



Research Article

Detection of bacterial infection of the eye chalazion in Mosul City

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Abstract

Chalazion is a common infection of the meibomian gland in all ages' eyelids. The current study sought to isolate and identify bacteria linked with chalazion in patients hospitalized to Mosul hospitals between 10/1/2021 and 1/3/2022. Samples were obtained using swabs soaked in the transport media (amies), and bacteria were identified using traditional methods with API20E. Vitek-2 and molecular approaches based on the 16 S rRNA gene verified the species-level diagnosis. Using BLAST software, the nitrogen base sequences were determined and compared to those of the NCBI. The results showed that *Staphylococcus aureus* was isolated by 52.2% and 62.5% among women, notably in the age (21-40) years, and by 27.8% for females and 19.2% for the same age group in males, and upper eyelid infection predominated. The infection rate in people with oily skin was 50.9% in females and 34.6% in men, with the presence of the *agr A*, *agrC*, and *agrD* genes in isolates that exhibited a positive result in the Congo red medium and microtiter plate.

Keywords: Chalazion, Meibomian gland, *Staphylococci*, *Agr* genes

Introduction

One of the most prevalent disorders affecting the eyelids is a chalazion. It is a meibomian gland inflammation caused by a blockage in one or more of these glands, as seen in Fig 1. As sebum secretions build up inside the glands, they form a cyst that develops to become a chalazion. It is not painful; in some situations, it is little and noticeable; in others, it is huge enough to push on the eyeball and impair vision. Many factors, including them, contribute to the occurrence of chalazion: Hormonal changes, bacterial infection, especially staphylococcus, viral infection, exposure to pollutants, chronic blepharitis, eyelid trauma, immunodeficiency, and others (Alsammahi et al., 2018).

Microbial alteration on the surface of the eye plays a significant function and is a contributing cause to the occurrence of chalazion. Exposure to many environmental factors, such as diet, toxins, and drugs such as antibiotics and infections, can disrupt the microbial equilibrium, resulting in dysbiosis (Napolitano et al., 2021). When the chalazion is

large in the center of the eyelid, it can put pressure on the cornea, causing astigmatism and refractive error (Jin et al., 2017), as well as eyelid ptosis (Ho & Lai, 2002).

Frequent chalazion is occasionally followed by more serious disorders, particularly in the elderly, such as the development of malignant tumors in the sebaceous glands (Silkiss et al., 2021). During the Corona epidemic, the use of face masks increased with the increase in temperature and humidity, leading to an increase in acne. Because wearing a non-breathable face mask often drives breathing upwards to the area above the eyes, it has been theorized that wearing such masks may also produce a favorable microenvironment for eyelid inflammation and contribute to the development of chalazion (Singh et al., 2017).

Sensing the regulatory quorum of additional genes accessory gene regulator (*agr*)

Staphylococci have many genetic regulators that govern virulence factor production. The Accessory Gene Regulator

(Agr) is a significant virulence program that controls cell activity in response to bacterial density.

When the AIP interacts with the AgrC kinase receptor, the response regulator AgrA is activated and binds to P2 and P3, resulting in transcriptional activation of AgrBDCA and RNAIII. RNAIII promotes the production of several virulence factors, including poisons and enzymes that regulate pathogen development and adaptability at infection sites. Furthermore, AgrA activates promoters of genes encoding phenol-soluble modulins (PSMs) (Nakamura et al., 2020).

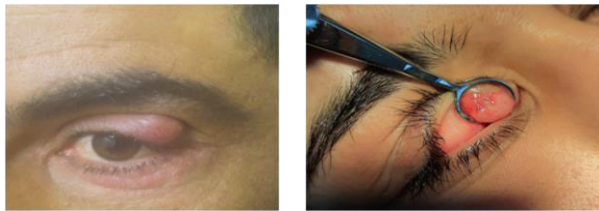


Fig. 1 Chalazion

Materials and Methods

From 10/1/2021 to 2/3/2022, 104 samples were obtained from patients who underwent minor surgery after being diagnosed by an ophthalmologist at Al Salam Teaching Hospital. Males and females aged less than 75 years were among the patients. A unique form was created for each patient that included information (age, gender, skin type, location of residence, recurrent chalazion) and samples were collected with a sterile cotton swab and placed in the center in transport media until they reach the laboratory for isolation and diagnosis.

Isolate bacteria

The samples were cultivated on blood agar, MacConky agar, and mannitol salt agar and incubated in aerobic conditions at 37°C for 48-24 hours. The structure and arrangement of cells, as well as the usage of certain diagnostic procedures (oxidase, catalase, coagulase).

