study delved into seasonal variations and gender disparities in positive culture rates, along with discussing characteristics like education, employment status, and hypertension, which were not addressed in our study. These comparisons highlight the diverse contexts and findings across different studies in the field.

In another study by Jiang M[18], analysis of conjunctival sac flora and drug susceptibility was done in normal children in East China. Our study focused on 196 students with a mean age of 21.26 years and found a higher proportion of Gram-positive bacteria (88%) among positive isolates, primarily Staphylococcus epidermidis (67%). In contrast, this study included 2516 children, with Gram-positive cocci (91.54%) dominating, led by Staphylococcus epidermidis (52.12%) and Streptococcus (12.09%). Gender distribution varied, with our study having more males, while this study initially had more males but a slightly higher concordance rate of binocular flora in females. Drug susceptibility profiles were not included in our study. Overall, there were discrepancies in sample sizes, age demographics, microbial prevalence, and drug susceptibility between the two studies.

Owji N[19] in his study focused on 72 operated eyes and normal fellow eyes, reporting higher bacterial growth rates in operated eyes (66.7%) compared to normal fellow eyes (43.1%), with nasal specimens showing growth in 90.3% of patients. These results are different from our study as we excluded those individuals who have undergone any treatment.

Adukwu BU[20] in his study determined the conjunctival fungal flora in a tertiary eye hospital in Nigeria. Our study focused on 196 young students, revealing a mean age of 21.26 years and a predominance of Gram-positive bacteria, particularly Staphylococcus epidermidis. In contrast, this study involved 225 patients with a broader age range (mean age 41 years) and a nearly equal gender distribution. While our study showed 43.88% growth with notable eye distribution data, this study had a 27.6% culture-positive rate, highlighting Aspergillus and Candida as common organisms and emphasizing the impact of age groups and occupations on culture results.

Toribio A[21] in his study evaluated the microbiological spectrum of Conjunctival flora in anophthalmic patients. Our study focused on 196 students, predominantly male, aged around 21 years, and found a growth rate of 43.88% with Staphylococcus epidermidis being the most prevalent bacterium among positive isolates. In contrast, this study involves 251 isolates from healthy eyes and those with

prostheses, noting Staphylococcus epidermidis as the most common organism but also identifying coagulase-positive Staphylococci, Streptococci, and Gram-negative bacteria. While our study lacks antibiotic sensitivity data, this study reveals no significant differences in sensitivities between isolates from sockets and healthy eyes, but notes increased resistance in Gram-positive microorganisms in subjects using self-prescribed antibiotic drops. Overall, the larger sample size and broader scope of eye conditions in the other study provide a more comprehensive understanding of ocular microbiology and antibiotic resistance patterns.

Conclusion:

This study showed that gram-positive bacteria, especially Staphylococcus epidermidis, are the most prevalent bacteria found in the conjunctiva. This finding highlights the microbial landscape among medical students in the region. Further research may delve into specific factors influencing bacterial prevalence and antimicrobial resistance patterns, aiding in tailored interventions for improved public health outcomes.

References:

1. Kim S, Lee S, Chang H, Kim M, Kim MJ, Kim KH. In vivo fluorescence imaging of conjunctival goblet cells. Scientific reports. 2019;9(1):15457.
2. Wang Z, Zhang P, Huang C, Guo Y, Dong X, Li X. Conjunctival sac bacterial culture of patients using levofloxacin eye drops before cataract surgery: a real-world, retrospective study. 2022;22(1):328.
3. Suzuki T, Sutani T, Nakai H, Shirahige K, Kinoshita S. The Microbiome of the Meibum and Ocular Surface in Healthy Subjects. Investigative ophthalmology & visual science. 2020;61(2):18.
4. Simina DS, Larisa I, Otilia C, Ana Cristina G, Liliana MV, Aurelian MG. The ocular surface bacterial contamination and its management in the prophylaxis of post cataract surgery endophthalmitis. Romanian journal of ophthalmology. 2021;65(1):2-9.
5. Ke R, Zhang M, Zhou Q, Yang Y, Shen R, Huang H, et al. Bacteriological profiles and drug susceptibility of Streptococcus isolated from conjunctival sac of healthy children. BMC pediatrics. 2020;20(1):306.
6. Petrillo F, Petrillo A, Marrapodi M. Characterization and Comparison of Ocular Surface Microbiome in Newborns. 2022;10(7).
7. McMonnies CW. The clinical and experimental significance of blinking behavior. Journal of optometry. 2020;13(2):74-80.
8. Pflugfelder SC, Stern ME. Biological functions of tear film. Experimental eye research. 2020;197:108115.
9. Aramă V. Topical antibiotic therapy in eye infections - myths and certainties in the era of bacterial resistance to antibiotics. Romanian journal of ophthalmology. 2020;64(3):245-60.
10. Chiang MC, Chern E. Ocular surface microbiota: Ophthalmic infectious disease and probiotics. Frontiers in microbiology. 2022;13:952473.
11. Ramani K, Kaliaperumal S, Sarkar S, Sistla S. Study of Conjunctival Microbial Flora in Patients of Intensive Care Unit. Korean journal of ophthalmology : KJO. 2021;35(4):318-24.
12. Peter VG, Morandi SC, Herzog EL, Zinkernagel MS, Zysset-Burri DC. Investigating the Ocular Surface Microbiome: What Can It Tell Us? 2023;17:259-71.
13. Mohamed YH, Toizumi M, Uematsu M, Nguyen HT, Le LT, Takegata M, et al. Prevalence of Streptococcus pneumoniae in conjunctival flora and association with nasopharyngeal carriage among children in a Vietnamese community. Scientific reports. 2021;11(1):337.
14. Gipson IK. Goblet cells of the conjunctiva: A review of recent

findings. Progress in retinal and eye research. 2016;54:49-63.
15. Zysset-Burri DC, Schlegel I, Lincke JB, Jaggi D, Keller I, Heller M, et al. Understanding the Interactions Between the Ocular Surface Microbiome and the Tear Proteome. Investigative ophthalmology & visual science. 2021;62(10):8.
16. Doan T, Akileswaran L, Andersen D, Johnson B, Ko N, Shrestha A, et al. Paucibacterial Microbiome and Resident DNA Virome of the Healthy Conjunctiva. Investigative ophthalmology & visual science. 2016;57(13):5116-26.
17. Xu S, Zhang H. Bacteriological profile of conjunctiva bacterial Flora in Northeast China: a hospital-based study. 2022;22(1):223.
18. Jiang M, Zhang J, Wan X, Ding Y, Xie F. Conjunctival sac flora and drug susceptibility analysis in normal children in East China. BMC ophthalmology. 2023;23(1):248.
19. wji N, Zareifard A. Bacterial Flora of the Conjunctiva One Year after Dacryocystorhinostomy. Middle East African journal of ophthalmology. 2018;25(1):35-9.
20. Adukwu BU, Nwosu SNN, Emele FE, Uba-Obiano C, Onyiaorah AA. Conjunctival Fungal Flora in a Tertiary Eye Hospital in Nigeria. Journal of the West African College of Surgeons. 2023;13(1):79-83.
21. Toribio A, Marrodán T, Fernández-Natal I, Martínez-Blanco H, Rodríguez-Aparicio L, Ferrero M. Conjunctival flora in anophthalmic patients: microbiological spectrum and antibiotic sensitivity. International journal of ophthalmology. 2019;12(5):765-73.

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