Estimation of the of Beta-Lactamase Production in Staphylococcus aureus Isolates from Blepharitis Patients

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Abstract:
In this study, 90 eyelid swabs were obtained from patients with blepharitis of both sexes and various age groups who were admitted to various hospitals, and other primary healthcare centers between September 2023 and February 2024, in Babylon governorate. It was found that blepharitis occurs in all age groups and affects both sexes without any differences between them, but it is clear that blepharitis is more prevalent in the age range of (20 to 49) years old. It was discovered that out of the 90 samples, 75 (83.3%) had positive bacterial cultures, and 15 (16.7%) had no growth at all. Also, Staphylococcus aureus was the most common bacteria 39 (52%), followed by Haemophilus influenzae 17 (22.7%), Streptococcus pneumoniae 10 (13.3%), Klebsiella pneumoniae 7 (9.3%), and only 2 isolates of Streptococcus pyogenes (2.7%). The rapid iodometric method has been employed to investigate the production of beta-lactamase enzyme by the common bacteria Staphylococcus aureus, which was isolated from the eyelid's margin of patients with blepharitis. It was found that 36 (92.3%) of the 39 Staphylococcus aureus isolates had the ability to produce β-lactamase. A number of common beta-lactam antibiotics, such as penicillin, amoxicillin, ampiclox cephalixin, and cefazolin have also been used to investigate the ability of Staphylococcus aureus to produce the β-lactamase enzyme. The results showed that all isolates of Staphylococcus aureus were 100% resistant to penicillin, cephalixin and cefazolin. whereas some isolates 30 isolates were resistant to ampiclox (77%), and only 23 isolates were resistant to amoxicillin (59%).

Keywords: Blepharitis, Staphylococcus aureus, β-lactamase, Antibiotic resistance.

Introduction
Blepharitis is the eyelid margin inflammation mainly involving its skin, associated structures, eyelashes, and Meibomian glands, it can affect people of all ages, possibly resulting from the abnormal overgrowth of bacteria that is normally found on our skin and other contributing factors often differ from person to person. However, it is commonly seen in people with rosacea, a common skin condition that causes red, inflamed lesions that sometimes look like acne. Other potential reasons are decreased oil production in the meibomian glands due to age or hormonal changes, lice that live on the eyelashes, allergies, certain types of bacterial infections, mites that live on the eyelashes or the
fungus caused by seborrheic dermatitis (eczema) (Mergen et al. 2023). Some people are more at risk of developing the condition than others, including people with dry eyes, meibomian gland dysfunction (a problem with the tiny glands in the eyelids that secrete an oily substance that is part of the tear film), dandruff on the scalp or a family history of blepharitis. (Li et al. 2023).

The most frequently isolated and characteristic organisms frequently associated with blepharitis include coagulase-negative Staphylococci, Propionibacterium acnes, Propionibacterium granulosum, Corynebacterium sp., Moraxella sp., Streptococcus pyogenes, Neisseria cinerea, Bacteroides sp., and Demodex sp. The Staphylococcus group, which currently contains approximately thirty different species, is the most frequently isolated microorganism (Mergen et al. 2023). In general, the ocular infections are frequently caused by endogenous flora of the ocular adnexa. This is because there is a potential to cause an excessive release of toxic substances, antimicrobial-resistant strains, and, most importantly, opportunistic acquisition of virulence factors. (Patel et al., 2023). Staphylococcus aureus colonizes more than one third of the general population, causing anterior blepharitis when it produces beta-lactamase, an enzyme that confers resistance to penicillins such as penicillin G and its derivatives (amoxicillin, ampicillin) and first-generation cephalosporins (cephalothin, cephaloridine, and cephalaxin). The infection with antibiotic-resistant bacteria may result in unresponsive disease and increases the demand for new antimicrobial agents in order to avoid the overuse and side effects of the use of systemic antibiotics in the absence of major complications (Hasanpour et al. 2023).

This study aims to isolate and identify bacterial pathogens associated with blepharitis, determine beta-lactamase enzyme production by the common bacterial isolates, and show that some beta-lactam antibiotics affect these common bacterial isolates.

Materials and Methods

Patients and Specimens

Eyelid swabs were taken from ninety individuals with blepharitis of age groups ranging from (7 to 52) years of both sexes in Babylon governorate. All patients being investigated in this study had the following clinical features: "redness of the eyes and eyelids, itching, pain, burning, and watering." Specimens were obtained from patients who did not receive any antibiotics. One sample of the eyelid's margin of each eye was taken by applying a sterile cotton swab to the eyelashes and margins of both top and bottom eyelids. The swabs were put into normal saline in the sterilized swab tubes and then sent to the investigating laboratory within two hours of collection. The eyelid's swabs were inoculated onto blood agar (enriched with 5% human blood), MacConky agar, and Mannitol salt agar, and the plates were then incubated at 37°C for 24 hours.

