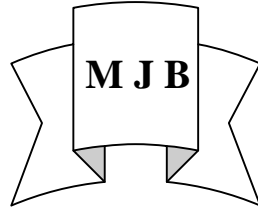


## Isolation of Bacteria from Patients With Blepharitis and Investigation of Beta-Lactamase Production

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### Abstract

In this study 31 eyelids swabs were obtained from patients suffering from blepharitis of different age groups and both sexes in Hilla province.

Bacterial infections were detected in 26 (83.9%) samples and no growth resulted in 5 (16.1%) samples . It was found that *Staphylococcus aureus* was the commonest bacteria ( 15 isolates ), followed by *Staphylococcus epidermidis* ( 7 isolates ) and only ( 4 isolates ) of *Streptococcus pneumoniae* .

The rapid iodometric method has been employed to investigate the production of beta-lactamase enzyme by common bacteria Staphylococci (*Staphylococcus aureus* and *Staphylococcus epidermidis* ) which isolated from eyelid's margin of patients with blepharitis . It was found that (13) isolates of *Staphylococcus aureus* and (5) isolates of *Staphylococcus epidermidis* have revealed positive results.

A number of common Beta-lactam antibiotics such as Penicillin, Ampiclox , Amoxicillin , Cephalexin and Cefazolin have also been used to investigate the ability of *Staphylococcus aureus* and *Staphylococcus epidermidis* to produce  $\beta$ -lactamase enzyme . The results showed that Staphylococci (*Staphylococcus aureus* and *Staphylococcus epidermidis* ) were 100% resistant to Penicillin , Cephalexin and Cefazolin , other antibiotics Ampiclox and Amoxicillin showed remarkable variations in respect of resistance ratio .

عزل البكتريا من المرضى المصابين بالتهاب الجفن والكشف عن إنتاج أنزيم البيتا لاكتاميز

### الخلاصة

في هذه الدراسة ، تم الحصول على ( 31 ) مسحة من المرضى المصابين بالتهاب الجفن في مدينة الحلة بفئات عمرية مختلفة ولكلا الجنسين . هي البكتريا *Staphylococcus aureus* تم الكشف عن الإصابات البكتيرية في ( 26 ) عينة ، وعدم وجود نمو في ( 5 ) عينات ، وقد وجد بان *Streptococcus pneumoniae* ( 7 ) عزلات ، و ( 4 ) عزلات فقط من *Staphylococcus epidermidis* السائدة ( 15 ) عزلة ، ثم تليها

( *Staphylococci* ) لقد استخدمت طريقة البود القياسية السريعة للكشف عن إنتاج أنزيم البيتا لاكتاميز المنتج من قبل البكتريا السائدة المعزولة من المرضى المصابين بالتهاب الجفن . وقد وجد بان ( 13 ) *Staphylococcus aureus epidermidis* , *Staphylococcus epidermidis* , *Staphylococcus aureus* قد أعطت نتيجة موجبة كذلك استخدم عدد من مضادات البيتا لاكتاميز الشائعة الاستخدام مثل البنسلين ، الاميكلوكس ، الاموكسيسيلين ، السيفالكسين والسيفازولين للكشف عن قابلية

على إنتاج أنزيم البيتا لاكتاميز . *Staphylococcus aureus* , *Staphylococcus epidermidis* . كانت مقاومة 100% *Staphylococcus aureus* , *Staphylococcus epidermidis* , *Staphylococci* ) *Staphylococcus aureus* وقد أظهرت النتائج أن بكتريا لكل من مضاد البنسلين ، السيفالكسين والسيفازولين وأما بقية المضادات فقد أظهرت اختلافات واضحة في نسب المقاومة .

### Introduction

**B**lepharitis is an inflammation of the eyelid margins , It is one of the most common causes of external ocular irritation , affecting people of all ages and of both sexes [1] . Blepharitis can be divided into anterior and posterior. Anterior blepharitis refers to inflammation mainly

centered around the eyelashes and follicles , while the posterior involves the meibomian gland orifices. Blepharitis usually is subdivided into Staphylococcal , Seborrhic variants and Meibomian Gland Dysfunction (MGD) [2] .

The symptoms of blepharitis include : redness of the eyes and eyelids, burning , watering , photophobia , pain and a feeling

that something is in the eyes . The most common causes of blepharitis are Staphylococcal infection ( usually caused by *Staphylococcus aureus* but occasionally other species ) and irritation from oily meibomian gland secretions , rosacea , seborrheic dermatitis , chalazion formation ,bacterial infections (dermatitis ),viral infections ,and may result from allergies [3].

The commonest types of bacteria implicated in bacterial blepharitis include *Staphylococcus aureus* , numerous amounts of coagulase negative *Staphylococci* , *Streptococcus viridans* , Diphtheroids , *Streptococcus pneumoniae* and *Streptococcus pyogenes* [4] .

