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# Microbiological Evaluation of Blepharitis: A Case-Controlled Study

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## ABSTRACT

**Background.** Blepharitis is a chronic inflammatory eyelid infection. The current study was designed to check the association of normal bacterial flora of normal eyelids with the potential pathogenic bacteria of blepharitis infected eyelids of blepharitis patients.

**Methodology.** A total of (n=50) blepharitis patients were recruited for this study. Then, 100 swab samples were taken from these patients. Sample distribution indicated that 50 swab samples were taken from infected eyelids and 50 from the uninfected part of eyelids of blepharitis patient. Bacterial flora was characterized in these samples through different biochemical tests and antibiotic resistance was checked by using the Kirby Bauer (KB) method.

**Results.** A total of 52 bacterial strains were isolated from 50 infected swab samples of 50 blepharitis patients including *Staphylococcus aureus* 31/52 (60%), *Staphylococcus epidermidis* 7/52 (13%), *Klebsiella* spp. 6/52 (11%), *E. coli* 2/52 (4%), *Acinetobacter* spp. 2/52 (4%), *Enterobacter* spp. 1/52 (2%), *Serratia marcescens* 1/52 (2%), *Shigella* spp. 1/52 (2%) and *Bacillus cereus* 1/52 (2%). Further, 48 bacterial strains were isolated from uninfected swab samples of 50 blepharitis patients including *Staphylococcus epidermidis* 24/48 (50%), *Staphylococcus aureus* 22/48 (42%), *Klebsiella* spp. 1/48 (2%), *Enterobacter* spp. 1/48 (2%), *Serratia* spp. 1/48 (2%), and *Acinetobacter* spp. 1/48 (2%). All Gram-positive bacteria isolated from both infected and uninfected eyelids were found to be sensitive to vancomycin antibiotics. The percentage of methicillin-resistant *Staphylococcus aureus* (MRSA) was 21% among the bacterial isolates obtained from infected eyelids. While, its percentage was 18.7% in the bacterial isolates obtained from the uninfected eyelids of the patients. Hence, the percentage of MRSA was higher in the infected eyelids of blepharitis patients as compared to their uninfected eyelids.

**Conclusion.** The identification of pathogens involved in blepharitis and performing their antibiotic susceptibility test (AST) are important steps in reducing bacterial resistance for blepharitis treatment.

**Keywords:** antibiotics, antibiotic susceptibility testing (AST), blepharitis, Gram-positive bacteria, methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin

## Highlights

- Blepharitis is more common in women as compared to men. Moreover, it is more common in patients of the age group 21-30. So, it varies in different sex and age cohorts.

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- The increased percentage of *S. aureus* followed by *Klebsiella spp., E. coli, Acinetobacter spp., Enterobacter spp., Serratia marcescens, Shigella spp*. and *Bacillus cereus* is associated with blepharitis.
- Gram-positive bacteria isolated from the infected eyelid of blepharitis patients were found to be 100% sensitive to vancomycin antibiotic.
- The percentage of MRSA isolated from infected eyelids was 21%. This percentage is higher than the percentage of MRSA isolated from the uninfected eyelids of blepharitis patients.

## 1. INTRODUCTION

Blepharitis is the chronic inflammation of eyelid margins which results in secondary changes in the conjunctiva and cornea regions of the eye [1]. This infection is usually ubiquitous and reoccurs often, and very difficult to manage [2]. Blepharitis is a complicated and poorly understood pathophysiology. It is thought to be a complex illness with a number of underlying causes [3]. Higher strain numbers and native bacterial abundances are indicative of blepharitis pathogenicity [4]. Its symptoms include irritation, sensation of foreign body, hyperemia, and eyelid crusting [5]. The alcohol consumption, retinoid medications, androgen antagonists, contact lenses, environmental factors, allergens exposure, and diet are the risk factors contributing to blepharitis [6].

