(e) ISSN Online: 2321-9599

(p) ISSN Print: 2348-6805

ORIGINAL ARTICLE

Comparative Evaluation of the Microbial Flora in Normal vs. Dry Eyes: A Clinical and Microbiological Approach

[1]Vishwajeet Bardoloi, [2]Nishad Navinchandra Dala

[1]Assistant Professor, Department of Microbiology, Azeezia Medical College and Hospital, Adichanalloor, Kerala, India;
[2]Assistant Professor, Department of Ophthalmology, KM Medical College and Hospital, Mathura, UP, India

ABSTRACT:
Aim: This study aims to conduct a comparative evaluation of the microbial flora in normal versus dry eyes using both clinical and microbiological approaches to better understand the microbial dynamics associated with dry eye syndrome. **Materials and Methods:** A total of 100 patients were enrolled, with 50 diagnosed with dry eye syndrome and 50 healthy controls. Dry eye was confirmed using clinical assessments such as the Schirmer's test, Tear Break-Up Time (TBUT), and ocular surface staining. Conjunctival swabs were collected from both groups and cultured on blood agar, MacConkey agar, and Sabouraud dextrose agar to assess bacterial and fungal growth. The microbial species were identified, and colony-forming units (CFU) per swab were calculated. Data analysis was performed using chi-square tests and t-tests. **Results:** The dry eye group exhibited significantly lower TBUT (5.2 ± 1.6 seconds) and Schirmer's test scores (6.4 ± 2.1 mm/5 min) compared to the normal group (11.4 ± 2.3 seconds, 18.2 ± 3.4 mm/5 min, respectively). A higher prevalence of *Staphylococcus aureus* (16% vs. 8%) and *Coagulase-negative staphylococci* (40% vs. 24%) was observed in the dry eye group. Additionally, *Pseudomonas aeruginosa* was found in 14% of dry eye patients, and the microbial load (CFU per swab) was significantly higher for these pathogens in the dry eye group compared to the normal group. **Conclusion:** This study demonstrates a significant association between dry eye disease and increased microbial load, particularly of *Staphylococcus aureus*, *Coagulase-negative staphylococci*, and *Pseudomonas aeruginosa*. The compromised tear film stability and reduced tear production in dry eye patients likely contribute to this increased microbial colonization, with a lower TBUT associated with a higher prevalence of microbial flora.
Keywords: Dry Eye Syndrome, Microbial Flora, Tear Break-Up Time, *Staphylococcus aureus*, *Pseudomonas aeruginosa*

Corresponding author: Nishad Navinchandra Dala, Assistant Professor, Department of Ophthalmology, KM Medical College and Hospital, Mathura, UP, India

This article may be cited as: Bardoloi V, Dala NN. Comparative Evaluation of the Microbial Flora in Normal vs. Dry Eyes: A Clinical and Microbiological Approach. J Adv Med Dent Scie Res 2017;5(1):208-213.

INTRODUCTION

The human eye, a complex and highly sensitive organ, is constantly exposed to a myriad of environmental factors such as dust, pollutants, allergens, and microorganisms. The cornea, conjunctiva, and other ocular surfaces are protected by a variety of defense mechanisms, including tear production, blinking, and a carefully balanced microbial ecosystem. This ecosystem, known as the ocular microbiota, plays a critical role in maintaining eye health and preventing ocular infections. However, alterations in the composition and diversity of this microbiota can lead to various eye diseases, including dry eye disease (DED).[1]

Dry eye disease is a multifaceted condition characterized by insufficient tear production or poor-quality tears, leading to discomfort, visual disturbance, and potential damage to the ocular surface. It is one of the most common complaints among patients presenting to ophthalmologists and optometrists. The pathophysiology of dry eye disease involves a complex interplay between the tear film, the ocular surface, inflammation, and the microbial flora present in the eye. Research into the ocular microbiota has unveiled its important role in maintaining the integrity of the ocular surface and modulating immune responses. Therefore, understanding the differences in the microbial composition of normal versus dry eyes could provide insights into the pathogenesis of dry eye disease and offer new avenues for diagnosis and treatment.[2]

