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Evaluation of Aerobic Conjunctival Flora in Patients with *Demodex* Blepharitis

Demodex Blefaritli Hastalarda Aerobik Konjonktival Floranın Değerlendirilmesi

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ABSTRACT

Objective: *Demodex* species are frequently found in blepharitis cases. This study aimed to compare the conjunctival flora of eyes with *Demodex-positive* blepharitis and *Demodex-negative* blepharitis with healthy individuals.
Methods: Eyelash epilation was performed to detect *Demodex* from 44 eyes of 44 patients with chronic blepharitis and 44 eyes of 44 healthy controls and examined under a microscope. A conjunctival swab was taken from the same eye and inoculated on eosin methylene blue agar, Sabouraud dextrose agar, chocolate agar, and 5% sheep blood agar. Aerobic conjunctival flora was evaluated among *Demodex-positive* blepharitis, *Demodex-negative* blepharitis and healthy eyes.
Results: *Demodex* spp. was detected in 3 (6.8%) of 44 healthy controls and 24 (54.5%) of 44 patients with blepharitis. The most frequently isolated bacteria in healthy controls were coagulase-negative *Staphylococci* (CNS) spp. (n=32, 72.7%), *Streptococcus* spp. (n=16, 36.4%), *Corynebacterium* spp. (n=13, 29.5%). The most frequently isolated bacteria in *Demodex-positive* blepharitis were CNS spp. (n=14, 58.3%), *Staphylococcus aureus* (n=11, 45.8%), *Corynebacterium* spp. (n=7, 29.2%). In *Demodex-negative* blepharitis, CNS (n=10, 50.0%), *S. aureus* (n=10, 50.0%), *Corynebacterium* spp. (n=5, 25.0%) were most commonly isolated. *S. aureus* growth was significantly increased in the *Demodex* negative and positive blepharitis groups compared with the healthy group (p=0.001 and p=0.002, respectively). Although CNS spp. growth decreased in both groups with *Demodex-negative* and positive blepharitis compared with the healthy group; the decrease was significant only in those with *Demodex-negative* blepharitis (p=0.045). In terms of other bacterial growth, there was no significant difference between healthy eyes and *Demodex* positive and negative eyes with blepharitis.
Conclusion: We found that *Demodex* blepharitis has no significant effect on conjunctival flora. Blepharitis itself may be the main factor in changes in the conjunctival flora.
Keywords: *Demodex,* blepharitis, conjunctiva, flora

ÖZ

Amaç: *Demodex* türleri blefarit olgularında sıklıkla bulunmaktadır. Bu çalışmanın amacı, D*emodex* pozitif ve *Demodex* negatif blefaritli gözlerin konjonktival florasını sağlıklı bireylerle karşılaştırmaktır.
Yöntemler: Kronik blefarit tanısı almış 44 hastanın 44 gözünden ve 44 sağlıklı kontrolün 44 gözünden *Demodex* spp. tespiti için kirpik epilasyonu yapıldı. Kirpikler mikroskopta incelendi. Ayrıca aynı gözlerden konjonktivadan sürüntü alındı ve eozin metilen blue agar, sabouraud dekstroz agar, çikolata agar and %5 kanlı agara ekim yapıldı. *Demodex* pozitif blefarit, *Demodex* negatif blefarit ve sağlıklı gözler arasında aerobik konjonktival flora değerlendirildi.
Bulgular: Kırk dört sağlıklı kontrolün 3'ünde (%6,8) ve 44 blefaritli hastanın 24'ünde (%54,5) *Demodex* spp. saptandı. Sağlıklı gözlerde en sık izole edilen bakteriler, koagülaz negatif stafilokok (CNS) (n=32, %72,7), *Streptococcus* spp. (n=16, %36,4) ve *Corynebacterium* spp. (n=13, %29,5) türleriydi. *Demodex* pozitif blefaritte en sık izole edilen bakteriler CNS (n=14, %58,3), *Staphylococcus aureus* (*S. aureus*) (n=11, %45,8), *Corynebacterium* spp. (n=7, %29,2) idi. *Demodex* negatif blefaritte ise en sık *CNS* spp. (n=10, %50,0) ve *S. aureus* (n=10, %50,0), *Corynebacterium* spp. (n=5, %25,0) izole edildi. *Staphylococcus aureus* üremesi *Demodex* negatif ve pozitif blefarit gruplarında sağlıklı gruba göre anlamlı olarak artmıştı (sırasıyla p=0,001 ve p=0,002). *Demodex* negatif blefaritli grupta ve *Demodex* pozitif blefaritli grupta koagülaz negatif *Staphylococcus* üremesi sağlıklı gruba göre azalırken, bu azalma sadece *Demodex* negatif blefaritli grupta anlamlıydı (p=0,045). Diğer bakteri üremeleri açısından, sağlıklı gözler ile *Demodex* pozitif ve negatif blefaritli gözler arasında anlamlı bir fark yoktu.