Resistance to antibiotics is considered one of the most common world problems economically as well as medically . The excessive wrong use of antibiotics has resulted in the appearance of new strains of bacteria characterized by their resistance to a variety of antibiotics , such as beta-lactam [5] .

There are many different mechanisms by which microorganisms might exhibit resistance to drugs , i.e., producing destructive enzymes that are able either to hydrolyze the molecules of the antibiotics or modify them in such a way as to make them inactive . Among these enzymes are the Beta-Lactamase which are associated with resistance to beta-lactam antibiotics and these enzymes are produced by gram-positive and gram-negative bacteria [6] .

Many studies have been conducted to study these enzymes , especially those related to genetic factor controlling their production . The gene encoded for these enzymes either located on bacterial chromosomes or on the plasmids , however, these genes are also seen to be found on transposons [7] .

## **Materials and Methods**

### **Patients and Specimens**

Eyelids swabs were obtained from ( 31 ) patients with blepharitis of age groups ranging from ( 7 to 52 ) years of both sexes in Hilla province .

All patients being investigated in this study were with the following clinical features (redness of the eyes and eyelids , itching , pain , burning , watering ) . Specimens were obtained from patients who did not receive

any antibiotics . One sample of the eyelids' margin of each eye was taken by applying rolling 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 eyelids' swabs were inoculated onto Blood agar ( enriched with 5 % human blood ) , MacConky agar and Mannitol salt agar , and incubated at 37 C<sup>0</sup> for 24 hours.

### **Laboratory Diagnosis :**

According to the diagnostic procedures recommended by Bergy's manual for determinative bacteriology [8]. a single colony was taken from each primary positive culture and investigated by gram stain , and specific biochemical tests such as (catalase test , oxidase test and coagulase test for differentiated between *Staphylococcus aureus* and coagulase negative *Staphylococci* *Staphylococcus epidermidis* ) . Also , bile solubility test was done for differentiated between *Streptococcus pneumoniae* and *Streptococcus viridans*.

### **Beta- Lactamase Production Test**

The rapid iodometric method was used for detection of the ability of the *Staphylococci* (*Staphylococcus aureus*, *Staphylococcus epidermidis* ) bacteria to produce  $\beta$ -lactamase .[9]

1. Prepare a new bacterial culture ( 24 hours ) later , transfer some colonies to an Ependrof tube which contained (100 ml ) of penicillin G solution and incubated the tube at 37 C<sup>0</sup> for ( 30 minutes ) then ( 50 ml ) of starch solution added and mixed it well with the tube content .
2. ( 20 ml ) of iodine solution was added to this tube ; the resulting colour was dark blue to the reaction between starch and iodine .
3. The tube is finally shaking for one minute : if the colour changes from dark blue into white after less than one minute from adding the indicator , the result is considered positive .
- 4 . The test is repeated if the white colour appears later .

### **Sensitivity Test For Beta-Lactam Antibiotics On Solid Media**

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<sup>0</sup> [10] .

### **Results and Discussion**

Blepharitis is one of the most common eyelid problems , it is occurs with chronic bacterial lid infection , meibomian gland dysfunction, seborrhea and acne rosacea that affects the eye , known as ocular rosacea .Blepharitis is affecting people of all age groups of both sexes . If severe , blepharitis may result in corneal infiltrates or ulcers. Rarely , sebaceous cell carcinoma may masquerade as unilateral or bilateral intractable blepharitis [11] .In this study , from the total 31 patients with blepharitis of age groups ranging from ( 7 to 52 ) years , 14 male and 17 female The results are shown in Table (1) .

As shown in table (1) that blepharitis occurs in all age groups and it was affecting both sexes without any differences between them , but it is clear that blepharitis is common in the age groups ranging from ( 40 – 49 ) years old .This is probably to the fact that associated conditions have role in the causation of blepharitis such as keratoconjunctivitis sicca , dermatologic conditions associated with seborrheic blepharitis and Meibomian Gland Dysfunction (MGD) ,tear dysfunction.The results agreed with several results obtained by [2,12] who demonstrated that blepharitis occurs in all age groups specially in the age groups ( 42 – 52 ) and could affecting patients with both sexes .but the results were contrary to the results obtained by [13] , who demonstrated that blepharitis occurs in age group less than 10 years because infections are more commonly seen in children , associated conditions like dandruff , tonsillitis , pharyngitis have role in the causation of blepharitis .

In this study , 31 eyelid swabs were subjected for culturing on Blood agar , MacConky agar , and Mannitole salt agar . It was found out that the total of (31) samples , only (26) samples showed positive bacterial cultures, and no growth was seen in (5)

samples which could indicate the presence of microorganisms other than bacteria such as viruses , or bacteria require special growth factors such as Chlamydia .

Bacteria are more predominant microorganisms than other agents could affecting eyelid 's margin of patients with blepharitis .

The most common types of bacteria isolated in this study was shown in Table ( 2 ) .