Confirm the diagnosis for the type level

Vitek-2 compact (Biomereux - French) was used to diagnose positive bacteria in the AmrLab medical laboratories in Dohuk city. The negative bacteria were identified using the Analytical profile index) API2E.

Determination of 16 S rRNA primers

The genome of bacteria was isolated using the DNA Extraction kit supplied by Geneaid Company, according to the proven method, Table 1.

The PCR amplification products were forwarded to Korea's Micro laboratory. Using the Basic Local Alignment Search Tool (BLAST) program, the 16s rRNA sequences were determined and compared to the sequences in NCBI.

Table 1. Primers sequence and size of the 16S rRNA gene

Gene	Size	Primer
16S rRNA	1500bp	F 5- AGAGTTTGATCMTGGCTCAG-3 R 5-AAGGAGGTGATCCARCCGCA-3

Detection of agr A, agr C, agr D

The DNA purity and concentration were determined using NanoDrop 2000 spectrometer, as shown in Table 2 (Cafiso et al., 2007). The product was then determined using agarose gel electrophoresis at 0.8/40ml concentration. A UV trans-illuminator was used to show DNA bands.

Table 2. The PCR program used for the detection of genes

No	Stage	Temp. °C	Time	No. of Cycle
1	Initial denaturation	94	5min	1
2	Denaturation	93	1min	30
3	Annealing		1min	
	Agr A	60		
	Agr C	55		
	Agr D	56		
4	Final annealing	68	1min	
5	Extension	68	1min	
6	Final extension	68	1min	1

Determination forming biofilms

Composition of Congo red medium: Brain Heart infusion agar 52g/L, sucrose 36 g/L, Congo red dye 0.8g/L (Khudhur, 2013).

Detection of biofilm by microtiter plate method

The microtiter plate method was used to test bacteria's ability to create biofilms. Bacteria were inoculated with heart brain infusion broth and cultured for 4-6 hours. The bacterial suspension was then diluted and compared to the 0.5 McFarland tube at a 1:100 ratio from the medium. Then, 200µl was transferred to each pit in the plate. The sterile broth was added without any bacterial suspension and served as a negative control. The plate was incubated at 37°C for 48 hours. The pits were gently rinsed 2-3 times with PBS. The plate was dried by inverting it at room temperature. For 20 minutes, a 0.1% crystal violet dye was applied to the plate (Triveni, et al., 2018).

To assess the results, statistical analysis was performed using the Chi-Square test. The outcomes were examined at the probability level (P < 0.05).

Results and Discussion

The current study detected bacteria-positive isolates in females (69.11%) and males (30.88%), as shown in Table 3. This is consistent with Iqbal et al. results (Iqbal et al., 2022). He stated that 70% of females were infected with chalazion and 30% of males were affected. According to Shah et al., females are more prone to infection due to hormonal changes, puberty, and the use of cosmetics (Shah et al., 2020).

Table 3. Percentages of isolates in both sexes

Gender	Positive	Negative
Female	46	19
65(62.5%)	69.11	27.94
Male	21	18
39(37.5%)	30.88	26.47
Total	67	37
104(100%)	64.42	35.57

This study found that *S. aureus* had the largest percentage, 38.23% in females and 13.23% in males, Table 4. This finding is consistent with the findings of Otulana et al., who conducted that chalazion infection is typically associated with *S. aureus* (Lee et al., 2020). According to the findings of Alsammahi et al. and Balamurugan & Udayakumar, *S. aureus* aids in the formation of a chalazion (Alsammahi et al., 2018; Balamurugan and Udayakumar, 2019).

Non-fermenting bacteria of mannitol included *S. pidermidis* (31.3%), *S. haemlyticus* (7.24%), *S. saprophyticus* (4.47%), and *S. lentus* (1.49%). *Pseudomonas aregenosa* was isolated at a rate of 4.47%. According to Jackson, the most frequent eye disorders caused by bacteria affect the skin, eyelashes, and meibomian glands (Jackson, 2008). Because of changes in the secretions of the meibomian glands in persons with blepharitis, the amount of polar fat circulating with the lacrimal layer decreases, and aqueous component evaporation increases.

The concentration of fat increases with duct blockage and enlargement of the meibomian glands, increasing dryness of the eye. The most prevalent bacteria isolated in individuals with chronic blepharitis are *S. pidermidis* and *S. aureus*, as well as Corynebacteria, which can move from the edge of the eyelids to the inside of the meibomian glands. In a study, Hou et al. discovered that many patients have bacterial infections (*S. epidermidis*, *S. aureus*, and *P. aergenosa*) (Hou et al., 2012). Because of these bacteria's capacity to build biofilms, the majority of infections occur from contact lenses or lens implants worn by people suffering from cataract illness.