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Türkiye PARAZİTOLOJİ Dergisi

Sonuç: *Demodex* blefaritinin konjonktival flora üzerinde anlamlı bir etkisi olmadığını tespit ettik. Blefaritin kendisi, konjonktival floradaki değişimi belirlemede ana faktör olabilir.
Anahtar Kelimeler: *Demodex,* blefarit, konjonktiva, flora

INTRODUCTION

Blepharitis is a condition characterized by inflammation of the eyelid margin, which is very common in ophthalmology practice. The effects of bacteria on the pathogenesis of blepharitis have been demonstrated frequently (1,2). Determining the types of bacteria is important for an effective treatment. *Demodex* spp. infestation is also common in blepharitis (3). However, *Demodex* blepharitis has its own characteristics (4). It is stated that *Demodex* spp. acts as a vector for some microorganisms in blepharitis and may cause changes in the ocular flora (3,5). Especially the symbiotic relationship with *Bacillus oleronius* and its effects on blepharitis has been reported (5).

The possible effects of *Demodex* spp. on other microorganisms are essential for determining the treatment strategy, given the importance of bacteria in blepharitis treatment. We conducted this study to compare the conjunctival flora of *Demodex* blepharitis, blepharitis without *Demodex* spp. and healthy controls to determine whether there is any difference.

METHODS

This study was conducted with 44 patients with chronic blepharitis who admitted to ophthalmology outpatient clinic and 44 age- and sex-matched healthy controls. There were 14 (31.8%) women and 30 (68.2%) men in both groups with and without blepharitis. The mean age of the group with and without blepharitis was 49.5±17.4 (20-80) and 49.1±16.9 (21-78), respectively (p=0.916). Microbiological sample analysis of the patients was performed in the microbiology and parasitology laboratory. Biomicroscopic examination was performed for the diagnosis of blepharitis in the patient and control group participating in the study. Blepharitis was accepted as the presence of erythema, telangiectasia, thickening, dandruff and choleret on the eyelid margin or tarsal conjunctiva. Severity of blepharitis was roughly equal in both eyes in all patients. Those who used topical or systemic antibiotics in the last month, those who had eye surgery, and those with other infectious or inflammatory diseases on the eyelid or eye surface were excluded from the study. The control group was selected from individuals who applied to the ophthalmology clinic only for refractive reasons and were otherwise healthy. The principles of the Declaration of Helsinki were adhered to in all steps of the study. Written and verbal consent was obtained from all patients before the study. Ethical approval was obtained from the Malatya Turgut Özal University Clinical Research Ethics Committee (decision no: 2020/206).

A minimum sample size of 20 individuals in each group was calculated for 0.80 power, 0.05 type-1 error and 0.84 effect size to identify a 40% difference in bacterial growth between controls and *Demodex* blepharitis.

Firstly, flora sample was taken from right eye of the patients and controls, and then eyelash epilation was performed for *Demodex* spp. detection from upper eyelid of the same eye.