In a normal, healthy eye, the microbial flora primarily consists of commensal microorganisms, including various species of bacteria, fungi, viruses, and mites. The most common bacteria found on the ocular surface are *Staphylococcus epidermidis*, *Corynebacterium*, and *Propionibacterium acnes*, which usually coexist in a balanced, non-pathogenic manner. The tear film plays an essential role in preventing the overgrowth of these microorganisms, while also facilitating the removal of harmful pathogens. The composition of the ocular microbiota in normal eyes remains relatively stable over time, with only minor fluctuations due to factors such as age, hormonal changes, diet, and environmental exposure. This stability is vital for maintaining ocular health and preventing infections such as bacterial keratitis or conjunctivitis.[3]

However, in dry eye disease, the equilibrium of the ocular microbiota can be disrupted. DED is often associated with inflammation of the ocular surface, leading to a compromised tear film and the

development of an altered microbial composition. Recent studies have highlighted an increase in pathogenic microorganisms, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*, in the conjunctiva and cornea of individuals with dry eye disease. These pathogenic organisms may contribute to the symptoms of DED by exacerbating inflammation, increasing tear film instability, or directly damaging the ocular surface. Furthermore, some research has suggested that the overgrowth of certain bacterial species could be linked to the activation of the immune response, which in turn may further exacerbate dry eye symptoms. In addition to bacterial changes, the ocular microbiota in dry eye disease may show alterations in the diversity and abundance of fungal species, viruses, and other microorganisms, although these findings are still under investigation.[4]

The microbial imbalance in dry eye disease, known as dysbiosis, can be influenced by several factors. One of the most prominent factors is the tear film instability that characterizes DED. Inadequate tear production or poor-quality tears can result in a dry, desiccated ocular surface that is more prone to microbial colonization. Furthermore, the inflammation associated with dry eye disease can alter the local immune environment, providing a more favorable niche for the growth of pathogenic organisms. Environmental factors such as prolonged use of digital devices, exposure to air conditioning or heating, and pollution can further exacerbate the condition by inducing dryness and contributing to microbial imbalances. Moreover, systemic conditions like autoimmune diseases, hormonal changes, and medications (e.g., antihistamines, antidepressants) may also play a role in altering the ocular microbiota.[5]

In addition to its potential role in the pathogenesis of dry eye disease, the ocular microbiota may also have therapeutic implications. Understanding the microbial profile of both normal and dry eyes could lead to the development of microbiome-based treatments, such as probiotics, prebiotics, or bacteriophage therapy, to restore microbial balance and alleviate the symptoms of DED. Targeting the microbiota could provide a novel, non-invasive treatment strategy for dry eye disease, reducing the reliance on traditional therapies such as artificial tears, anti-inflammatory medications, and immunosuppressive agents. Furthermore, exploring the role of the microbiota in other ocular conditions, such as conjunctivitis or corneal ulcers, could expand the scope of microbiome-based interventions in ophthalmology.[6,7]

A comprehensive comparative evaluation of the microbial flora in normal versus dry eyes requires both clinical and microbiological approaches. Clinically, the evaluation of dry eye disease typically involves the assessment of symptoms, tear break-up time, Schirmer test, and ocular surface staining. These assessments help diagnose and categorize the severity of DED. Microbiologically, the analysis of ocular samples—such as conjunctival swabs, tear samples, or corneal scrapings—can provide insights into the composition and diversity of the microbial flora. Advanced molecular techniques, such as next-generation sequencing (NGS), have revolutionized our ability to identify and quantify the microorganisms present in ocular samples, allowing for a more detailed and accurate understanding of the ocular microbiome. By combining these clinical and microbiological methods, researchers can gain valuable insights into the differences in the ocular microbiota between normal and dry eyes and explore the potential role of microbial dysbiosis in the pathogenesis of dry eye disease.

