

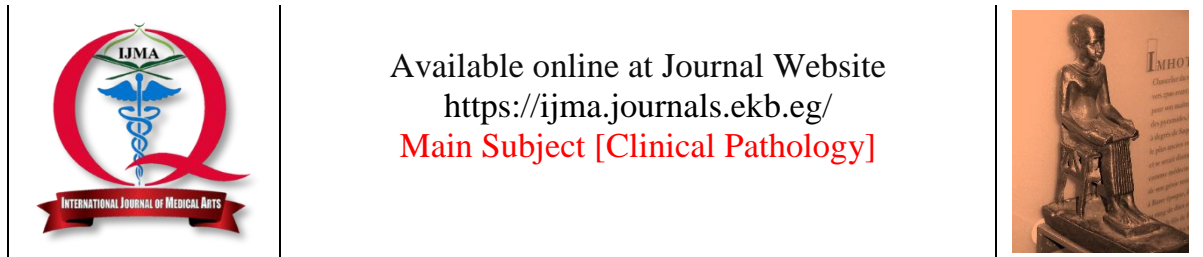
IJMA



INTERNATIONAL JOURNAL OF MEDICAL ARTS

VOLUME 6, ISSUE 6, JUNE 2024

P- ISSN: 2636-4174
E- ISSN: 2682-3780



Available online at Journal Website
<https://ijma.journals.ekb.eg/>
 Main Subject [Clinical Pathology]



Original Article

Bacteriological Study of Conjunctival Swab Cultures in Diabetic Patients

Moustafa Alkot ^{*1}, Wael Refaat Hablas ¹, Mohamed Ahmed Anwar Shaheen ¹,
 Mohammed Eid Abd El-Salam ²

¹ Department of Clinical Pathology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt.

² Department of Ophthalmology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt.

ABSTRACT

Article information

Received: 09-04-2024

Accepted: 23-06-2024

DOI:
 10.21608/IJMA.2024.217439.1707.

*Corresponding author
 Email: dr.moustafa1994@gmail.com

Citation: Alkot M, Hablas WR, Shaheen MAA, Abd El-Salam ME. Bacteriological Study of Conjunctival Swab Cultures in Diabetic Patients. IJMA 2024 June; 6 [6]: 4607-4611. doi: 10.21608/IJMA.2024.217439.1707.

Background: Diabetic patients are known to be at an increased risk of ocular infections due to compromised immune functions and altered ocular surface microenvironment. Conjunctival infections in diabetic individuals can lead to severe complications, highlighting the importance of understanding the bacteriological profile in this population.

The aim of the work: This study aimed to investigate the bacteriological profile of conjunctival swab cultures in diabetic patients to identify common pathogens and their relation to clinical and laboratory data.

Patients and Methods: A case-control study was conducted involving 160 eyes of eighty patients attending ophthalmology outpatients' clinic at Al-Azhar University Hospitals. Conjunctival swab cultures were collected aseptically and processed for bacterial isolation and identification.

Results: In this study, 80 diabetic patients with a mean age of 51.2 ± 7.05 years were included. Bacterial growth was observed in 45% of the patients, with Coagulase-negative Staphylococci being the most commonly isolated pathogen at 30%, followed by Staphylococcus aureus. The prevalence of positive conjunctival cultures was slightly higher among patients receiving insulin compared to oral hypoglycemic drugs [OHDs], although the presence of Staph coagulase-negative, Staph aureus, and non-hemolytic streptococcus was more prominent in patients undergoing insulin therapy. However, these differences did not reach statistical significance. Furthermore, no significant correlation was found between the culture results and the levels of HbA1C among the diabetic patients.

Conclusion: The findings of this study underline the importance of regular surveillance of conjunctival infections in diabetic patients to guide appropriate antimicrobial therapy and prevent potential complications. Understanding the bacteriological spectrum and resistance patterns can aid in the development of targeted treatment strategies to improve ocular health outcomes in this vulnerable population.

Keywords: Diabetes Mellitus; Conjunctiva; Culture; Staphylococcal Infections.