Eltwisy et al. and Panda & Singh agree with the current study's findings, stating that *S. hemolytic* is the second most common species causing eye infections (Eltwisy et al., 2022; Panda and Singh, 2018). It was also isolated with *S.*

epidermidis due to its potential to build biofilms. In one investigation, Wang et al. discovered that *S. lentus* was isolated from dry eye and meibomian gland illness at a rate of 12.2%. In people with dry eyes, normal eye bacteria may contribute to disease progression (Wang et al., 2022). Priya et al. confirmed in a study that *S. saprophyticus* was identified from a 6% eye condition (Priya et al., 2014).

Table 4. The proportions of bacteria isolated from both sexes

Isolate	Female	Male	Total
	No. (%)		
<i>Staph. aureus</i>	(38)26	9(13)	35(51)
<i>Staph. epidermidis</i>	11(16)	10(14)	21(30)
<i>Staph. haemolyticus</i>	4(5)	1(1.4)	5(7)
<i>Staph. saprophyticus</i>	3(4)	Zero	3(4)
<i>Staph. lentus</i>	Zero	1(1.4)	1(1.4)
<i>Pseudomonas aregenosa</i>	3(4)	Zero	3(4)



Fig. 2 Biochemical assays for Gr- bacteria and positive for the oxidase test of pseudomonas aerugenosa using the API system

Fig. 2 depicts the results of biochemical tests using the API system for Gram-negative and positive bacterial species to confirm the diagnosis at the species level.

Vitek-2 was used to validate the identification of Gr+ bacteria at the species level, and the results and percentages of purity are reported in Table 5.

Table 5. Results of positive isolates by Vitek-2 and their probability

Bacteria	Probability%
<i>Staphylococcus aureus</i>	97%
<i>Staphylococcus haemlyticus</i>	%94
<i>Staphylococcus epidermidis</i>	%94
<i>Staphylococcus saprophyticus</i>	%99
<i>Staphylococcus lentus</i>	%87

The results of 16SrRNA matching with API20E Vitek-2 are provided in Appendix.

Detecting the slime layer using the red Congo's center

The results revealed a total of 31 isolates with a success rate of 45.58%, comprised of black to reddish black colonies. This demonstrates the cells' ability to produce a slime layer. *S. epidermidis*, *S. hemolyticus*, *S. aureus*, *S. saprophyticus*, and *P. aeruginosa* were among the isolates. The negative isolates 37 rate of 54.41% produced red colonies. These findings were similar to those of Triveni, *et al.* who said that the percentage of isolates developing strong and moderate biofilms was 7.97% and 21.27%, respectively, with 17.19% displaying colonies on Congo red isolated from pus. There were 133 non-productive isolates with a proportion of 70.74% (Triveni *et al.*, 2018). The study's findings contradicted what was stated by Haddad *et al.*, who stated that the percentage of biofilm-forming staphylococci was 69.8% (Haddad *et al.*, 2018).

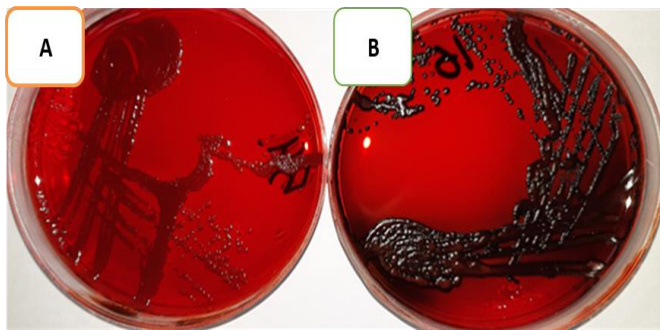


Fig. 3 Growth in the center of Congo red, A: Negative, B: Positive

Detection of biofilm formation using the microtiter plate method

In comparison to the control, the percentage of isolates producing biofilm in the plate was 9.67%, while 58.22% developed a moderate membrane and 65.62% created a weak biofilm. This finding is virtually identical to Chen's, who stated that the percentage of bacteria *S. aureus* creating a strong biofilm was 3.09, moderate at 14.34%, and created a weak membrane was 45.64% (Chen *et al.*, 2020).

Detection of agr genes

The results revealed the presence of *agrA*, *agrC*, and *agrD* genes in all isolate that showed a positive ability to form biofilms in the Congo red media and the microtiter plate, Fig 4. This is consistent with Tan *et al.* and Dufour *et al.*, who demonstrated the existence of these genes in *S. aureus* and *S. epidermidis* (Tan *et al.*, 2022; Dufour *et al.*, 2002).