MATERIALS AND METHODS

This study aimed to conduct a comparative evaluation of the microbial flora in normal versus dry eyes, utilizing both clinical and microbiological approaches. A total of 100 patients were enrolled, with 50 patients diagnosed with dry eye syndrome and 50 healthy controls. The inclusion criteria for the dry eye group consisted of patients presenting with symptoms of dryness, irritation, and foreign body sensation, confirmed through clinical assessments such as the Schirmer's test, Tear Break-Up Time (TBUT), and ocular surface staining with fluorescein. The control group was composed of individuals with no history of dry eye disease, exhibiting normal tear production and ocular health. Ethical approval for the study was obtained from the institutional review board, and all participants provided written informed consent.

For microbiological analysis, conjunctival swabs were collected from both eyes of all patients using sterile cotton-tipped applicators. The swabs were immediately transported to the microbiology laboratory and inoculated on various culture media, including blood agar, MacConkey agar, and Sabouraud dextrose agar, to assess both bacterial and fungal flora. After incubation at 37°C for 24-48 hours, microbial growth was identified using standard microbiological techniques, including Gram staining, biochemical tests, and automated identification systems. The microbial species were classified and compared between the dry eye and normal eye groups. In addition, the quantitative microbial load was evaluated by calculating colony-forming units (CFU) per swab. All data were analyzed using appropriate statistical methods, including chi-square tests for categorical variables and t-tests for continuous variables, to determine significant differences in the microbial flora between the two groups.

RESULTS

Table 1: Demographic Characteristics of the Study Groups

The demographic characteristics of the study groups were compared to determine any significant differences in age, gender, tear break-up time (TBUT), and Schirmer's test results between dry eye

patients and healthy controls. Regarding age, the average age in the dry eye group was 47.2 ± 12.4 years, while the normal eye group had a slightly younger average age of 45.1 ± 13.0 years. The p-value of 0.45 indicates that there was no statistically significant difference in age between the two groups.

When examining gender distribution, there was no significant difference. In the dry eye group, 18 (36%) were male and 32 (64%) were female, while in the normal eye group, 20 (40%) were male and 30 (60%) were female. The p-value of 0.68 suggests the gender distribution was similar between both groups.

Mean Tear Break-Up Time (TBUT), a key diagnostic tool for dry eye syndrome, showed a significantly lower result in the dry eye group (5.2 ± 1.6 seconds) compared to the normal eye group (11.4 ± 2.3 seconds), with a p-value of 0.0001. This significant difference supports the notion that dry eye patients typically have a reduced tear film stability, which is a hallmark of the condition. Similarly, Schirmer's Test for tear production revealed a marked difference between groups, with the dry eye group having a much lower mean of 6.4 ± 2.1 mm/5 min, compared to 18.2 ± 3.4 mm/5 min in the normal eye group. The p-value of 0.0001 further confirms the statistical significance of this difference, indicating reduced tear secretion in dry eye patients.

Table 2: Microbial Flora Present in Dry Eye vs. Normal Eye Groups

Table 2 outlines the comparison of microbial flora found in the eyes of dry eye patients versus normal controls. Staphylococcus aureus was present in 8% of the normal eye group and 16% of the dry eye group. This difference was statistically significant (p-value = 0.04), suggesting that dry eye patients are more likely to harbor this opportunistic pathogen.

Similarly, Coagulase-negative staphylococci, another common ocular pathogen, were found in 40% of dry eye patients compared to 24% of normal eye patients, with a p-value of 0.01 indicating a significant increase in prevalence among dry eye sufferers. This microorganism is often associated with ocular infections, particularly in compromised eye conditions such as dry eye disease.

The prevalence of Streptococcus pneumoniae was comparable between the two groups, with 6% in the dry eye group and 4% in the normal eye group. The p-value of 0.53 indicates no statistically significant difference between these two groups for this pathogen.

There was a notable difference in the occurrence of Pseudomonas aeruginosa, which was present in 14% of the dry eye group but only 4% of the normal eye group, with a p-value of 0.03, showing a significant association between dry eye disease and this pathogen. Fusarium species were found in 4% of the dry eye group, whereas no growth was observed in the normal eye group. However, the p-value of 0.09 suggests that this result may not be statistically significant.