This is an open-access article registered under the Creative Commons, ShareAlike 4.0 International license [CC BY-SA 4.0] [<https://creativecommons.org/licenses/by-sa/4.0/legalcode>].

INTRODUCTION

The conjunctiva, a thin mucous membrane covering the inner surface of the eyelids and the outer surface of the eyeball, is inhabited by a diverse microbial community known as the conjunctival flora^[1]. This natural flora consists of various microorganisms, including bacteria such as Staphylococci, Streptococci, Corynebacteria, and Propionibacteria. These commensal bacteria play a crucial role in maintaining ocular surface health by outcompeting potential pathogens, producing antimicrobial substances and modulating the local immune response^[2].

In diabetes mellitus, when the immune system is compromised, certain members of the conjunctival flora that are typically non-pathogenic can become opportunistic pathogens, potentially resulting in severe complications and illness^[3].

Diabetes mellitus is a chronic metabolic disease characterized by high blood glucose levels as a result of defects in insulin production or insulin action. It affects millions of people worldwide and can lead to serious medical complications if not well controlled^[4].

Diabetic patients are predisposed to ocular infections due to compromised immunity and altered ocular microenvironment. Understanding the bacterial profile of conjunctival infections in this population is crucial for effective management^[5].

In the realm of diabetic patients, particularly those with ocular complications, the understanding of the specific bacterial flora present in conjunctival infections remains an area with significant gaps in existing literature. While prior research has shed light on the correlation between diabetes and increased susceptibility to ocular infections, there is a notable scarcity of comprehensive studies focusing on the detailed bacteriological profile of conjunctival swab cultures in diabetic individuals. Understanding the bacteriological dynamics in diabetic conjunctival infections is crucial for guiding evidence-based clinical decisions and optimizing patient outcomes^[6]. By elucidating the microbial flora and resistance patterns in this vulnerable population, the study aims to enhance our understanding of ocular infections in diabetic patients and inform strategies for better management and prevention of ocular complications associated with diabetes.

This study aimed to investigate the bacteriological profile of conjunctival swab cultures in diabetic

patients to identify common pathogens and their relation to clinical and laboratory data.

PATIENTS AND METHODS

Eighty patients, contributing 160 eyes, were sourced from the Ophthalmology Clinic at Al-Azhar University Hospital. They were invited and included in the research between November 1, 2022, and April 30, 2023. This case-control study centered on bacteriological cultures from the unaffected conjunctiva of diabetic patients attending the Eye Outpatient Department.

Inclusion criteria: Patients with diabetic retinopathy, whatever type 1 [insulin dependent] or type 2 with Hemoglobin A1C test.

Exclusion Criteria: Patients experiencing an active ocular infection and/or take topical antibiotics.

The control group comprising 20 subjects without diabetes was selected randomly from individuals without any history of ocular infections or systemic diseases affecting the conjunctiva.

The research adhered to the ethical guidelines outlined by the ethical committee of Al-Azhar University hospitals. All participants underwent assessment involving HbA1C test, medical history examination, Conjunctival Swab culture, and laboratory identification of microorganisms.

Study Procedure: Specimens were collected using disposable sterile cotton swabs and placed in tubes containing Stuart transport medium. Each specimen was appropriately labeled with the participant's name, age, gender, as well as the date and time of specimen collection. Collection of conjunctival swabs from diabetic retinopathy patients attending ophthalmic clinic was performed after informed consent. Patients were instructed to gaze upwards and delicately lower the lower eyelid to reveal the conjunctiva. The cotton swab stick was gently moved along the lower fornix from the inner to the outer corner of the eye, ensuring no contact with the eyelids. The swab was promptly transferred into a container with bacterial medium, after which the patient was instructed to close their eye briefly.

