Investigation of the antibiotic resistance genes *mecA* and *ermA* of resistant *Staphylococcus aureus* isolated from Ocular bacterial infections in the holy city of Karbala.

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ABSTRACT

Back ground: Staphylococcus aureus is leading to ocular infection worldwide among gram –positive bacteria. Out of 169 swabs were collected from patients, with eye infections and from three areas: conjunctiva, cornea and eyelids, when they visited the ophthalmology consultant at Al-Hassan Al-Mujtaba Hospital, Al-Hindiya General Hospital and Al-Sayeda Zainab Specialized Center for Eyes in the Holy Karbala Governorate

Aims: to investigating the most important microscopic bacterial neighborhoods that cause the Eye infection and molecular study of antibiotic resistance genes in the most common bacterial isolated from ocular infection: the patients age ranged between (1-75) years, of both sexes, males and females, for the period from August 2022 to January 2023. Seventy-six bacterial isolates were obtained based on the phenotypic identification, biochemical tests: the diagnosis was confirmed by the Vitek2 system. Distribution of bacterial isolates were of follows: 41 Gram-positive bacterial isolates and 35 Gram-negative bacterial isolates, No Bacterial growth was found in 93 samples. The results showed that Staphylococcus aureus was dominant 22 (28.94%) samples, followed by Staphylococcus epidermidis with 19 (25%) samples, then Pseudomonas aeruginosa 18 (23.68%) samples and 12(15.78%) isolates belonging to Escherichia coli, 4(% 5.26) isolates of Klebsiella pneumoniae and 1(1.31%) isolates of Serratia marcescens. The sensitivity test was performed using the Vitek2 system, and using the polymerase chain reaction (PCR) technique,

In conclusion: Staphylococcus aureus was dominant and presence of antibiotic resistance genes mecA and ermA was confirmed in Staphylococcus aureus bacteria that infect the eye. by 95.4% and 72.7%, respectively.

Keywords: eye infections - *Staphylococcus aureus* - antibiotic resistance genes

التحري عن جينات مقاومة المضادات الحيوية *mecA* و *ermA* للمكورات العنقودية الذهبية المقاومة المعزولة من الالتهابات البكتيرية العينية في مدينة كربلاء المقدسة.

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الكلمات المفتاحية: التهابات العين - المكورات العنقودية الذهبية - الجينات المقاومة للمضادات الحيوية

الخلفية: بكتريا المكورات العنقودية الذهبية هي التي تقود الى الإصابات العينية حول العالم بين البكتريا الموجبة لصبغة غرام. تم جمع 169 مسحة من مرضى التهابات العين ومن ثلاث مناطق: الملتحمة والقرنية والجفون، وذلك عند زيارة استشاري طب وجراحة العيون بمستشفى الحسن المجتبى ومستشفى الهندية العام ومركز السيدة زينب التخصصي. للعيون في محافظة كربلاء المقدسة

الهدف: للتحقيق في أهم الأحياء البكتيرية المجهرية المسببة لعدوى العين والدراسة الجزيئية للجينات المقاومة للمضادات الحيوية في البكتيريا الأكثر شيوعا المعزولة عن عدوى العين: تراوحت أعمار المرضى بين (1-75) سنة، من كلا الجنسين، ذكور وإناث، للفترة من أغسطس 2022 إلى يناير 2023Vitek2. تم الحصول على 76 عزلة بكتيرية بناءً على تحديد النمط الظاهري، الاختبارات البيو كيميائية: تم تأكيد التشخيص بواسطة نظام كان توزيع العزلات البكتيرية كالتالي: 41 عزلة بكتيرية موجبة الجرام و35 عزلة بكتيرية سالبة الجرام، لم يتم العثور على نمو بكتيري في 93 عينة. عينة، 19 بـ (25٪) *Staphylococcus epidermidis*، تليها 22 كانت سائدة (28.94٪)*Staphylococcus aureus*أظهرت النتائج أن بكتيريا عزلات من (5.26%)4، *Escherichia coli*عزلة تنتمي إلى 12عينة و (15.78٪) (23.68%) 18 *Pseudomonas aeruginosa*ثم ، وباستخدام Vitek2تم إجراء اختبار الحساسية باستخدام نظام .*Serratia marcescens* و 1 (1.31٪) عزلات من *Klebsiella pneumoniae* (PCR)تقنية تفاعل سلسلة انزيم البلمرة

الاستنتاج: كانت بكتريا Staphylococcus *aureus* هي السائدة وتم تأكيد وجود جينات مقاومة المضادات الحيوية *mecA* و *ermA*في بكتيريا *Staphylococcus aureus* التي تصيب العين. بنسبة 95.4٪ و72.7٪ على التوالي.