Anterior blepharitis (AB) is contracted usually due to staphylococcal bacteria, viral infection, or seborrheic disease. On the contrary, posterior blepharitis (PB) occurs due to meibomian gland dysfunction, rosacea acne, or hormonal changes [7]. A cross-sectional study was conducted at the University of Gondar Hospital, Ethiopia in 2017 to identify the bacterial pathogens associated with external ocular infections. It was found that *Staphylococcus aureus* (50.6%) was most commonly isolated among blepharitis cases followed by Coagulase-negative Staphylococci (Co-NS) (32.9%) [8]. Another study conducted at the same institution using 210 ocular samples in 2018 demonstrated that the most prevalent isolated bacteria were coagulase-negative *Staphylococcus* (Co-NS) 27.5% (36), and *S. aureus* 26.7% (35), followed by other bacteria [9]. The strains of *S. epidermidis* along with other bacteria isolated from the eyelids of blepharitis patients have overgrown and produced the virulence factors [10]. Patients with blepharitis had an ocular surface microbiota that was identical to that of healthy individuals in terms of composition, but there were variations in the amount of each microbe [11].

Methicillin-resistant *S. aureus* (MRSA) has emerged as a significant problem [12]. Methicillin resistance is a widespread issue and MRSA strains have developed *mecA* gene that renders them immune to all beta-lactam antibiotics [13]. These strains should be treated with vancomycin, while Gram-positive bacteria may develop resistance against vancomycin in the future [14, 15].

The current research aimed to isolate and compare bacterial flora from both infected and uninfected eyelids of blepharitis patients in Lahore, Pakistan. Furthermore, antibiotic resistance of the isolated bacterial strains was checked by using the Kirby Bauer (KB) method.

## 2. METHOD

### 2.1. Sample Collection

The current study was a hospital based cross-sectional study of the blepharitis patients. A total of 100 swab samples of 50 blepharitis patients were collected from eye outpatients department (OPD) of Jinnah hospital, Lahore. A set of two swab samples were obtained from each patient. A total of 50 swab samples (such as oil, crust, and fluid discharge) was taken from the eyelid of the infected eye and another 50 swab samples from the normal uninfected part of the eyelids (as control), if one eye of the blepharitis patient was affected. If both eyes of the patient were affected, the samples were taken from the infected eye as well as normal part of the eyelid (as control). The history of the patients was recorded by using a consent form. Out of 50 patients, there were (n=28) female patients, while remaining were male patients (n=22). The age of these patients ranged from 1 to 80 years. Details about marital status, socioeconomic status, onset of blepharitis symptoms, signs and symptoms, risk factors, and occupation of the patients were also recorded.

### 2.2. Enrichment of Samples

The eyelid skin swab samples were taken with the help of moistened cotton swab immersed in normal sterile saline (0.9%). The samples were immediately inoculated into nutrient broth containing test tubes (2 ml) and incubated for 24 hours at 37℃. Afterwards, the samples were streaked on blood agar plates. The plates were then examined for bacterial growth after incubation for 24 hours at 37℃.

### 2.3. Isolation of Pure Bacterial Strains

Bacterial strain purification was performed by quadrant streaking each morphologically different bacterial colony on nutrient agar plates. Pure bacterial cultures were sub-cultured on selective and differential media, such as Mannitol salt agar and MacConkey agar. Bacterial growth was examined after incubation for 24 hours at 37℃.

### 2.4. Morphological and Biochemical Characterization of Bacterial Isolates

Gram staining was performed to identify and characterize bacterial strains. Different biochemical tests such as Catalase, Oxidase, Deoxyribonuclease (DNase), Mannitol fermentation, Citrate, Urease, Indole, Motility, Triple Sugar Iron (TSI), and Methyl Red-Voges Proskauer (MR-VP) tests were performed for the characterization of the isolated bacterial strains.

### 2.5. Antibiotic Susceptibility Testing

Kirby-Bauer (KB) method was used for antibiotic susceptibility testing (AST). It is also known as disc diffusion method, modified by Clinical & Laboratory Standards Institute (CLSI). Mueller Hinton (MH) agar was utilized to check antibiotic susceptibility of the isolates. The tested bacterial strain was swabbed on MH agar and antibiotic discs were placed on agar surface. The growth of an organism is reduced or diminished by antibiotic. This results in absence of growth around disc, known as the zone of inhibition (ZOI). The diameter of zone was measured in millimeters (mm) [16].

The size of the zone was compared to the standard chart to determine results, such as sensitive, intermediately susceptible, or resistant. Oxacillin, Cefoxitin, and Vancomycin were used for Gram-positive isolated bacteria, whereas Gentamicin, Ampicillin, Tetracycline, Oxacillin, Cefoxitin, and Vancomycin were used for the Gram-negative isolated bacteria. The

concentrations of these antibiotics based on CLSI guidelines are given in (Table 1).