Conjunctival swab culture: Swabs were directly placed onto blood agar, chocolate agar, and MacConkey agar. Blood and MacConkey agar plates were then incubated at 36 ± 1 °C in aerobic conditions, while chocolate agar plates

were incubated at 36 ± 1 °C in jars with 10% CO₂. Following a 24-hour incubation period, all agar plates were examined. Any visible colonies on the culture agar plates were subjected to standard microbiological identification methods. If no growth was observed on the culture plates after 24 hours of incubation, it was recorded as no growth

Statistical analysis: The data was analyzed with SPSS version 24. For quantitative data, the mean and standard deviation were reported. Qualitative data were presented as frequencies and percentages. The Mann-Whitney U test was employed for comparing two means in the case of non-normally distributed data, whereas the Chi-square test was utilized for comparing categorical variables. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Table [1] shows that the mean age of patients with diabetes was 51.2 years. Females [56%] were more frequent than males. The mean duration of diabetes was 16.5 years, and most of them received insulin therapy [57.5%].

Regarding culture results, 45% of patients with diabetes had positive culture. The most frequent organism was CON staph [30%] followed by Staph aureus [8.75%]. Only one [5%] case in control group had positive culture [Table 2].

Table [3] shows that there were no significant differences between males and females with diabetes in relation to culture results.

There were no significant differences regarding culture results according to type of treatment or HbA1C levels [Tables 4, 5].

Table [1]: Demographic, clinical and laboratory data of studied cases

		Patients [N = 80]		Control [N = 20]		Rest	P-value
Age [years]	Mean \pm SD	51.2 \pm 7.05		47.9 \pm 6.7		MW = 576	0.053
Sex	Male	35	43.7%	8	40%	X ² = 0.9	0.761
	Female	45	56.3%	12	60%		
HbA1C [%]	Mean	7.6		5.5		MW = 0.0	< 0.001
	\pm SD	0.6		0.5			
Duration of DM [years]	Mean \pm SD	16.5 \pm 2.5					
	Min – Max	2 – 30					
Therapy	OHDs	34	42.5%				
	Insulin	46	57.5%				

Table [2]: Comparison between studied groups as regard culture results

		Patients [N = 80]		Control [N = 20]		Stat. test	P-value
Culture results	No growth	44	55%	19	95%	X ² = 11.06	0.011
	CON staph	24	30%	1	5%		
	Staph aureus	7	8.75%	0	0%		
	Non-hemolytic strept.	5	6.25%	0	0%		

Table [3]: Relation between culture results and sex distribution in patients' group

		Male [N = 35]		Female [N = 45]		Stat. test	P-value
Culture results	No growth	18	51.4%	26	57.8%		
	CON staph	11	31.4%	13	28.9%		
	Staph aureus	4	11.4%	3	6.7%		
	Non-hemolytic strept.	2	5.7%	3	6.7%		

Table [4]: Relation between culture results and treatment in patients' group

		Treatment				Stat. test	P-value
		OHDs [N = 34]		Insulin [N = 46]			
Culture results	No growth	19	55.9%	25	54.3%	X ² = 0.88	0.83
	CON staph	9	26.5%	15	32.6%		
	Staph aureus	3	8.8%	4	8.7%		
	Non-hemolytic strept.	3	8.8%	2	4.3%		

Table [5]: Relation between culture results and HbA1C in patients' group

Culture results	HbA1C [%]	Stat. test	P-value
No growth [n = 38]	7.63 ± 0.54	X ² = 1.68	0.431
CON staph [n = 27]	7.48 ± 0.53		
Staph aureus [n = 8]	7.73 ± 0.77		
Non-hemolytic strept. [n = 7]	7.7 ± 0.5		

DISCUSSION

Conjunctival flora acts as a layer of defense against infection and includes significant pathogens of eye infections. In well persons, the conjunctival flora contains several of the same microorganisms as the skin flora [7]. The normal bacterial flora restricts the proliferation and entrance of pathogenic microbes by limiting their source of nutrition and the space available for enzyme growth and secretion [8].

Diabetes is one disorder that might impair immune system function. Diabetics have a conjunctival flora sequence with higher bacteria, which is a common cause of diabetic infections [5].

For evaluating culture data acquired under pathologic environments, detailed understanding of normal conjunctival flora is required. Our study included 80 patients, 35 were male and 45 were female and 34 were under OHDs treatment, 46 were on insulin therapy who met the inclusion criteria.