1.INTRODUCTION

The eye is one of the organs of the human body with a complex structure located within a protective skeleton of the skull [1]. Although the eye is protected by a number of natural defense mechanisms through the continuous flow of tears that contain antibacterial compounds. Many people suffer from a number of infections due to the fact that the ocular surface is rich in nutrients and therefore supports a variety of microorganisms that make up the normal eye flora and whose growth is regulated and thus prevent infection, but these microorganisms under different conditions can cause eye pathogens [2]. The eye is exposed to air that carries dust and microorganisms such as bacteria that cause infections, especially when there is a scratch or wound in the tissues of the lining of the eye, which leads to significant damage to the eye [3]. Gram-positive bacteria are the main cause of eye infections, especially *Staphylococcus aureus* [4]. *Staphylococcus aureus* has the ability to encode the production of beta-lactamase enzymes by *mecA* and anti-erythromycin resistance by the *ermA* gene [5,6]. This study focused on researching the origins of bacterial eye infections such as conjunctivitis, blepharitis, and keratitis, and the effect of demographic factors on bacterial eye infections with a molecular study of antibiotic resistance genes of *Staphylococcus aureus* bacteria isolated from eye infections of patients with Ocular infections and hospitalized patients in the holy city of Karbala.

2.MATERIALS AND METHODS

Sample Collection and bacterial identification:

A total of patients one hundred sixty-nine eye swabs were collected from patients attending Karbala hospitals from August 2022 to January 2023. Information was collected directly from the patient and his consent was obtained to carry out this research.(1-75) years old, for both sexes, and then a swab was taken from the infected eye using sterile cotton swabs containing a culture carrier medium, after which it was transferred to the microbiology laboratory in Imam Al-Hassan Al-Mujtaba Hospital, and it was inoculated on general media with blood agar and differential with MacConkey agar and selectivity (Mannitol salt agar) and incubated at 37°C for 24 hours [7]. Then different biochemical tests were performed according to the methods described by [8] and confirmed using the Vitek-2 diagnostic system.

Antimicrobial susceptibility test:

The Vitek2 system was used to estimate the sensitivity and resistance of *Staphylococcus aureus* bacteria isolates according to [9] by using the antibiotic sensitivity test card for a number of antibiotics as shown in table (1) according to the instructions of the BioMerieux company.

Table (1): antibiotics used in study

No.	antibiotics	No.	antibiotics
1	Amoxicillin	16	Levofloxacin

2	Amoxicillin\Clavulanic acid	17	Moxifloxacin
3	Benzylpenicillin	18	Norfloxacin
4	Ticarcillin\Clavulanic acid	19	Azithromycin
5	Oxacillin	20	Clarithromycin
6	Cefadroxil	21	Erythromycin
7	Cefalexin	22	Clindamycin
8	Cefixime	23	Linezolid
9	Cefpodoxime	24	Teicoplanin
10	Cefoperazone	25	Vancomycin
11	Cefotaxime	26	Tetracycline
12	Cefepime	27	Tigecycline
13	Meropenem	28	Fusidic Acid
14	Gentamicin	29	Rifampicin
15	Ciprofloxacin	30	Trimethoprim\Sulfamethoxazole

MOLECULAR STUDY

DNA extraction was carried out from the isolates of *Staphylococcus aureus* bacteria using the kit prepared from the Korean company (ADD BIO) and according to the instructions of the equipped company. Specialized primers designed for this purpose were used. The primers were prepared in a lyophilized form from (Chromogen) and dissolved in deionized water to obtain Working Solution with a volume of 100 microliters. The following table (2) represents the primers used in this study, while table (3) shown in components of PCR mixture and table (4) shown Conditions for thermal reaction cycles

Table (2): Primers of *Staphylococcus aureus* bacteria

Primers gene	**The sequences of the nitrogenous bases**		**Amplicon (bp)**	**References**
MecA	F	GTAGAAATGACTGAACGTCCGATAA	310	[10]
	R	CCAATTCCACATTGTTTCGGTCTAA		
ermA	F	CTTCGATAGTTTATTAATATTAGT	645	[11]
	R	TCTAAAAAGCATGTAAAAGAA		

Preparation of the polymerase chain reaction mixture

Table (3) Components required for the polymerase chain reaction (PCR).