According to Le and Otto, the most investigated quorum sensing system in staphylococci is *agr*. It plays an important role in pathogenesis and resistance and is made up of two

distinct transcripts, RNAII and RNAIII, produced by the P2 and P3 promoters, respectively. RNAII is made up of four genes: *agrA*, *agrB*, *agrC*, and *agrD*. Because the concentration of AIP outside the cell is low in the presence of a single bacterium, RNAIII is inactive. When the extracellular AIP concentration rises, it is delivered to the receptors on the histidine kinase *agrC*, which acts on the phosphorylation of *agrA*. RNAII stimulates the transcription of *agrB*, which serves as an extracellular AIP transporter protein (Le and Otto, 2015).

RNAII stimulates the transcription of *agrB*, which operates as extracellular AIP transporter proteins. The AIP-inducible receptor and the autoinducing peptide are encoded by *agrC* and *agrD*, respectively (AIP). Simultaneously. It activates *agrA*, which binds to the repressor P3 to start cloning and expressing a 514-nucleotide transcript representing virulence factors. Da *et al.* identified phenol-soluble modulins PSM in *S. haemolyticus*, which is the obligatory regulator of the *agr* gene found in *S. aureus* and *S. epidermidis*, therefore its existence in *S. haemolyticus* was taken into account (Da *et al.*, 2017).

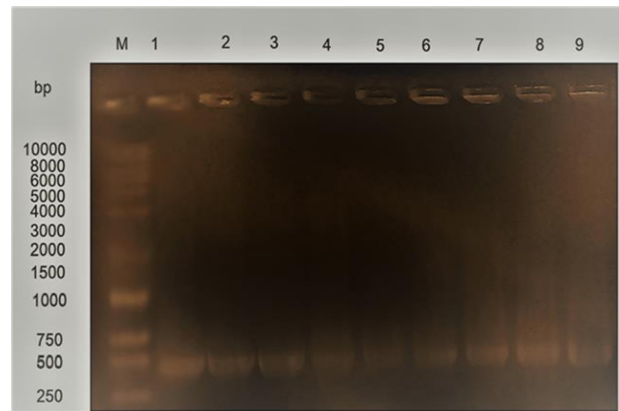


Fig. 4 The migration of the *agrA* for the bacteria under study, Lane 1: *P. aeruginosa*, lane 2: *S. saprophyticus*, lane 3: *S. haemolyticus*, lane 4: *S. saprophyticus*, lane 5: *S. lentus*, lane 6: *S. epidermidis*, lane 7: *S. aureus*, lane 8: *S. epidermidis*, lane 9: *S. aureus*

The effect of gender, age, skin type, and some other variables on the patients under study

The relationship between genders and ages

The study found an increase in female infection 62.5%, but a decrease in male infection by 37.5%, with participants ranging in age from 1 to 75 years. The largest infection rate was in the age group (21-40) years, with females infected at 27.8% and males infected at 19.23%. According to the Li *et al.* study, the percentage of females who received treatment for chalazion was 65% and those who underwent surgery was 60%, while the percentage of males who received treatment

was 35% and those who underwent procedures to remove the chalazion was 40% (Li et al., 2020).

In a study, Otulana et al. discovered that the male-to-female infection ratio is 1:1.1. According to him, a chalazion is one of the most common eyelid swellings encountered by ophthalmologists, and it is more common in teens and young adults, as indicated in Table 6 (Otulana et al., 2008).

Table 6. The relationship between sex and age of the patients under study

Gender	1-20 y	21-40 y	41-60 y	61-80 y	Total
Female No(%)	28(26)	29(27)	7(6)	1(0.9)	65(62.5)
Male No(%)	10(9)	20(19)	8(7)	1(0.9)	39(37.5)

Relationship between genders and skin type of the patients under study

The study found that oily skin has a role in chalazion infection, with females with oily skin having a higher frequency of infection 50.9% than males 34.6%, Table 7. This is congruent with the findings of Alsoudi et al., who discovered that females were impacted at a higher rate than males 58% (Alsoudi et al., 2022). According to Petricek et al., sex hormones, particularly androgens, are vital in controlling the lacrimal, meibomian, and sebaceous glands, the imbalance of which is related to dry eye illness. Several hormones are also involved in the release of sebum from the meibomian glands (Petriček et al., 2021). Androgen receptors are known to increase sebaceous gland secretion and viscosity, as well as to encourage the growth and balance of all sebaceous glands in the body. Because the synthesis of lipids enhances the viscosity of the meibomian generated, chalazion is more common in teens and pregnant women (Wagle, 2021).