In our research, bacterial growth was observed in 45% of the 80 patients examined. The most frequently identified bacteria was Coagulase-negative Staphylococcus [CONS] at 30%, followed by Staphylococcus aureus at 8.75% and non-hemolytic streptococcus at 6.25%.

In our research, we considered the impact of hypoglycemic therapy on conjunctival flora. We found that the rate of positive cultures was higher in patients using insulin [57.5%] compared to oral hypoglycemic drugs [OHDs] [42.5%], but this difference was not statistically significant. Additionally, the presence of CONS was slightly elevated in patients on insulin [32.6%] than in those on OHDs [26.5%], also without statistical significance.

The key finding in our study is the increased occurrence of gram-positive bacterial isolates among patients with diabetes.

In the study by **Bilen et al.** [9], it was found that 35.4% of patients with type 1 diabetes had no bacterial growth, while 21.2% of patients with type 2 diabetes and 50% of control subjects showed no growth. The most commonly isolated organisms

in patients with type 1 diabetes were Staphylococcus epidermidis [11.79%] and Staphylococcus aureus [11.7%], while in patients with type 2 diabetes, Staphylococcus epidermidis [24.2%] and Staphylococcus aureus [21.2%] were predominant. In control subjects, the most frequently isolated organisms were Staphylococcus epidermidis [22%], Staphylococcus aureus [12%], and Corynebacterium spp [10%].

These findings were in line with the results documented by **Karimsab and Razak** [10], where Staphylococcus epidermidis was the predominant organism isolated in both diabetic and non-diabetic cohorts. It was identified in 86.66% of diabetic individuals compared to 36% in non-diabetic individuals.

In a study conducted by **Arbab et al.** [11], parallel results were observed, indicating that diabetic patients exhibited a markedly elevated rate of positive conjunctival cultures. The study also noted a correlation between the presence of diabetic retinopathy, an increase in positive cultures, and a higher prevalence of Staphylococcus epidermidis.

Contrary to the results of **Adam et al.** [12], our study revealed distinct microbial profiles in the diabetic group. Specifically, we identified Staphylococcus aureus in 6 cultures [30%], Escherichia coli [gram-negative bacilli] in 4 cultures [20%], CONS in 2 cultures [10%], Klebsiella pneumoniae in 2 cultures [10%], and multiple bacterial species in 6 cultures [30%].

Antibiotic susceptibility differs between gram-positive and gram-negative microorganisms. Although no antibiogram was conducted in this study, there are other investigations in the literature. In the study by **Coşkun et al.** [13], it was found that a high percentage of Staphylococcus aureus conjunctival isolates were susceptible to ofloxacin [91.1%] and ciprofloxacin [86.6%], while only a small portion showed sensitivity to penicillin G [8.8%]. Additionally, around 28.8% of the isolates were identified as methicillin-resistant Staphylococcus aureus, with 38.5% of these being susceptible to ofloxacin or ciprofloxacin. Similarly, Staphylococcus epidermidis

cultures demonstrated high sensitivity to ofloxacin [92.5%] and ciprofloxacin [91.5%] in the same study.

The primary limitation of this study is the lack of longitudinal data, which would have provided insights into the temporal dynamics of conjunctival infections in diabetic individuals. Furthermore, the study was conducted at a single center, which may introduce institutional biases and limit the diversity of the participant population. Future studies should aim to include larger, multi-center cohorts to enhance our understanding of the relationship between diabetes and conjunctival infections.

Conclusion

In diabetic patients, the elevated presence of bacteria in the conjunctival flora is a common factor contributing to numerous ocular infections associated with diabetes. Conjunctivitis was found to take place more repeatedly in people with diabetes but incidence was not related to the degree of glycemic control. It is essential for ophthalmologists to remember that there are variations in conjunctival flora between diabetic and non-diabetic individuals. Diabetic patients show a higher prevalence of gram-positive bacteria in their conjunctival flora. It is crucial to recognize that these flora components could serve as significant pathogens in ocular infections.

Financial disclosures: None

Conflict of Interest: None.