Components	**Quantity**	**Concentration**
DNA sample	3	100-200 ng

Primer(F)	1.5	10 Pico mole
Primer(R)	1.5	10 Pico mole
Nuclease free water	9	-
Master Mix	10	-
Total volume	25	-

Table (4) Conditions for thermal reaction cycles

Stage	Temperature (°C)		Time	The number of cycles
Initial denaturation	95		5 minute	1
Denaturation	95		30 seconds	35
Annelaling	ermA	mecA	45 seconds	
	54.6	55		
Extension	72		55 seconds	
Final extension	72		5 minute	1

PCR products were obtained after electrophoresis using a 1.5% agarose gel supplemented with ethidium bromide dye and photographed under UV light.

3. RESULTS

Among the 169 swabs, the results showed that 76 (45%) samples have bacterial growth, while no growth appeared in 93 (55%) samples. Gram-positive bacterial isolates were found in 41 (53.94%), from which the highest frequency was *Staphylococcus aureus* 22(28.94%) isolates, followed by *Staphylococcus epidermidis*19 (25%), while Gram-negative bacterial isolates were appeared in 35(46.05%) samples, from which *P. aeruginosa* 18(23.68%). was the most frequently isolated Gram-negative bacterial, followed by *E. coli* 12(15.70%) and *K. pneumoniae* 4(5.20%), while the only one bacterial isolate was *S. marcescens* with (1.3%) Figure (1).