**Table 1.** List of Antibiotics with Their Specific Concentrations Based on CLSI Guidelines.

| Serial no. | Antibiotics | Concentrations used |
|---|---|---|
| 1 | Oxacillin | 1 µg |
| 2 | Vancomycin | 30 µg |
| 3 | Cefoxitin | 30 µg |
| 4 | Gentamicin | 10 µg |
| 5 | Ampicillin | 10 µg |
| 6 | Tetracycline | 30 µg |

## 3. RESULTS

### 3.1. Socio-demographic Factors

Out of a total of 50 blepharitis patients, 28(56%) patients were female while the remaining were male patients 22(44%). More samples were obtained from female patients as compared to male patients. The highest concentration 14(28%) of patients was in the age group 21-30 years, followed by the age group 51-60 years 10(20%). The concentration of patients in other age groups was 2(4%) in the age group of 1-10 years, 6(12%) in the age group of 11-20 years, 3(6%) in the age group of 31-40 years, 7(14%) in the age group of 41-50 years, and 8(16%) in the age group of >60 years. The tendency to develop blepharitis was found to be more common in poor (66%) patients and among the married people (74%). About 30% of patients were not educated in this study. Common symptoms among the patients were red, swollen, watery, and painful eyes (with the highest percentage of 50%). In the current study, diet, environmental factors (smoke, wind and humidity), poor hygiene, advanced age, cosmetic makeup, contact lenses, allergen exposure and seborrheic dermatitis were determined as the main risk factors of blepharitis. The distribution of all blepharitis-associated risk factors indicated that 15 patients had poor hygiene, 12 were of advanced age, 8 patients made excessive use of cosmetic makeup, 7 suffered environmental influences, 4 had diet issues, 2 were exposed to an allergen, 1 made excessive use of contact lens and, the remaining 1 patient was found to have seborrheic dermatitis. The high risk factors patients were poor hygiene (30%) and advanced age (24%). The details of all these socio-demographic factors influencing the blepharitis patients were shown in the (Figure 1 and 2).

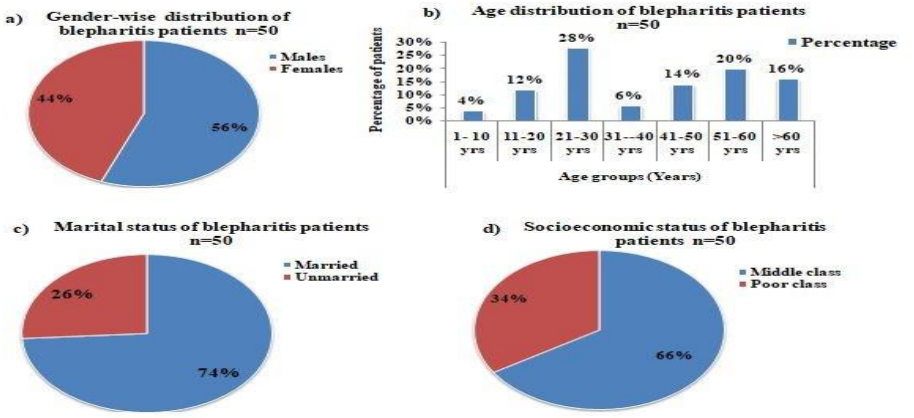


**Figure 1.** Details of Socio-Demographic Factors of Blepharitis Patients a) Distribution of

Blepharitis Patients with respect to Gender b) Distribution of Blepharitis Patients with respect to Age c) Marital Status of Blepharitis Patients d) Socio-Economic Status of Blepharitis Patients (N = Number of Blepharitis Patients).