Sampling and Examination for *Demodex*

The eyelash samples were examined by a specialist medical microbiologist (A.G). Two or three eyelashes (especially those with scale) taken from all patients with sterile forceps were quickly delivered to the laboratory in sterile sponges. Eyelashes brought to the laboratory were placed on the slide and then 2-3 drops of immersion were dropped on them and covered with a lamella. The area was scanned with x4 magnification of the microscope, and then the area was scanned with the diaphragm slightly closed at x10 and x40 magnification and the findings were noted. If one or more parasites are detected in this examination, *Demodex* spp. evaluated as positive. This review was done within an hour.

Sampling and Evaluation for Flora

The lower eyelid of the patients was pulled down slightly and flora sample was taken from the lower fornix conjunctiva with sterile swab. While taking the swab sample, care was taken to avoid touching the eyelids and eyelashes. Topical anesthetic medication was not applied to the patient for this procedure. The samples were planted in the thioglycollate broth in a short time, avoiding contamination. Then, in the microbiology laboratory, eosin methylene blue agar, sabouraud dextrose agar, chocolate agar and 5% sheep blood agar were inoculated. Media were incubated at 37 °C for 24 hours. Non-growth plates were incubated for an additional 24 hours. The incubation period of the samples planted on Sabouraud dextrose agar was extended to three weeks. Growing microorganisms were identified by standard microbiological methods.

Statistical Analysis

Statistical analysis was done with SPSS 25.0 program (Chicago, IL, USA). Results are presented as mean ± standard deviation (minimum-maximum) in measurable data. Nominal data are presented as frequency and percent. Normal distribution was evaluated with the Kolmogorov-Smirnov test. Normally distributed data were evaluated using the unpaired t-test, and non-normally distributed data were evaluated using the Mann-Whitney U test. Nominal data were evaluated using Pearson's chi-square test or Fisher's Exact test. A p-value of less than 0.05 was considered significant.

RESULTS

Demodex was observed in 3 (6.8%) samples in the group without blepharitis and in 24 (54.5%) samples in the group with blepharitis ($p<0.001$). Bacterial growth occurred in 36 (81.8%) and 38 (86.3%) samples in the group with and without blepharitis, respectively (p=0.560). The mean growth was 1.77±1.31 (0-5) and 2.00±1.29 (0-6) from each sample in the group with and without blepharitis, respectively (p=0.411). *Staphylococcus aureus* growth was significantly less in the group without blepharitis than with blepharitis ($p<0.001$). In other bacteria, growth numbers were similar between groups with and without blepharitis ($p>0.05$) (Table 1).

There were 61 (69.3%) samples with no *Demodex* spp. and 27 (30.7%) samples with *Demodex* spp. One to three *Demodex* parasites were detected in all patients except one patient with four *Demodex* parasites. There were 18 (29.5%) females and 43 (70.5%) males in the non-*Demodex* group, while there were 10 (37.0%) females and

17 (63.0%) males in the *Demodex* group (p=0.484). The mean age of the non-*Demodex* and *Demodex* groups was 48.3±17.7 (21-80) and 51.5±15.9 (20-71), respectively (p=0.419). The mean growth was 1.86±1.31 (0-6) and 1.93±1.29 (0-5) from each sample in the non-*Demodex* and *Demodex* groups, respectively (p=0.787). In the non-*Demodex* group, there were 51 (83.6%) growths in the conjunctiva samples, while in the *Demodex* group, there were growth in 23 (85.2%) samples (p=0.560). There was no significant difference in the number of growth of any bacteria in conjunctival flora between the *Demodex* and non-*Demodex* groups (p>0.05) (Table 2).

The mean age was 46.7±19.4 (21-80) and 51.7±15.6 (20-71) in non-*Demodex* blepharitis and *Demodex* blepharitis groups, respectively (p=0.341). There were 5 (25.0%) women and 15 (75.0%) men in the group with non-*Demodex* blepharitis, while there were 9 (37.5%) women and 15 (62.5%) men in the group with *Demodex* blepharitis (p=0.375). There was growth in 16 (80.0%) and 20 (83.3%) samples in non-*Demodex* and *Demodex* blepharitis groups (p=1.000). The mean growth number from each sample was 1.60±1.23 (0-4) and 1.91±1.38 (0-5) in non-*Demodex* and *Demodex* blepharitis groups, respectively (p=0.437). Yeast growth occurred in two patients with *Demodex* blepharitis and two without blepharitis. There was no yeast growth from other samples.