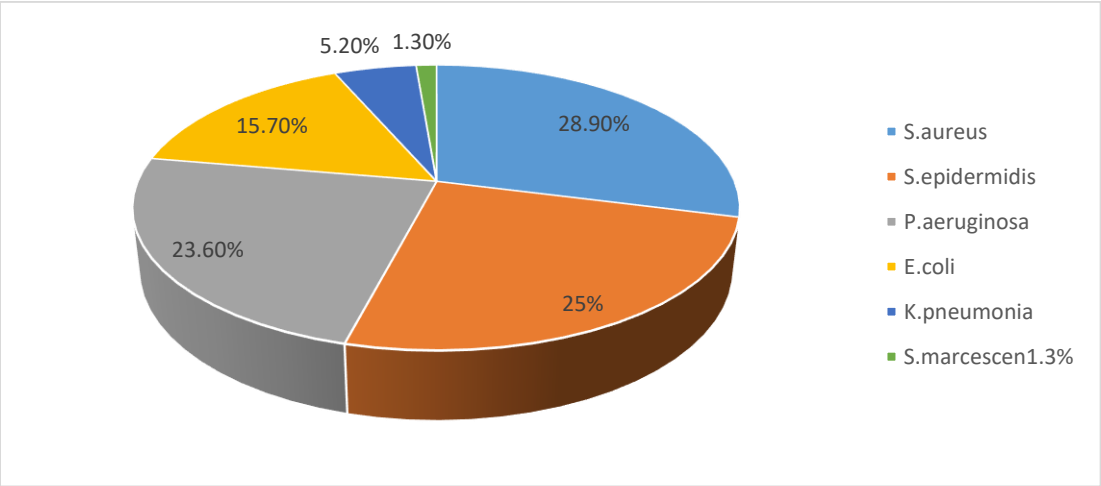
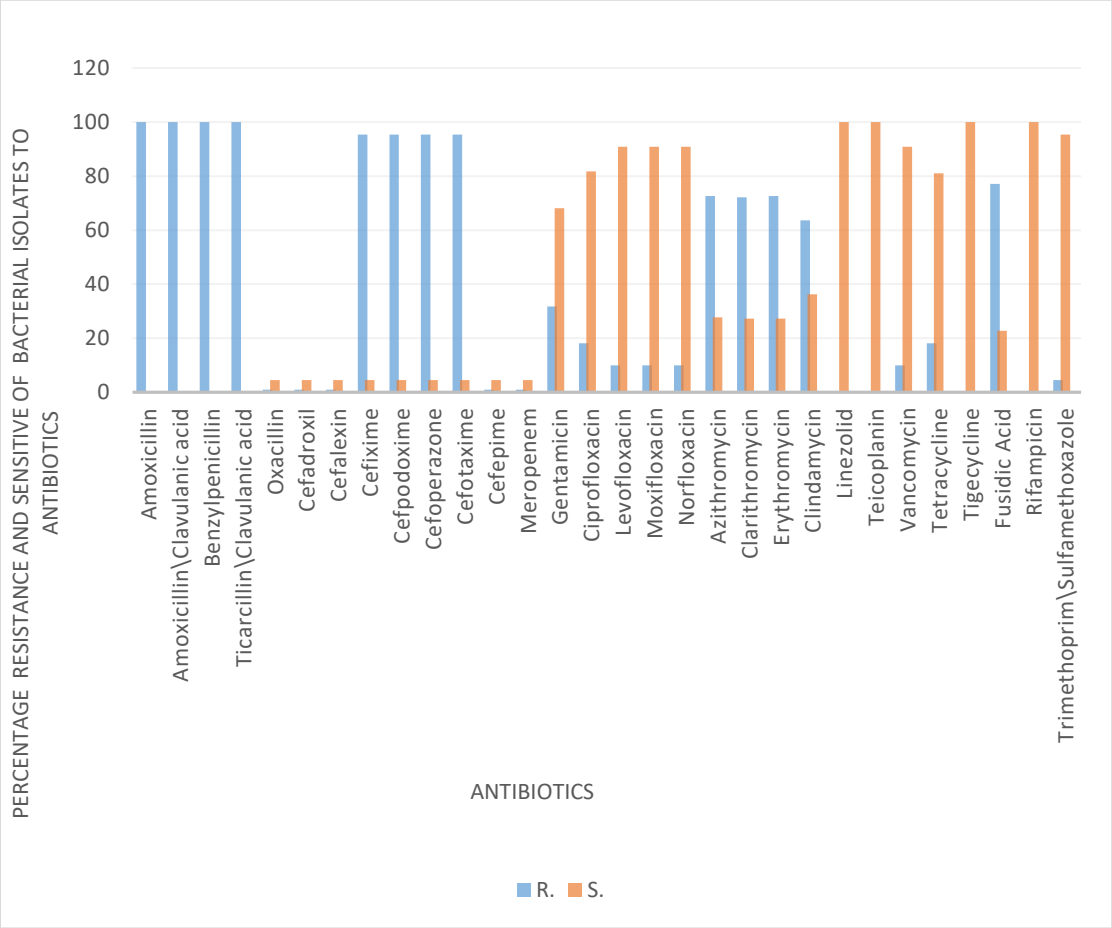


Figure 1: Bacterial species isolated from different eye infections

Staphylococcus aureus isolates showed high sensitivity 100% to Rifampicin, Linezolid, Teicoplanin, and Tigecycline, 95.4% sensitivity to Trimethoprim\Sulfamethoxazole, and 90.9% sensitivity to Levofloxacin, Moxifloxacin, Norfloxacin, and Vancomycin, with sensitivity 81.1%. % for Tetracycline and Ciprofloxacin antibiotics and 68.1% for Gentamicin.

On the other hand, they were varied in their resistance to the antibiotics under study, as the resistance rate reached 100% for each of Amoxicillin, Amoxicillin\Clavulanic acid, Benzylpenicillin, and Ticarcillin\Clavulanic acid, and by 95.4% for each of Oxacillin and Cephalosporins of the first, second, third, and fourth generation used in this study, with a percentage of 77.2 for Fusidic Acid, 72.7% for Azithromycin, Clarithromycin, and Erythromycin, and 63.6% for Clindamycin. As shown in figure (2)



R: resistance to antibiotics, S: sensitive to antibiotics

Figure 2: The sensitivity of *S. aureus* isolates isolated from eye infections towards a number of antibiotics.