It was found that most common bacteria which isolated from eyelids patients with blepharitis was *Staphylococcus aureus* (15) isolates in all age groups specially in patients that relatively younger ( 40 – 49 ) years old .

It is possible that a decrease in local lysozyme and immunoglobulin levels associated with tear deficiency may alter resistance to bacteria predisposing to the development of staphylococcal Blepharitis [12]. The same result was obtained by [14,13,2] .

Also (7) isolates of *Staphylococcus epidemidis* were isolated from eyelids patients with blepharitis . Both *Staph. aureus* and *Staph. epidemidis* are believed to play a role in the development of Staphylococcal blepharitis , but the mechanism of disease production remain poorly understood . Toxin production has been reported to correlate with the presence of blepharoconjunctivitis [15,12] .

Furthermore ,only 4 isolates of *Str. pneumoniae* were isolated from eyelids of children with blepharitis in the age groups ranging from ( < 10 – 12 ) years old of both sexes .

In this study , the ability of common bacteria Staphylococci (*Staphylococcus aureus* and *Staphylococcus epidermidis* ) which have been isolated from patients with blepharitis to produce β-Lactamase enzyme was studied by rapid iodometric method .It was found that 18 isolates of Staphylococci (13 isolates of *Staphylococcus aureus* and 5 isolates of *Staphylococcus epidermidis*) had the ability to produce β-lactamase in the presence of the penicillin G( inducer ) .

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  $\beta$ -Lactamase gene expression may be induced by exposure to  $\beta$ -Lactam antibiotics [16].

Some Beta-Lactam antibiotics ( Penicillin , Ampiclox , Amoxicillin , Cephalixin and Cefazolin ) were used to show their effects on Staphylococcal isolates 22 isolates ( 15 isolates of *Staphylococcus aureus* and 7 isolates of *Staphylococcus epidermidis* ) . The results of sensitivity test are shown in Table ( 3 ) .

It has been found that all Staphylococcal isolates 22 isolates were resistant (100%) to Penicillin , Cphalexin and Cefazolin .Also, 77% of these isolates were resistant to Ampiclox ; whereas 59% were resistant to Amoxicillin .

The resistance of Staphylococci can be described according to their ability to produce  $\beta$ -Lactamase or penicillinase enzymes that break down the beta lactam ring and render it to inactive products .This is mediated by extra-chromosomal piece of DNA (plasmid ) [17] .

The same results have been reported by [18] who found that *Staphylococcus aureus* isolates were resistant 100% to penicillin .Also the results are agrees with those of some researchers as [19] who have found that all *Staphylococcus aureus* isolates were resistant 100% to penicillin and 58% of these were amoxicillin resistant .

On the other hand , [20] have showed that *Staphylococcus aureus* and *Staphylococcus epidermidis* were resistant 100% to Cphalexin and Cefazolin and 73% of these were resistant to Ampiclox .

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**Table 1** Frequency of patients according to age groups

Age groups ( year )	No. of patients %	Sex	
		Male	Female
< 10	1 (3.2)	0	1
10 – 19	3 (9.7)	1	2
20 – 29	5 (16.1)	2	3
30 – 39	4 (12.9)	2	2
40 – 49	12 (38.7)	5	7
≥ 50	6 (19.4)	4	2
<b>Total</b>	<b>31 (100)</b>	<b>14 (45.2%)</b>	<b>17 (54.8%)</b>

**Table 2** Frequency of patients according to the type of bacteria

Age groups ( year )	<i>Staph. aureus</i>		<i>Staph.epidermidis</i>		<i>Str. pneumoniae</i>		Total
	No.	%	No.	%	No.	%	
< 10	2	(13.3)	0	0	2	(50)	4 (15.4)
10 – 19	0	0	0	0	2	(50)	2 (7.7)
20 – 29	0	0	1	(14.3)	0	0	1 (3.8)
30 – 39	3	(20)	2	(28.6)	0	0	5 (19.2)
40 – 49	7	(46.7)	3	(42.9)	0	0	10 (38.5)
≥ 50	3	(20)	1	(14.3)	0	0	4 (15.4)
<b>Total</b>	<b>15</b>	<b>(57.7)</b>	<b>7</b>	<b>(26.9)</b>	<b>4</b>	<b>(15.4)</b>	<b>26 100</b>

**Table 3** The percentage of resistance of *Staphylococcus aureus* and *Staphylococcus epidermidis* to some Beta-Lactam antibiotics

<i>Staphylococci</i> isolates	No. of isolates	PN	AMC	AMO	CL	CFZ
<i>Staphylococcus aureus</i>	15	15	12	9	15	15
<i>Staphylococcus epidermidis</i>	7	7	5	4	7	7
<b>Total %</b>	<b>22 (100)</b>	<b>22 (100)</b>	<b>17 (77)</b>	<b>13 (59)</b>	<b>22 (100)</b>	<b>22 (100)</b>

PN : Penicillin    AMC : Ampiclox    AMO : Amoxicillin    CL : Cphalexin    CFZ : Cefazolin