Candida albicans was present in 8% of the dry eye group and 2% of the normal eye group, but the p-value of 0.10 indicates no significant difference between the groups. Lastly, no microbial growth was observed in 58% of the normal eye group compared to only 12% in the dry eye group, with a highly significant p-value of 0.0001, further indicating that dry eye patients tend to have a more diverse ocular microbiota.

Table 3: Bacterial and Fungal Load (CFU per Swab) in Dry Eye and Normal Eye Groups

Table 3 compares the bacterial and fungal load, as measured by colony-forming units (CFU), between the two groups. Staphylococcus aureus showed a significantly higher CFU in the dry eye group (16.5 ± 5.3) compared to the normal eye group (5.2 ± 2.1), with a p-value of 0.0001, indicating a statistically significant higher microbial load in the dry eye group. Similarly, Coagulase-negative staphylococci had a higher CFU in the dry eye group (21.4 ± 6.8) compared to the normal eye group (10.5 ± 3.4), and the p-value of 0.0002 confirms that this difference is statistically significant.

The CFU of Streptococcus pneumoniae was higher in the dry eye group (5.3 ± 1.4) compared to the normal eye group (3.0 ± 1.2), with a p-value of 0.04, further confirming that the dry eye group harbors a higher microbial load for this organism as well. For Pseudomonas aeruginosa, the CFU count was significantly higher in the dry eye group (12.2 ± 4.6) compared to the normal eye group (4.4 ± 2.2), with a p-value of 0.0001, indicating a significant association between dry eye disease and this pathogen's increased load.

Fusarium species were present in the dry eye group with a CFU of 1.8 ± 0.6, but no data were available for the normal eye group (N/A). The p-value of 0.12 suggests that this finding may not be statistically significant. Lastly, Candida albicans had a higher CFU in the dry eye group (3.2 ± 1.1) compared to the normal eye group (1.4 ± 0.7), with a p-value of 0.03, indicating a significant difference in fungal load between the two groups.

Table 4: Statistical Comparison of Microbial Flora Between Dry Eye and Normal Eye Groups

In Table 4, the statistical comparison of the prevalence of various microorganisms between the dry eye and normal eye groups is presented. Staphylococcus aureus was present in 16% of the dry eye group compared to 8% in the normal eye group (p-value = 0.04), demonstrating a statistically significant higher prevalence in the dry eye group. Similarly, Coagulase-negative staphylococci were found in 40% of the dry eye group and 24% of the normal eye group, with a p-value of 0.01 indicating a significant difference.

The prevalence of Streptococcus pneumoniae was similar between the two groups (6% in the dry eye group and 4% in the normal eye group), and the p-value of 0.53 suggests that this difference was not statistically significant. On the other hand, Pseudomonas aeruginosa was found in 14% of the dry eye group and 4% of the normal eye group (p-value = 0.03), confirming a significant association with dry eye disease. Fusarium species were found in 4% of dry eye patients and none in the normal eye group, but the p-value of 0.09 suggests that the difference was not statistically significant.

The presence of Candida albicans was observed in 8% of dry eye patients and 2% of normal patients, with a p-value of 0.10, suggesting no significant difference. Lastly, no microbial growth was observed in 58% of normal eye patients and only 12% of dry eye patients, with a highly significant p-value of 0.0001, showing that dry eye patients tend to harbor more microorganisms.

Table 5: Tear Film Quality and Microbial Flora Correlation

Table 5 examines the relationship between tear film quality (as measured by TBUT) and microbial flora. For patients with TBUT ≤ 5 seconds, indicating poorer tear quality, there was a higher prevalence of microorganisms: 25% tested positive for *Staphylococcus aureus*, 48% for *Coagulase-negative staphylococci*, 18% for *Pseudomonas aeruginosa*, and 12% for *Candida albicans*.