Figure (3) shows the electrophoresis of the PCR products, through which it can be seen that the primer of the gene *mec A* was successful in amplifying this gene through the appearance of a product of size 310 bp When observing the product of the migration, it turns out that 21 bacterial isolates from *Staphylococcus aureus* are under study It contains the *mecA* gene 95.4%,

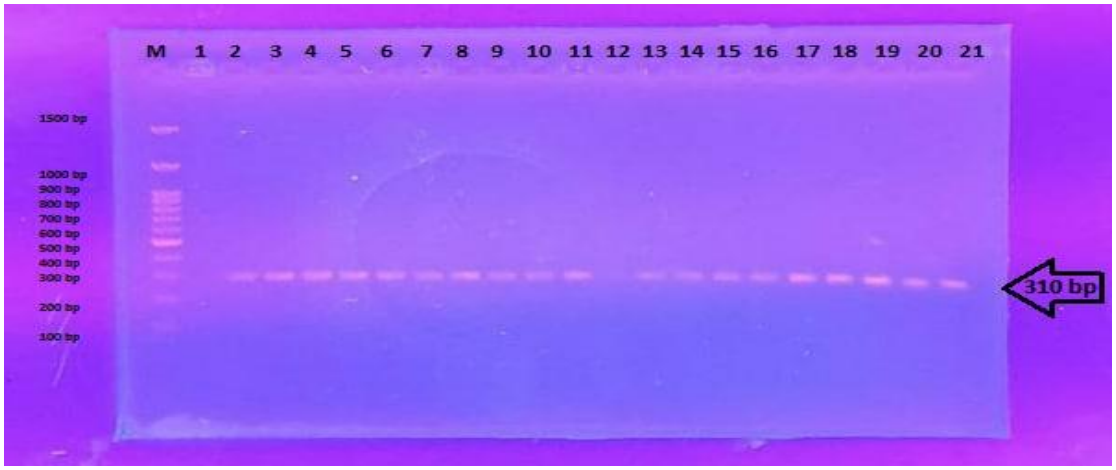


Figure (3): Electrophoresis of the product of the PCR reaction of *S. aureus* using a specific primer for the *mecA* gene (310bp), at a gel concentration of (1.5%), and a voltage of (70) volts for (50) minutes.

Figure (4) shows the electrophoresis of the PCR products, through which it was shown that the primer of the *ermA* gene was successful in amplifying this gene through the appearance of a 645bp PCR product. The *ermA* gene appeared in 16 bacterial isolates, with a percentage of 72.7%.

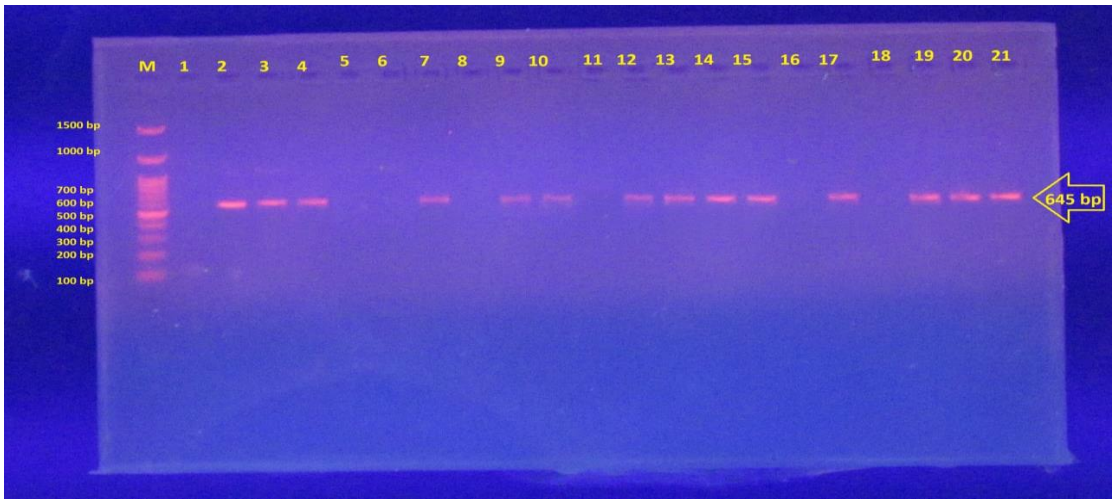


Figure (4): Electrophoresis of the product of the PCR reaction of *S. aureus* using a specific primer for the *ermA* gene (645 bp), at a gel concentration of (1.5%), and a voltage of (70) volts for (50) minutes.