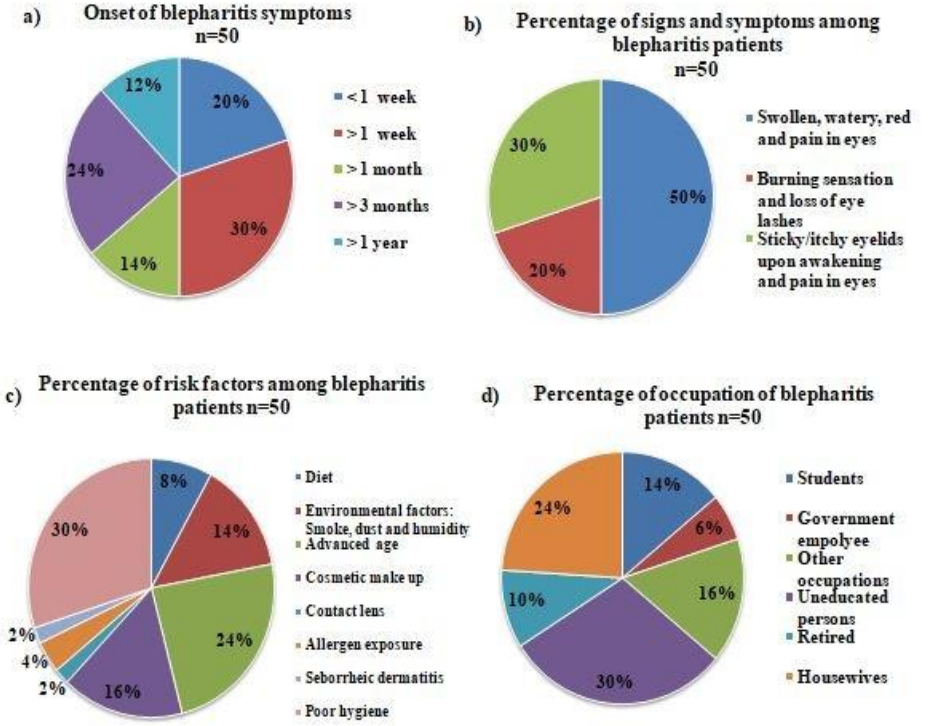


**Figure 2.** Details of Socio-Demographic Factors of Blepharitis Patients a) Onset of Symptoms Related to Blepharitis Patients b) Signs and Symptoms in Blepharitis Patients c) Risk Factors Related to Blepharitis Patients d) Occupations Related to Blepharitis Patients (n=Number of Blepharitis Patients)

### 3.2. Isolated Bacterial Strains from Infected and Uninfected Parts of the Patients' Eyelids

The total 100 bacterial strains were isolated from blepharitis patients. Of these, 52 bacterial strains including *Staphylococcus aureus* 31(60%), *Staphylococcus epidermidis* 7(13%), *Klebsiella* spp. 6(11%), *E. coli* 2(4%), *Acinetobacter* spp. 2(4%), *Enterobacter* spp. 1(2%), *Serratia marcescens* 1(2%), *Shigella* spp. 1(2%) and *Bacillus cereus* 1(2%) were isolated from the infected eyelids of blepharitis patients (Figure 3a). The remaining 48 bacterial strains including *Staphylococcus epidermidis* 24(50%), *Staphylococcus aureus* 22(42%), *Klebsiella* spp. 1(2%), *Enterobacter* spp. 1(2%), *Serratia* spp. 1(2%), and *Acinetobacter* spp. 1(2%) were isolated from the uninfected eyelid of blepharitis patients (Figure 3b).

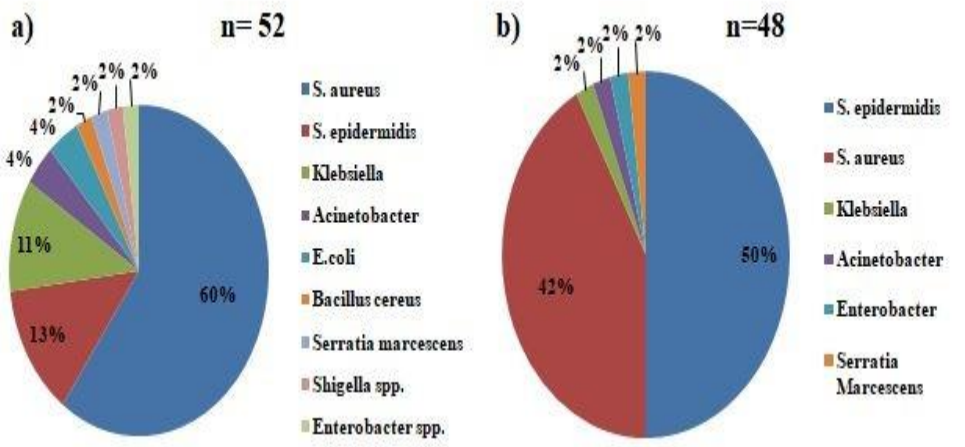


**Figure 3.** Bacterial Isolation (n=100) from Infected (n=52) and Uninfected Eyelids (n=48) of Blepharitis Patients a) Percentage of Bacterial Isolates from Infected Eyelids of Blepharitis Patients b) Percentage of Bacterial Isolates from Uninfected Eyelids of Blepharitis Patients (as Control Subjects) (n=Number of Bacterial Isolates)