Table 1. Distribution of microorganisms in conjunctival flora according to blepharitis status

	B- n=44	B+ n=44	p
Demodex, n (%)	3 (6.8)	24 (54.5)	**<0.001***
CNS, n (%)	32 (72.7)	24 (54.5)	0.076*
S. aureus, n (%)	5 (11.4)	21 (47.7)	**<0.001***
Corynebacterium spp., n (%)	13 (29.5)	12 (27.3)	0.813*
Streptoccocus spp., n (%)	16 (36.4)	9 (20.5)	0.098*
Bacillus spp., n (%)	7 (15.9)	4 (9.1)	0.334*
Neisseria spp., n (%)	5 (11.4)	3 (6.8)	0.713ϒ
Micrococcus spp., n (%)	5 (11.4)	2 (4.5)	0.434ϒ
Haemophilus spp., n (%)	3 (6.8)	1 (2.3)	0.616ϒ

S. aureus: Staphylococcus aureus, CNS: Coagulase negative *Staphylococci*, B-: Blepharitis negative, B+: Blepharitis positive, *Pearson chi-square test, ϒ Fisher's Exact test

Table 2. Distribution of organisms in conjunctival flora according to *Demodex* status

	D- n=61	D+ n=27	p
CNS, n (%)	41 (67.2)	15 (55.6)	0.294*
S. aureus, n (%)	15 (24.6)	11 (40.7)	0.126*
Corynebacterium spp., n (%)	16 (26.2)	9 (33.3)	0.496*
Streptoccocus spp., n (%)	17 (27.9)	8 (29.6)	0.866*
Bacillus spp., n (%)	9 (14.8)	2 (7.4)	0.492ϒ
Neisseria spp., n (%)	4 (6.6)	4 (14.8)	0.243ϒ
Micrococcus spp., n (%)	6 (9.8)	1 (3.7)	0.431ϒ
Haemophilus spp., n (%)	4 (6.6)	0 (0.0)	0.308ϒ

S. aureus: Staphylococcus aureus, CNS: Coagulase negative *Staphylococci*, D-: *Demodex* negative, D+: *Demodex* positive, *Pearson chi-square test, ϒFisher's Exact test

The four most frequently bacteria isolated in the conjunctiva of healthy eyes were coagulase-negative *Staphylococci* (CNS) (n=32, 72.7%), *Streptococcus* spp. (n=16, 36.4%), *Corynebacterium* spp. (n=13, 29.5%), *Bacillus* spp. (n=7, 15.9%). The four most frequently isolated bacteria in the conjunctiva of eyes with non-*Demodex* blepharitis are CNS (n=10, 50.0%), *S. aureus* (n=10, 50.0%), *Corynebacterium* spp. (n=5, 25.0%), *Streptococcus* spp. (n=3, 15.0%). The four most frequently isolated bacteria in the conjunctiva of eyes with *Demodex* blepharitis are CNS (n=14, 58.3%), *S. aureus* (n=11, 45.8%), *Corynebacterium* spp. (n=7, 29.2%), *Streptococcus* spp. (n=6, 25.0%), respectively (Figure 1).

S. aureus growth was significantly increased in *Demodex* and non-*Demodex* blepharitis groups compared to healthy group (p=0.001 and p=0.002, respectively), but there was no significant difference between *Demodex* and non-*Demodex* blepharitis groups (p=0.783). Although CNS growth decreased in both groups with *Demodex* and non-*Demodex* blepharitis compared to the healthy group, the decrease was significant only in the group with non-*Demodex* blepharitis (p=0.045). There was no significant difference between the groups with *Demodex* and non-*Demodex* blepharitis groups in CNS in conjunctival flora (p=0.580). In terms of other bacteria, there was no significant difference between healthy eyes and the groups with blepharitis with and without *Demodex* spp. (p>0.05) (Table 3).