In contrast, for patients with TBUT > 5 seconds, the prevalence of these microorganisms was significantly lower: 7% for *Staphylococcus aureus*, 16% for *Coagulase-negative staphylococci*, 4% for *Pseudomonas aeruginosa*, and 2% for *Candida albicans*. These findings suggest a clear correlation between reduced tear film stability (lower TBUT) and a higher likelihood of microbial colonization in the ocular surface. The data indicates that poorer tear film quality may contribute to an increased microbial load in dry eye patients.

Table 1: Demographic Characteristics of the Study Groups

Characteristic	Dry Eye Group (n=50)	Normal Eye Group (n=50)	p-value
Age (mean ± SD)	47.2 ± 12.4	45.1 ± 13.0	0.45
Gender (Male/Female)	18 (36%) / 32 (64%)	20 (40%) / 30 (60%)	0.68
Mean Tear Break-Up Time (TBUT)	5.2 ± 1.6 seconds	11.4 ± 2.3 seconds	0.0001
Schirmer's Test (mm/5 min)	6.4 ± 2.1	18.2 ± 3.4	0.0001

Table 2: Microbial Flora Present in Dry Eye vs. Normal Eye Groups

Microorganism	Dry Eye Group (n=50)	Normal Eye Group (n=50)	p-value
Staphylococcus aureus	8 (16%)	4 (8%)	0.04
Coagulase-negative staphylococci	20 (40%)	12 (24%)	0.01
Streptococcus pneumoniae	3 (6%)	2 (4%)	0.53
Pseudomonas aeruginosa	7 (14%)	2 (4%)	0.03
Fusarium species	2 (4%)	0 (0%)	0.09
Candida albicans	4 (8%)	1 (2%)	0.10
No growth	6 (12%)	29 (58%)	0.0001

Table 3: Bacterial and Fungal Load (CFU per Swab) in Dry Eye and Normal Eye Groups

Microbial Species	Dry Eye Group (mean ± SD)	Normal Eye Group (mean ± SD)	p-value
Staphylococcus aureus	16.5 ± 5.3	5.2 ± 2.1	0.0001
Coagulase-negative staphylococci	21.4 ± 6.8	10.5 ± 3.4	0.0002
Streptococcus pneumoniae	5.3 ± 1.4	3.0 ± 1.2	0.04
Pseudomonas aeruginosa	12.2 ± 4.6	4.4 ± 2.2	0.0001
Fusarium species	1.8 ± 0.6	N/A	0.12
Candida albicans	3.2 ± 1.1	1.4 ± 0.7	0.03

Table 4: Statistical Comparison of Microbial Flora Between Dry Eye and Normal Eye Groups

Microorganism	Dry Eye Group (%)	Normal Eye Group (%)	p-value
Staphylococcus aureus	8 (16%)	4 (8%)	0.04
Coagulase-negative staphylococci	20 (40%)	12 (24%)	0.01
Streptococcus pneumoniae	3 (6%)	2 (4%)	0.53
Pseudomonas aeruginosa	7 (14%)	2 (4%)	0.03
Fusarium species	2 (4%)	0 (0%)	0.09
Candida albicans	4 (8%)	1 (2%)	0.10
No growth	6 (12%)	29 (58%)	0.0001

Table 5: Tear Film Quality and Microbial Flora Correlation

Tear Film Quality (TBUT)	*Staphylococcus aureus* (%)	*Coagulase-negative staphylococci* (%)	*Pseudomonas aeruginosa* (%)	*Candida albicans* (%)
TBUT ≤ 5 seconds	25% (12/48)	48% (23/48)	18% (9/48)	12% (6/48)
TBUT > 5 seconds	7% (3/42)	16% (7/42)	4% (2/42)	2% (1/42)

DISCUSSION

The results from this study reveal significant findings regarding microbial colonization in dry eye patients compared to normal controls. In our study, the dry eye group exhibited a significantly reduced **Tear Break-Up Time (TBUT)** and **Schirmer's Test** scores, which is consistent with prior research that highlights diminished tear production and poor tear film stability as key features of dry eye syndrome. For instance, a study by **Schein et al. (1997)** showed that dry eye patients often demonstrate significantly lower TBUT values, which was corroborated by our study where the dry eye group had an average TBUT of 5.2 seconds, significantly lower than the normal group's 11.4 seconds (p-value = 0.0001). [6]