REFERENCES

1. Leal SM Jr, Rodino KG, Fowler WC, Gilligan PH. Practical Guidance for Clinical Microbiology Laboratories: Diagnosis of Ocular Infections. *Clin Microbiol Rev.* 2021 Jun 16;34[3]:e0007019. doi: 10.1128/CMR.00070-19.
2. Peter VG, Morandi SC, Herzog EL, Zinkernagel MS, Zysset-Burri DC. Investigating the Ocular Surface Microbiome: What Can It Tell Us? *Clin Ophthalmol.* 2023 Jan 19;17:259-271. doi: 10.2147/OPTH.S359304.
3. Petrillo F, Pignataro D, Lavano MA, Santella B, Folliero V, Zannella C, et al. Current Evidence on the Ocular Surface Microbiota and Related Diseases. *Microorganisms.* 2020 Jul 13;8[7]:1033. doi: 10.3390/microorganisms8071033.
4. Rahman MS, Hossain KS, Das S, Kundu S, Adegoke EO, Rahman MA, Hannan MA, Uddin MJ, Pang MG. Role of Insulin in Health and Disease: An Update. *Int J Mol Sci.* 2021 Jun 15;22[12]:6403. doi: 10.3390/ijms22126403.
5. Amorim M, Martins B, Fernandes R. Immune Fingerprint in Diabetes: Ocular Surface and Retinal Inflammation. *Int J Mol Sci.* 2023 Jun 6;24[12]:9821. doi: 10.3390/ijms24129821.
6. Grandi G, Bianco G, Boattini M, Scalabrin S, Iannaccone M, Fea A, Cavallo R, Costa C. Bacterial etiology and antimicrobial resistance trends in ocular infections: A 30-year study, Turin area, Italy. *Eur J Ophthalmol.* 2021 Mar;31[2]:405-414. doi: 10.1177/1120672119896419.
7. Nadăș GC, Novac CȘ, Matei IA, Bouari CM, Gal ZM, Tamas-Krumpe OM, Macri AM, Fiț NI. Prevalence of Antimicrobial Resistant Bacteria from Conjunctival Flora in an Eye Infection Prone Breed [Saint Bernard]. *Molecules.* 2021 Apr 12;26[8]:2219. doi: 10.3390/molecules26082219.
8. Aragona P, Baudouin C, Benitez Del Castillo JM, Messmer E, Barabino S, Merayo-Llodes J, et al. The ocular microbiome and microbiota and their effects on ocular surface pathophysiology and disorders. *Surv Ophthalmol.* 2021 Nov-Dec;66[6]:907-925. doi: 10.1016/j.survophthal.2021.03.010.
9. Bilen H, Ates O, Astam N, Uslu H, Akcay G, Baykal O. Conjunctival flora in patients with type 1 or type 2 diabetes mellitus. *Adv Ther.* 2007 Sep-Oct;24[5]:1028-35. doi: 10.1007/BF02877708.
10. Karimsab D, Razak SK. Study of aerobic bacterial conjunctival flora in patients with diabetes mellitus. *Nepal J Ophthalmol.* 2013 Jan-Jun;5[1]:28-32. doi: 10.3126/nepjoph.v5i1.7818.
11. Arbab TM, Qadeer S, Iqbal S, Mirza MA. Aerobic bacterial conjunctival flora in diabetic Patients. *Pak J Ophthalmol.* 2010 Dec 31;26[4]:177-181. doi: 10.36351/pjo.v26i4.534.
12. Adam M, Balcı M, Bayhan HA, İnkaya AÇ, Uyar M, Gürdal C. Conjunctival Flora in Diabetic and Nondiabetic Individuals. *Turk J Ophthalmol.* 2015 Oct;45[5]:193-196. doi: 10.4274/tjo.33230.
13. Coşkun M, Koçak AG, Simavlı H, Anayol MA, Toklu Y, Çelikbilek N. Analyzing normal conjunctival flora and detecting antibiogram sensitivity to fluoroquinolones and penicillin derivatives. *Glo-Kat.* 2007;2:167-70.