### 3.3. Antibiotic Susceptibility Testing (AST)

AST was performed by using KB method was performed on the bacterial strains (n=59) isolated from both the infected eyelid (n=31) and the uninfected parts of the eyelids (n=28) of blepharitis patients (Figure 4a and 4b). ZOI was measured in 'mm' and compared by using its standard table. The AST results of Gram-positive and Gram-negative bacterial strains isolated from infected and uninfected eyelid of blepharitis patients are shown in the form of graphs (Figure 5 to 8). Gram-positive bacterial strains (n=24) isolated from the infected eyelid of blepharitis patients showed 88% resistance and 12% sensitivity to oxacillin, 100% sensitivity to vancomycin, and 83% resistance and 17% sensitivity to cefoxitin respectively (Figure 5). Gram-negative bacterial strains (n=7) isolated from the infected eyelids of blepharitis patients illustrated 86% resistance and 14% intermediately sensitive to ampicillin, 86% resistance and 14% sensitive to gentamicin, 43% resistance and 57% sensitivity to tetracycline, 100% resistance to oxacillin, 100% resistance to vancomycin, and 100% resistance to cefoxitin, respectively (Figure 6). Gram-positive bacterial strains isolated from uninfected eyelid of blepharitis patients (n=26) showed 92% resistance and 8% intermediately sensitive to oxacillin, 100% sensitive to vancomycin, and 77% resistance and 23% sensitivity to cefoxitin respectively (Figure 7). Gram-negative bacterial strains isolated from uninfected eyelids of blepharitis patients (n=2) illustrated 100% resistance to ampicillin, 100% resistance to gentamicin, 50% intermediately sensitive and 50% sensitivity to tetracycline, 100% resistance to oxacillin, 100% resistance to vancomycin, and 100% resistance to cefoxitin, respectively (Figure 8).

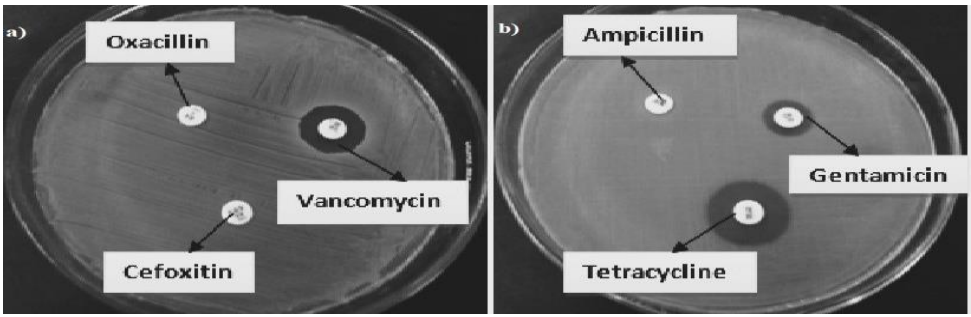


**Figure 4.** Antibiotic Susceptibility Testing (AST) Results of Isolated Bacterial Strains a) Antibiotics Used for Gram-Positive Bacteria (Oxacillin, Vancomycin, and Cefoxitin) b) Antibiotics Used for Gram-Negative Bacteria (Ampicillin, Gentamicin, and Tetracycline)