4. DISSCUSION

The results of current study show ocular infection caused by bacteria Similar was dominated by [12] finding in a study to isolate bacterial causes of eye infections whom they attended to the hospital in Sana'a in Yemen, in which the percentage of isolation of this bacteria was 30.1%, and it represents the dominant bacteria. While it does not agree with Nigeria study in which Gram-negative bacteria were dominant in 68.2% [13], *S. aureus* is the main cause of eye infections among Gram-positive bacteria, and this is due to its natural presence on the skin and nose of most healthy individuals, as it is 15% of the population carry it in the front openings, and it can bear growth at

high temperatures of up to 40°C It may become pathological under certain conditions such as immunodeficiency or systemic diseases to which humans are exposed, and the emergence of bacterial strains with resistance to many antibiotics, which increases the rate of its spread [14]. it is clear that all isolates obtained from this study were sensitive to Linezolid, and this study agrees with what was mentioned by [15] as *S. aureus* isolates obtained from patients with conjunctival infection in a hospital in Egypt were 100% sensitive to the antigen under study, and the same sensitivity was obtained for *S. aureus* isolated from eyes in Iran [16]. It was also 100% resistant to Amoxicillin\Clavulanic acid, and this result is consistent with a previous study conducted in Italy, where the same percentage was obtained [17]. The ability of bacterial isolates to resist penicillin antibiotics is achieved by several mechanisms, including their ability to produce β-lactamase enzymes that hydrolyze the beta-lactam ring and change the penicillin-binding proteins present in the group of penicillins that inactivate the antibody [18]in addition to the mechanism of changing the target. With a decrease in affinity for the antibiotic, or a change in the antibiotic through the bacteria through the flow pumps [19], the *Staphylococcus aureus* isolates were resistant by 72.7% to each of the antibiotics Erythromycin, Azithromycin, and Clarithromycin. These antibiotics belonging to the group of macrolides inhibit protein synthesis. In the bacterial cell by binding to the 50S ribosomal subunit, this linking process prevents the activity of the enzyme Peptidyl transferase and interferes with the transfer of amino acids during the translation and assembly of proteins. The ribosomal nucleic acid (rRNA) around the binding site at the exit tunnel through which the nascent protein migrates until it exits the ribosome or through additional resistance mechanisms that are deletion or insertion mutations in the ribosomal protein that alter the shape of the protein-producing tunnel [20].PCR assay is ideal for determining methicillin resistance in isolates of *S. aureus* bacteria and for identifying multi-antibiotic resistant strains (MRSA) by encoding the PBP-2a protein, which is a penicillin-binding protein or an essential enzyme of the bacterial cell wall that stimulates the production of peptidoglycan in the bacterial cell wall. Therefore, PBP-2A continues to stimulate bacterial cell wall synthesis even in the presence of several antibiotics. As a result, *S. aureus* strains that make PBP-2A can grow in the presence of many antibiotics. MRSA strains tend to be resistant to methicillin, oxacillin and cephalosporins. This high percentage came close to many studies that confirmed the increase in the prevalence of MRSA strains, which are the main cause of eye infections, from 34% to 53% in different countries of the world, including Iran, the United States, India and Taiwan, which caused an increase in the rates of resistance to commonly used antibiotics [21,22,23]. While it does not correspond to a local study in Najaf, in which the presence of the *mecA* gene was only 45.45% in the bacterial isolates causing eye infection [24].

The presence of the *ermA* gene of *S. aureus* was also detected, it encodes for ribosomal DNA methylation (23s rRNA -methylation), which prevents the antibiotic from binding to the ribosomal target, thus producing a modification in the target site [25].Many studies have confirmed the presence of the *ermA* gene in bacterial isolates isolated from eye infections, but at different rates from one country to another, as it amounted to 67.9% in Egypt [26] and by 57.1% in a previous study in Iran [27]. The result of the current study was inconsistent with a Polish study in which the *ermA* gene was present in only 15.6% of *S. aureus* isolates isolated from hospitalized and non-hospitalized patients with eye infections [28].

5.CONCLUSIONS

Bacterial ocular infection one of an eye problem and if left without treated cause damage structure of eye in the current study that *Staphylococcus aureus* is the most common cause among Gram-positive bacteria of various eye infections in the Holy Karbala governorate. The result of the molecular study confirmed the sensitivity of *S. aureus* to a number of antibiotics. Through it, it possesses *mecA*, the most important and widespread antibiotic resistance gene, and the *ermA* gene, which makes it the main threat in eye infections through the presence of resistance genes at a high rate.

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