Laboratory Diagnosis

According to the diagnostic procedures recommended by Bergy’s manual for determinative bacteriology (Holt et al. 1994), a single colony was taken from each primary positive culture and investigated by gram stain, as well as specific biochemical tests such as "the catalase test, oxidase test, and coagulase tests, which allowed for the differentiation of Staphylococcus aureus from coagulase-negative Staphylococci." A bile solubility test was performed to distinguish between Streptococcus pneumoniae and Streptococcus viridans. Additionally, a satellitism test was done for the identification of H. influenzae isolates.

Beta-Lactamase Production Test

The ability of Staphylococcus aureus isolates to produce the β-lactamase enzyme was determined using the rapid iodometric method, as described by (Isenberg, 2004). Penicillin solution prepared in 100 μl volume was dispensed in microtitre wells, and test organisms were suspended in wells to a concentration of 109 cells/ml (McFarland’s number 4). Two drops of 1% freshly prepared starch indicator were added to each well and thoroughly mixed,
following which the tray was incubated at room temperature for 30-60 minutes. One drop of iodine reagent was dispensed in each well. Rapid decolorization of blue color within ten minutes was regarded as positive for β-lactamase activity and persistence of blue color as negative.

**Sensitivity Test for Beta-Lactam Antibiotics**

Muller-Hinton agar was prepared and sterilized by autoclave and then supplemented by the required antibiotics at final concentrations (100µg / ml), the plates were then inoculated by the bacteria by using (picking and patching method) and incubated for 24h at 37°C and the zone inhibition diameters were determined as suggested by the Clinical and Laboratory Standard Institute (CLSI) 2014 guideline.

**Results & Discussions**

*Staphylococcus aureus* blepharitis is a sight-threatening ocular disease, which accounts for approximately four to six million eye disorders every year. It is the most prevalent disease of the eyelids and anterior segment tissues and has the capability to affect patients with diseases such as chronic blepharoconjunctivitis, staphylococcal marginal keratitis, chalazion, intraocular abscesses and systemic diseases like central retinal vein occlusion and endophthalmitis. (Kumar and Mah, 2022)

In this study, from the total 90 patients with blepharitis of age groups ranging from (7 to 52) years, 43 male and 47 female. The results are shown in Table 1.

As illustrated in Table 1, blepharitis occurs in all age groups and affects both sexes without any differences between them, but it is clear that blepharitis is more prevalent in the age range of (20 to 49) years old. This is probably due to the fact that associated conditions play a role in the causation of blepharitis, such as keratoconjunctivitis sicca, dermatologic conditions associated with seborrheic blepharitis, and Meibomian Gland Dysfunction (MGD), or tear dysfunction. The findings corresponded with those of Rhumaid et al. (2022), who showed that blepharitis affects patients of both sexes and occurs in all age groups, particularly in the age groups (20 – 49).

**Table 1. Distribution of Patients with Blepharitis According to Age Groups and Gender**

<table>
<thead>
<tr>
<th>Age Groups (years)</th>
<th>No. Patients</th>
<th>%</th>
<th>Gender</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10</td>
<td>4 (4.4)</td>
<td>1</td>
<td>Male</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>10 – 19</td>
<td>8 (8.9)</td>
<td>3</td>
<td>Female</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>20 – 29</td>
<td>18 (20)</td>
<td>10</td>
<td>Male</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>30 – 39</td>
<td>20 (22.2)</td>
<td>11</td>
<td>Female</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>40 – 49</td>
<td>23 (25.6)</td>
<td>12</td>
<td>Male</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>≥ 50</td>
<td>17 (18.9)</td>
<td>7</td>
<td>Female</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>90 (100%)</td>
<td>44</td>
<td>Male</td>
<td>44</td>
<td>46</td>
</tr>
</tbody>
</table>

On the other hand, in this study, 90 eyelid swabs were subjected to culturing on Blood agar, MacConky agar, and Mannitol salt agar. It was discovered that out of the 90 samples, 75 (83.3%) had positive bacterial cultures, and 15 (16.7%) had no growth at all. It would suggest the existence of microorganisms other than bacteria, like viruses, or that some bacteria, like chlamydia, need certain growing conditions. Bacteria are more prevalent microorganisms than other agents that could affect the eyelid margin of patients with blepharitis.