DISCUSSION

Since the etiopathogenesis of blepharitis is not fully clarified, the relationship between blepharitis and microorganisms continues to be investigated. Various forms of bacteria, fungi and parasites have been isolated in blepharitis (3,6,7). The frequency and types of isolated organisms vary even in the same type of blepharitis (1,2,8). Although the increase in *S. aureus* in our study is consistent with the literature, the CNS decrease differs in general from the literature (1,2,8). The more frequent reporting of bacteria found in the skin flora in blepharitis indicates that organisms such as *Staphylococci* and *Demodex* spp. may invade the area (2). On the other hand, it is reported that environmental bacteria are frequently isolated in flora studies performed with blepharitis (3,8). Our results show that *S. aureus* and *Demodex* spp. are the most frequent organisms in blepharitis. This finding is consistent with the result often found in blepharitis (2,8,9). This situation indicates that no matter what mechanism is dominant, the microenvironment in blepharitis is most advantageous for *Demodex* spp. and *S. aureus*.

Demirkazık and Koltaş (10) detected *Demodex* in 143 (42.6%) of 335 patients with a preliminary diagnosis of blepharitis, conjunctivitis, and visual impairment. In addition, it was reported in this study that the incidence of *Demodex* increased significantly with increasing age. Tanrıverdi et al. (11) detected *Demodex* in 69 (45.1%) of 153 chronic blepharitis cases, but they did not find an age-related *Demodex* increase. In our study, *Demodex* was found in 54.5% of blepharitis patients and 6.8% of healthy controls. Although the mean age of cases with *Demodex* blepharitis was higher than that of cases with non-*Demodex* blepharitis, the difference was not significant.

The fact that *Demodex* spp. may cause inflammatory effects in blepharitis with *Demodex* (12,13) and carry bacteria with it (5,14)

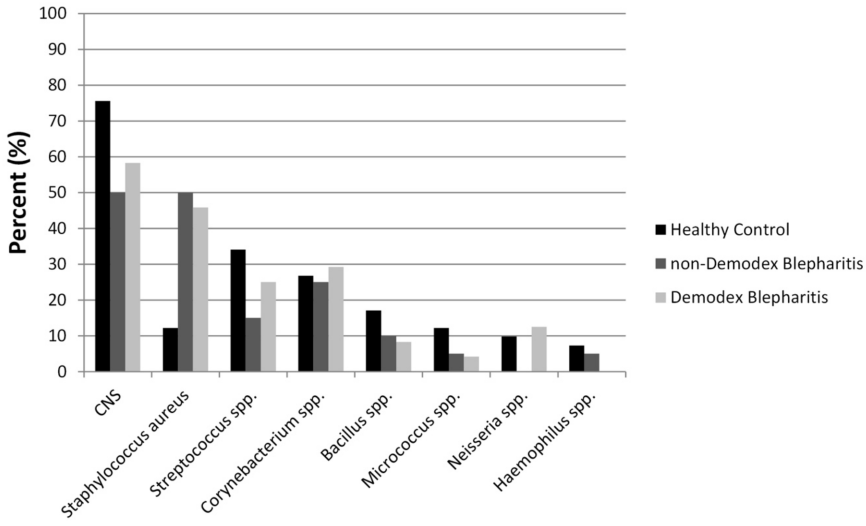

Figure 1. Growth percentages of bacteria from conjunctival samples of *Demodex* positive blepharitis cases and *Demodex* negative blepharitis cases and healthy eyes
CNS: Coagulase negative Staphylococci

Table 3. Comparison of aerobic conjunctival flora in healthy eyes, in blepharitis without *Demodex* infestation, and in blepharitis with *Demodex* infestation