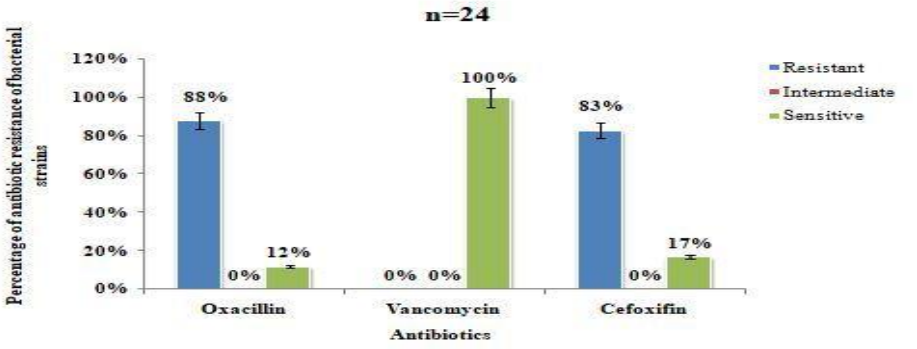


**Figure 5**. Percentage of Antibiotic Resistance of Gram-Positive Isolates from Infected Eyelids of Blepharitis Patients. Gram-Positive Bacterial Strains Showed 88% Resistance, and 12% Sensitivity to Oxacillin, 100% Sensitivity towards Vancomycin, 83% Resistance, and 17% Sensitivity to Cefoxitin (n=Number of Bacterial Isolates)

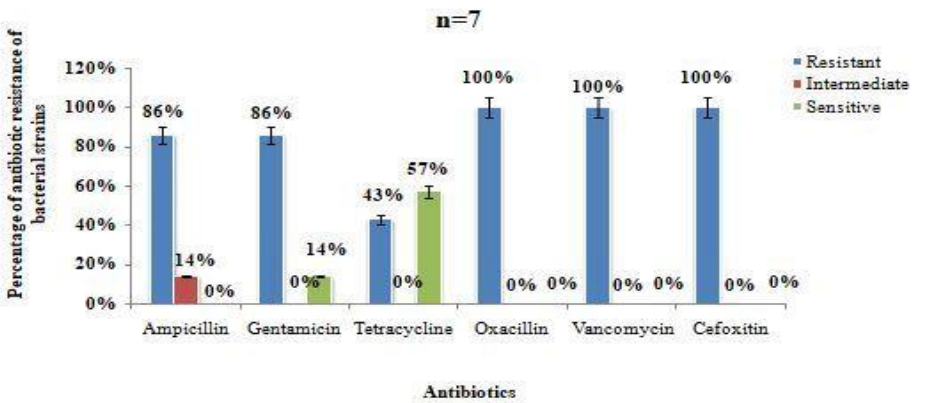


**Figure 6.** Percentage of Antibiotic Resistance of Gram-Negative Isolates from Infected

Eyelids of Blepharitis Patients. Gram-Negative Bacterial Strains Showed 86% Resistance, and 14% Intermediately Sensitive to Ampicillin, 86% Resistance, and 14% Sensitivity to Gentamicin, 43% Resistance, and 57% Sensitivity to Tetracycline, 100% Resistance to Oxacillin, 100% Resistance to Vancomycin, and 100% Resistance to Cefoxitin (n=Number of Bacterial Isolates)

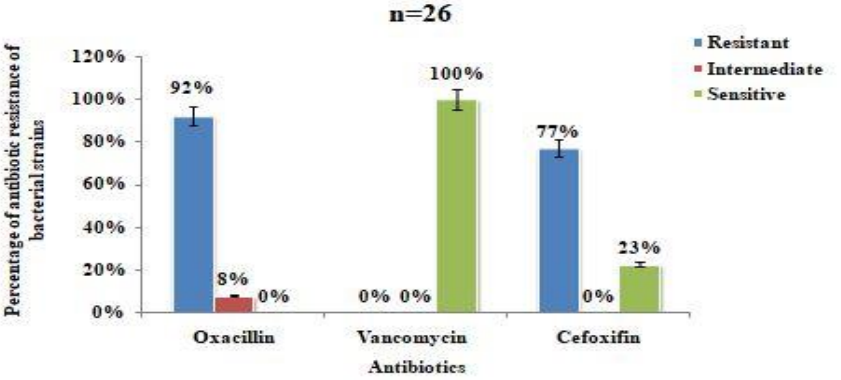


**Figure 7.** Percentage of Antibiotic Resistance of Gram-Positive Isolates from Uninfected Parts of the Eyelids of Blepharitis Patients (as Control). Gram-Positive Bacterial Strains Showed 92% Resistance, and 8% Intermediately Sensitive to Oxacillin, 100% Sensitivity to Vancomycin, and 77% Resistance, and 23% Sensitivity to Cefoxitin (n=Number of Bacterial Isolates).