Table 7. Relationship between sex and skin type of the patients under study

Gender	Oily	Dry	Total
	No (%)		
Female	53 (50)	12 (11)	65 (62.5)
Male	36 (34)	3 (2.8)	39 (37.5)

The relationship between gender and housing of the patients under study

The current study found that females inside the city had the highest percentage of infection (33.6%), while males' rate

were (25.9%). Females outside the city had a percentage of (28.84%) and males had a percentage of (11.43%), Table (8). These findings are consistent with Das and Dave's findings that (44%) of respondents were from rural areas and (55%) were from Urban areas. They also stated that the chalazion is more prevalent among the middle and upper social and economic groups than in the lower class (85%). The findings differed from those reported by Singh et al. in the western Indian state of Uttar Pradesh, where the incidence of chalazion was (0.37%) and (0.16%) in rural and city schools, respectively. 13 patients suffered from chalazion of all patients with eye problems in the school included in the study. In the Taiwan region, Li et al. discovered a link between air pollution and outpatient visits for nonspecific conjunctivitis (Li et al., 2020). According to Alsammahi et al., exposure to polluted air might cause foreign particle obstruction of the meibomian glands and the formation of chalazion. It can impact people of all ages and genders. It is especially common when there is a lack of proper eye hygiene (Alsammahi et al., 2018).

Table 8. The relationship between sex and housing of the patients under study

Gender	Inside City	Outside city	Total
	No (%)		
Female	35(33)	30(28)	65(62.5)
Male	27(26)	12(11)	39(37.5)

The relationship between genders and the type of affected eyelid

The current study found that the upper eyelids were more impacted than the lower eyelids, with the upper right eyelid afflicted in females (18.26%) and males 8.65% (a total of 26.9% in both sexes). Table 9 shows the percentage of females with upper left eyelids (18.26%) and males with 11.53% (a total of 29.8% for both sexes). This is consistent with Mansour et al. mention that upper eyelid infections are more common than lower eyelid infections, with (20) (27) samples from the upper eyelid (Mansour et al., 2006). It also agrees with Alsoudi et al. study, which found a 53.2% incidence of infection in the upper eyelids (Alsoudi et al., 2022). The reason for this is that the upper eyelid has more sebaceous glands than the lower eyelid, with 25-40 and 20-30, respectively. Upper eyelid glands are denser, thinner, and longer than lower eyelid glands, making them more vulnerable to injury (Zheng et al., 2022).

The relationship between sex and recurrence of infection for the injured under study

According to the study, females (38.4%) and males (26.9%) experienced their first chalazion.

Recurrent infections occurred at a rate of (24.03%). Three of them were female children. 4 out of 11 males (10.56%) were children, [Table 10](#). According to Roheen et al., the rate of infection recurrence in females was (10.9%) and in males was (8.4%) of the total 148 cases ([Roheen et al., 2022](#)). According to Zheng et al., children are more prone to have multiple chalazion ([Zheng et al., 2022](#)). Patients with

rosacea, seborrheic dermatitis, and blepharitis, according to Gilchrist and Lee, are more recurrent and have chalazion ([Gilchrist and Lee, 2009](#)). Treatment of hypothyroidism, as Bipat and Toelsie observed in a study of chalazion recurrence related to hypothyroidism, may minimize the recurrence of a chalazion ([Bipat et al., 2020](#)).

Table 9. Relationship between sex and the affected eyelid of the patients used in the study

Gender	upper right eyelid	lower right eyelid	upper left eyelid	Lower left eyelid	Total
	No (%)				
Female	19 (18)	15 (14)	19 (18)	12 (11)	65 (62.5)
Male	9(8)	10 (9)	12 (11)	8 (7)	39 (37.5)

Table 10. Relationship between sex and recurrence of infection for the injured under study

Gender	First time infection No. (%)	Recurrent infection No (%)		Total
		2times	More than 2	
Female	40 (38)	12 (11)	13 (12)	65 (62.5)
Male	28 (26)	6 (5)	5 (4)	39 (37.5)

Conclusions

According to the study, females have the highest infection than males, and those with oily skin had the highest infection rate. Upper eyelids were damaged more than lower eyelids. Children and females had a higher rate of recurrence and multiplicity of chalazion. Residents within the city had the highest infection rates. *S. aureus* had the highest rate of isolation, followed by *S. epidermidis*, *S. heamolyticus*, *S. saprophyticus*, *P. aerugenosa*, and *S. lentus*. The presence of the genes *agrA*, *agrC*, and *agrD* in the isolates exhibited a positive result in the Congo red media and Microtiter plate.

Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication.

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Ethical considerations

The study was approved by the institutional ethical committee

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