	D-B-		**D-B+**		**D+B+**		
	(-)	**(+)**	**(-)**	**(+)**	**(-)**	**(+)**	
CNS, n (%)	10 (24.4)	31 (75.6)	10 (50.0)	10 (50.0)	10 (41.7)	14 (58.3)	**P_1:0.045*** P_2:0.145* P_3:0.580*
S. aureus, n (%)	36 (87.8)	5 (12.2)	10 (50.0)	10 (50.0)	13 (54.2)	11 (45.8)	**P_1:0.001*** **P_2:0.002*** P_3:0.783*
Coryne., n (%)	30 (73.2)	11 (26.8)	15 (75.0)	5 (25.0)	17 (70.8)	7 (29.2)	P_1:0.879* P_2:0.839* P_3:0.757*
Strep., n (%)	27 (65.9)	14 (34.1)	17 (85.0)	3 (15.0)	18 (75.0)	6 (25.0)	P_1:0.117* P_2:0.441* P_3: 0.477^{Ÿ}
Bacillus, n (%)	34 (82.9)	7 (17.1)	18 (90.0)	2 (10.0)	22 (91.7)	2 (8.3)	P_1:0.704^{Ÿ} P_2:0.466^{Ÿ} P_3:1.000^{Ÿ}
Neisseria, n (%)	37 (90.2)	4 (9.8)	20 (100)	0 (0.0)	21 (57.5)	3 (12.5)	P_1:0.293^{Ÿ} P_2:0.703^{Ÿ} P_3: 0.239^{Ÿ}
Micrococ., n (%)	36 (87.8)	5 (12.2)	19 (95.0)	1 (5.0)	23 (95.8)	1 (4.2)	P_1:0.653^{Ÿ} P_2:0.400^{Ÿ} P_3:1.000^{Ÿ}
Haemo., n (%)	38 (92.7)	3 (7.3)	19 (95.0)	1 (5.0)	24 (100)	0 (0.0)	P_1:1.000^{Ÿ} P_2:0.290^{Ÿ} P_3:0.455^{Ÿ}

S. aureus: Staphylococcus aureus, CNS: Coagulase negative *Staphylococci*, *Strep.*: *Streptococcus* spp., *Coryne.*: *Corynebacterium* spp., *Micrococ.*: *Micrococcus* spp., *Haemo.*: *Haemophilus* spp., D-B-: *Demodex* negative and blepharitis negative, D-B+: *Demodex* negative and blepharitis positive, D+B+: *Demodex* positive and blepharitis positive, (-): No growth, (+): Growth, P_1: Significance value in comparison between D-B- and D-B + groups, P_2: Significance value in comparison between D-B- and D+B + groups, P_3: Significance value in comparison between D-B+ and D+B + groups, *Pearson chi-square test, ^{Ÿ}Fisher's Exact test

brings to mind its effects on other microbiological species. Zhu et al. (8) reported that the number of *Propionibacterium acnes* colonies on the lid margin and eyelashes increased significantly in patients with blepharitis and those with *Demodex* in the control group compared to those without *Demodex*. Yan et al. (3), in their study comparing the flora of patients with *Demodex* blepharitis and healthy controls through bacterial 16S RNA, identified potential bacterial genera in *Demodex* blepharitis as *Bacilli, Firmicutes, Cyanobacteria, Lactobacillus* and *Streptophyta* Lee et al. (15) could not find a correlation between *Demodex* and ocular microbiota,

but emphasized that the number of cases with *Demodex* was low. In our study, the growth rate of *S. aureus* was significantly increased among the *Demodex* group with blepharitis compared to the healthy group. Similarly, *S. aureus* was increased in the group with non-*Demodex* blepharitis compared to the healthy group, but the CNS were decreased. These results may indicate that the presence of *Demodex* spp. in eyes with blepharitis has no effect on bacterial flora and that the bacterial distribution is more related to blepharitis than the presence of *Demodex* spp.

In our study, *S. aureus* was found at a rate of 12% in the conjunctiva of healthy eyes, while it was detected in approximately 50% of eyes with blepharitis regardless of the status of *Demodex*. Previously, *S. aureus* was isolated as a floral element of healthy conjunctivae (16,17), while this bacterium was reported to appear at higher rates in eyes with blepharitis (8,9,15). The presence of this bacterium was not affected by *Demodex* status in our study and was isolated at a significantly increased rate in eyes with blepharitis.