Additionally, the most common types of bacteria isolated in this study are shown in Table 2. *S. aureus* was the most common bacteria, 39 (52%), followed by *H. influenzae* 17 (22.7%), *S. pneumoniae* 10 (13.3%), *K. pneumoniae* 7 (9.3%), and only 2 isolates of *S. pyogenes* (2.7%).
Table 2. Frequency of Patients with Blepharitis According to The Type of Bacteria

<table>
<thead>
<tr>
<th>Age Groups (years)</th>
<th>S. aureus No. %</th>
<th>H. influenzae No. %</th>
<th>S. Pneumoniae No. %</th>
<th>K. pneumoniae No. %</th>
<th>St. pyogenes No. %</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10</td>
<td>4 (10.3)</td>
<td>0</td>
<td>5 (14.3)</td>
<td>1 (50)</td>
<td>1 (50)</td>
<td>11 (14.7)</td>
</tr>
<tr>
<td>10 – 19</td>
<td>1 (2.6)</td>
<td>1 (5.9)</td>
<td>4 (28.6)</td>
<td>2 (50)</td>
<td>9 (12)</td>
<td></td>
</tr>
<tr>
<td>20 – 29</td>
<td>2 (5.1)</td>
<td>3 (17.6)</td>
<td>1 (10)</td>
<td>4 (57.1)</td>
<td>0</td>
<td>10 (13.3)</td>
</tr>
<tr>
<td>30 – 39</td>
<td>7 (17.9)</td>
<td>4 (23.5)</td>
<td>0</td>
<td>0</td>
<td>11 (14.7)</td>
<td></td>
</tr>
<tr>
<td>40 – 49</td>
<td>17 (43.6)</td>
<td>7 (41.2)</td>
<td>0</td>
<td>0</td>
<td>24 (32)</td>
<td></td>
</tr>
<tr>
<td>≥ 50</td>
<td>8 (20.5)</td>
<td>2 (11.8)</td>
<td>0</td>
<td>0</td>
<td>10 (13.3)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>39 (52)</td>
<td>17 (22.7)</td>
<td>10 (13.3)</td>
<td>7 (9.3)</td>
<td>2 (2.7)</td>
<td>75 (100)</td>
</tr>
</tbody>
</table>

It is evident that S. aureus was the most often isolated bacterium from blepharitis patients’ eyelids with blepharitis in all age groups, particularly in patients that were relatively younger (20 – 49) years old. It is possible that a decrease in locallysozyme and immunoglobulin levels associated with tear deficiency may alter resistance to bacteria, predisposing to the development of Staphylococcus aureus blepharitis. These results agreed with the other previous studies reported by Chaudhary et al. (2010) and Biradar et al. (2012), which stated that Staphylococcus aureus was the most predominant pathogen identified in blepharitis.

Furthermore, the ability of the common bacteria S. aureus to exhibit β-lactamase enzyme production was studied by the rapid iodometric method. The results indicated that 36 (92.3%) of the 39 S. aureus isolates had the ability to produce β-lactamase in the presence of penicillin G as an inducer. Pichichero (2005) stated that β-Lactamase enzymes will break open the β-Lactam ring of the antibiotics, rendering the antibiotics ineffective. The genes encoding these enzymes may be present on the bacterial chromosome or may be acquired via plasmid transfer, and β-Lactamase gene expression may be induced by exposure to β-Lactam antibiotics.

Moreover, some beta-lactam antibiotics including penicillin, amoxicillin, ampiclox, cephalixin and cefazolin, were used to show their effects on isolates of S. aureus. The results of the sensitivity test are shown in Figure 1.

It was indicated by Figure 1 that all 39 of the S. aureus isolates were resistant to penicillin, cephalexin, cefazolin (100%); whereas some isolates 30 isolates were resistant to ampiclox (77%), and only 23 isolates were resistant to amoxicillin (59%). S. aureus isolates appeared the highest resistance rate to cephalexin, cefazolin, and penicillin and this might be related to the synthesis of beta-lactamase enzymes and changes of the penicillin-binding proteins.

(Astley et al.2023) indicated that blepharitis is caused by the bacterium S. aureus, which has shown resistance to beta-lactam antibiotics, particularly penicillin. This bacterial infection commonly affects the eyelids, leading to irritation, redness, and swelling. It can also cause the formation of crusts and scales along the eyelash line.

Yang et al. (2023) revealed that Staphylococcal blepharitis, a type of eye infection caused by the bacteria S. aureus, is becoming more and more resistant to beta-lactam antibiotics. Specifically, the resistance is observed with two commonly used antibiotics, cephalexin and cefazolin. This resistance poses a significant challenge to treating this condition, requiring healthcare professionals to explore alternative antibiotic options. It is crucial to address this issue to prevent further complications and ensure effective treatment for patients suffering from Staphylococcus aureus blepharitis.
Conclusions

The prevalence of bacterial infection in patients suffering from blepharitis is 83.3%. This result refers to the fact that bacterial infection is higher than other infections and can affect both sexes without any differences between them, but it is clear that blepharitis is more prevalent in the age range of (20 to 49) years old. Also, the common type of bacteria that causes bacterial blepharitis in this study is *S. aureus*, and 36 (92.3%) of the 39 *S. aureus* isolates had the ability to produce β-lactamase. In addition, all isolates of *S. aureus* were 100% resistant to penicillin, cephalaxin, and cefazolin. However, the resistance ratios to other antibiotics like ampiclox and amoxicillin exhibited significant variations.

References


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