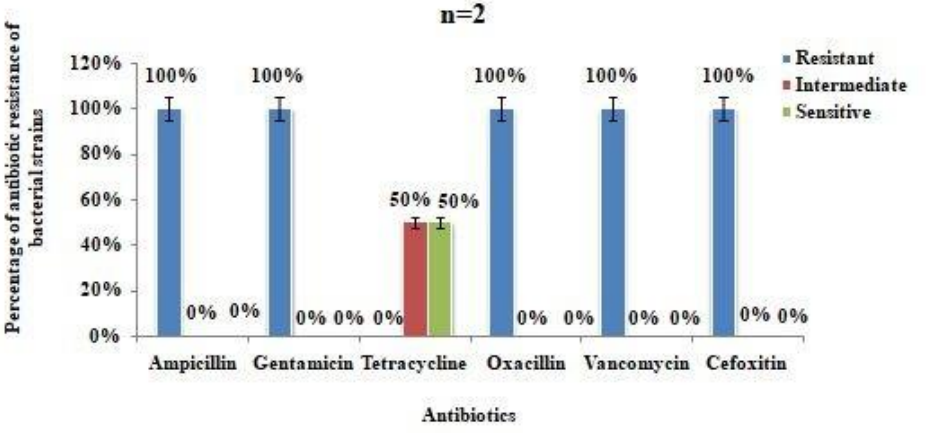


**Figure 8.** Percentage of Antibiotic Resistance of Gram-Negative Isolates from Un-Infected Parts of the Eyelids of Blepharitis Patients (as Control). Gram-Negative Bacterial Strains Illustrated 100% Resistance to Ampicillin, 100% Resistance to Gentamicin, 50% Intermediately Sensitive, and 50% Sensitivity towards Tetracycline, 100% Resistance to Oxacillin, 100% Resistance to Vancomycin, and 100% Resistance to Cefoxitin (n=Number of Bacterial Isolates)

### 3.4. MRSA Prevalence in Blepharitis Patients

The occurrence of MRSA isolated from the infected eyelids was 11/52 (21%) and the occurrence of MRSA isolated from the uninfected eyelids of blepharitis patients was 9/48 (18.7%).

## 4. DISCUSSION

Blepharitis is a chronic inflammatory eyelid infection characterized by remissions and flare-ups [17]. It is quite difficult to treat because there is not a reliable cure [18]. A study in Karachi determined that blepharitis (5%) is more common among students due to their poor hygiene habits [19]. In Lahore, a survey was conducted on 300 children aged up-to 5 years by using a self-designed questionnaire. The percentage of blepharitis was 5% among the selected 300 children [20]. Blepharitis is very common in Pakistan. The current study conducted in Lahore, found blepharitis to be more common in female patients (56%) as compared to (44%) male patients Figure 1 (a). The infection was found to be more common in patients of age group 21-30 years (28%) Figure 1 (b).

It was reported in previous studies that the anterior blepharitis (AB) is associated with Gram-positive cocci, such as *Staphylococcus aureus, Staphylococcus epidermidis,* and *Corynebacterium* [21, 22]. These studies produced similar results to the current findings. The current study found that blepharitis infection is caused by the increased percentage of *S.aureus* followed by *Bacillus cereus, Klebsiella spp., E.coli, Acinetobacter spp., Enterobacter spp., Shigella spp.* and *Serratia marcescens*.

A cross-sectional study was carried out in Misurata, Libya on 56 patients of AB. The isolated bacteria were viridians *Streptococci* (9%), *Staphylococcus epidermidis* (25%), *Staphylococcus aureus* (25%), *Pseudomonas aeruginosa* (9%), *Proteus sp.* (7%), *Enterobacter aerogenes* (5%), and *E. coli* (2%) obtained in the order of decreasing frequency [23]. This study produced results which showed some similarity to the current research. Although, in the current research, *Pseudomonas aeruginosa*, viridians *Streptococci*, and *Proteus* species were not isolated.