When comparing microbial flora between dry eye and normal eye groups, our results align with previous studies that have observed a higher prevalence of **Staphylococcus aureus** and **Coagulase-negative staphylococci** in dry eye patients. **McCulley et al. (1998)** demonstrated that ocular surface diseases like dry eye often lead to a higher bacterial load, particularly of staphylococci. In this study, we found that 16% of dry eye patients had *S. aureus* compared to 8% in the normal group (p-value = 0.04), and 40% of dry eye patients harbored*Coagulase-negative staphylococci* versus 24% in the normal group (p-value = 0.01). [7]

Additionally, our results found a significant association between **Pseudomonas aeruginosa** and dry eye patients, with 14% of dry eye patients and only 4% of normal eye patients testing positive (p-value = 0.03). This finding is consistent with **Lemp et al. (2004)**, who reported a higher prevalence of *Pseudomonas aeruginosa* in individuals with dry eye disease. The increased load of this pathogen in dry eye patients could be due to the prolonged exposure of the ocular surface to environmental factors, coupled with the lack of effective tear exchange and antimicrobial defense mechanisms.[8]

The finding of **Fusarium species** in 4% of dry eye patients but none in the normal eye group (p-value = 0.09) warrants further attention. While not statistically significant, this result is in line with studies by **Jones et al. (2011)**, who noted the presence of fungal organisms in certain dry eye patients, particularly those with significant ocular surface damage or immune suppression. Although our study's sample size might not have been large enough to fully establish this correlation, it suggests that dry eye patients with severe disease might be more susceptible to fungal infections.[9]

Interestingly, **Candida albicans** was present in 8% of the dry eye group compared to 2% of the normal group, though the difference was not statistically significant (p-value = 0.10). Our findings on *Candida* are similar to those of **Moss et al. (2011)**, who found that while fungal infections like *Candida* are relatively rare in the general population, they may occur more frequently in patients with dry eye disease, especially in cases with a compromised epithelial barrier.[10]

The **microbial load** in dry eye patients was notably higher across multiple organisms, including *S. aureus*, *Coagulase-negative staphylococci*, and *P. aeruginosa*, compared to normal controls. These results are consistent with a study by **Kara et al. (2009)**, who found that dry eye patients had a significantly higher CFU count for both bacterial and fungal species compared to healthy individuals. In our study, the CFU count for *S. aureus* in the dry eye group was 16.5 ± 5.3 compared to 5.2 ± 2.1 in the normal eye group (p-value = 0.0001). Similarly, *Coagulase-negative staphylococci* showed a higher CFU count in the dry eye group (21.4 ± 6.8) compared to the normal group (10.5 ± 3.4), with a p-value of 0.0002. These findings emphasize the impact of reduced tear film stability and tear production in promoting microbial overgrowth on the ocular surface.[11]

The **correlation between tear film quality and microbial load** was also highlighted in this study. Dry eye patients with a lower TBUT (≤5 seconds) showed a higher prevalence of microbial colonization, including *S. aureus* (25%), *Coagulase-negative staphylococci* (48%), and *P. aeruginosa* (18%). This relationship is consistent with findings by **Cheng et al. (2016)**, who demonstrated that a compromised tear film leads to increased microbial adherence, particularly of opportunistic pathogens. In contrast, patients with a higher TBUT (>5 seconds) showed significantly lower microbial prevalence, supporting the idea that improved tear film stability might protect against ocular infections.[12]

CONCLUSION

In conclusion, this study highlights a significant association between dry eye disease and microbial colonization, with dry eye patients exhibiting higher microbial load and diversity, particularly of *Staphylococcus aureus*, *Coagulase-negative staphylococci*, and *Pseudomonas aeruginosa*. The results suggest that compromised tear film stability and reduced tear production in dry eye patients may create a favorable environment for microbial overgrowth. Additionally, a lower Tear Break-Up Time (TBUT) was associated with increased microbial prevalence, emphasizing the role of tear film quality in maintaining ocular surface health.

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