The CNS is often isolated in the normal conjunctival flora (17,18). Many studies report increased CNS isolation at the conjunctiva and lid margin in blepharitis with or without *Demodex* compared to healthy controls (18). In our study, CNS isolation was found significantly higher in the conjunctiva of healthy individuals compared to those with blepharitis without *Demodex*. However, the number of CNS isolations in the conjunctiva of eyes with blepharitis with and without *Demodex* was similar. This result, which differs from other studies (9,19), indicates that the CNS, which is a normal flora component, decrease in case of blepharitis in our study, and it may indicate that the CNS as a normal flora component may be negatively affected in blepharitis. However, the finding of more pathogenic CNS in other studies may have caused this difference.

Although *Corynebacterium* spp. can be found in soil, water and plants in nature, non-pathogenic species can be found in skin and mucosa (15). Most of those found in normal conjunctiva and valve flora are lipophilic species (20). It has been isolated on the conjunctiva and eyelid margin in eyes with blepharitis (1,8,15). Bezza Benkaounha et al. (1) reported that *Corynebacterium* spp. increased significantly in eye conjunctiva with blepharitis compared to healthy controls, but stated that this increase may not necessarily be related to pathogenesis. In our study, it was the third most common bacterial in normal flora and was the least affected by blepharitis regardless of *Demodex* status. Lee et al. (15) suggested that in addition to the association of blepharitis with bacteria in the skin flora, an increased proportion of bacteria such as *Corynebacterium* species, which can be found in pollen, dust and soil particles in cases with blepharitis, may be associated with pollen, dust and soil contamination.

Although *Streptococcus* spp. are frequently isolated from the skin flora, no significant increase was found in our study. Although Zhu et al. (8) isolated it more frequently in eyes with blepharitis, they did not consider it as a pathogenic organism in blepharitis because the bacterial load of *Streptococcus* spp. did not increase significantly. *Streptococcus* spp. appear to be more associated with corneal infections and dacryocystitis than blepharitis (21).

Bacillus spp. was a relatively less common type of bacteria in our study. It has been previously reported that *Bacillus oleronius* may be in a symbiotic relationship with the *Demodex* parasite and can cause infection by being transported by the parasite and multiplying under suitable conditions (5). In other studies, it has been reported that *Bacillus* spp. associated with *Demodex* may have an effect on the development of blepharitis and facial rosacea (22). Kıvanç et al. (23) reported that *Bacillus* spp. isolated from the conjunctiva showed antimicrobial activity against methicillin-resistant *Staphylococcus* species. In our study, the frequency of *Bacillus* spp. isolation from samples with *Demodex* blepharitis was similar to that of non-*Demodex* blepharitis and healthy controls. In addition, the presence of *Bacillus* spp. was not affected by the presence of *Demodex*, and no change was observed in the isolation frequency of other organisms in the presence of *Bacillus* species.

Detection and treatment of *Demodex* spp. has an important place in the treatment of blepharitis. In our study, frequent bacterial growth in *Demodex* blepharitis requires antibiotic use in addition to *Demodex* treatment. However, since the presence of *Demodex* spp. does not make a difference in bacterial distribution with non-*Demodex* blepharitis, it may not be necessary to take additional measures in antibiotic treatment for blepharitis when planning antibiotic treatment.

Study Limitations

The limitation of our study is that the ocular findings of *Demodex* blepharitis were not evaluated in relation to the presence of *Demodex* spp. and flora.

CONCLUSION

We think that *Demodex* blepharitis has no significant effect on conjunctival flora and that blepharitis itself is the main factor in determining microbial diversity.

*Ethics

Ethics Committee Approval: Ethical approval was obtained from the Malatya Turgut Özal University Clinical Research Ethics committee (decision no: 2020/206).

Informed Consent: Written informed consent was taken from all the participants.

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*Authorship Contributions

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