In another study conducted in China in 2018, eyelid samples were taken from 56 blepharitis patients and 46 healthy controls. The bacteria isolated from eyelid margin were *S. aureus* 22(39.29%), *S. epidermidis* 22(39.29%), *Streptococcus spp.* 12(21.43%), *Corynebacterium spp.* 8(14.29%), *Propionbacterium acnes* 41(73.21%) *and Bacillus spp.* 11(19.64%). In healthy controls, the bacteria isolated from eyelid margin were *S. aureus* 12(26.09%), *S. epidermidis* 21(45.65%), *Streptococcus spp.* 2(4.35%), *Corynebacterium spp.* 4(8.70%), *Propionibacterium acnes* 13(28.26%) *and Bacillus spp.* 5(10.87%) in low percentage as compared to those isolated from the infected eyelid of patients [24]. However, in the current study, *Streptococcus spp.*, *Corynebacterium spp.*, and *Propionibacterium spp.* were not isolated.

The rise of antibiotic resistance is a main public health concern. MRSA is a superbug that causes both nosocomial infections and community-associated illnesses [25]. In the current study, the antibiotic susceptibility of the 31 bacterial strains isolated from infected eyelids of blepharitis patients and 28 bacterial strains isolated from the uninfected eyelids (as control) was tested and vancomycin was determined as the preferred medication to treat MRSA. In the treatment of MRSA

and multidrug resistant *S. aureus*, the development of vancomycin tolerance has become a severe problem [26–29].

The current research aligns with some previous researches. In a study conducted in Sudan in 2016, *S. aureus* illustrated 90% susceptibility and *S. epidermidis* showed 100% susceptibility towards vancomycin in case of Gram-positive bacteria. All *E. coli* (100%) and more than half (75%) of *Klebsiella spp.* totally were found to be resistant and 25% were found to be susceptible to ampicillin. Similarly, 50% *E. coli* were resistant and 50% *E. coli* were sensitive to tetracycline antibiotics. On the contrary, 100% of *Klebsiella spp.* were resistant to tetracycline antibiotics in case of Gram- negative bacteria [27].

The results also illustrated the percentage of MRSA as 18.7% among the microbiota isolated from uninfected eyelids (as control) and 21% MRSA among the microbiota isolated from the infected eyelid of blepharitis patients. The main reason behind high prevalence of MRSA might be misdiagnosis and inappropriate usage of antibiotics. In another research conducted in Brazil, the percentage of MRSA in blepharitis infection was found to be 5.96% [28]. Its high prevalence leads to development of antibiotic resistance against MRSA infections. Vancomycin has become ineffective against some MRSA strains due to its frequent usage for infectious diseases caused by pathogens other than MRSA. The number of therapeutic alternatives is constrained by vancomycin-resistant *Staphylococcus aureus* resistance (VRSA) to the majority of current antibiotics. Since *S. aureus* infections in both hospitalized and non-hospitalized patients can be fatal, the introduction of VRSA is concerning for the medical community [29]. Hence, new anti-microbial agents with high efficacy, low toxicity and decreased potential for the development of bacterial resistance are required [30, 31]. In case of Staphylococcal blepharitis treatment, topical antibiotics and topical steroids are used [32].

### 4.1. Conclusion

Blepharitis is very common in Pakistan. However, there is only a small amount of data available regarding MRSA prevalence among blepharitis patients and their antibiotic susceptibility testing. It was concluded that increased percentage of *S. aureus* followed by *Klebsiella spp., E. coli, Acinetobacter spp., Enterobacter spp., Serratia marcescens, Shigella spp*., and *Bacillus cereus* is associated with blepharitis. *S. aureus, S. epidermidis,* and *B. cereus* isolated from the infected and uninfected eyelid of blepharitis patients were found to be 100% sensitive to vancomycin. So, it should be the drug of choice to treat MRSA bacterial infections. The dissemination of antibiotic tolerant microbes can be slowed down by the use of proper contamination control techniques, adequate antimicrobial management, sanitary environment, and public awareness. The current study was limited by time constraints and data resources. This research could be upgraded to molecular level in the future in order to identify methicillin antibiotic resistance genes (*mecA* and *mecC genes*) among MRSA bacterial isolates.

## CONFLICT OF INTEREST

The author of the manuscript has no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

## DATA AVALIABILITY STATEMENT

The data associated with this study will be provided by the corresponding author upon request.

## FUNDING SOURCE

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## ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Research Ethics and Biosafety Committee of Institute of Microbiology and Molecular Genetics (IMMG), University of the Punjab, Lahore, Pakistan. Informed written consent was taken from each participating patient or from their parents prior to this study.

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