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Isolation and identification of bacteria causing conjunctivitis

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Abstract

The human eye is exposed to multiple types of microorganisms, and eye infections have serious consequences if neglected, as they can lead to blindness. Therefore, the current study was conducted to mitigate the most important bacterial microorganisms causing Bacterial conjunctivitis in the city of Samarra, where 150 samples were collected for this purpose, (130 of patients with eye inflammation after they were clinically diagnosed by a specialist doctor and 20 control), their ages ranged between 1-80 years, of both genders, from April 2022 to January 2023. The results of bacterial culture showed the presence of bacterial growth All samples belonged to Gram-positive it was *Staphylococcus aureus* bacteria were dominant, with a percentage of 28.57%, followed by *Staphylococcus lentus* bacteria, where their percentage was 11.42%, Then came the rest of the bacterial isolates, Control samples also gave 15 samples positive growth, and all samples belonged to Coagulase-negative staphylococci, as they were 5. Isolates of *Staphylococcus epidermidis*, 3 isolates of *Staphylococcus haemolyticus*, and two isolates of *Staphylococcus hominis*, *Staphylococcus lenutus*, and *Staphylococcus warneri*. In this study, it was found that there were clear differences in infection between age groups, as the age group of 40-59 years had the highest infection rate, and their infection rate reached 33.33%. Gender and place of residence also had a relationship in the distribution of bacterial infections, as the infection rate in males, was higher than in females, reaching 61%. Likewise, those living in the city had the highest share of infection with eye infections, with the infection rate reaching 76.19%.

Introduction

The eye is the primary organ responsible for the human sense of sight. The eye is the mirror through which one sees the outside world and can distinguish between objects and shapes and perceive what surrounds it, It is also one of the delicate organs in the body, as the eye consists of

very precise parts that work together in a manner It is integrated so that the process of seeing can be done clearly, and it cooperates with the nerves and the brain to get the complete picture [1].

Any pathogenic organism capable of reaching the eye can cause eye inflammation and may affect one or both of them. Cases of infection range from conjunctivitis, which involves inflammation being limited to the outer layer of the eye and the inner surface of the eyelid, to more serious cases that affect visual acuity, represented by. Keratoconjunctivitis and endophthalmitis, which may cause blindness. Eye inflammation is often the result of a bacterial, sometimes viral, or fungal infection or hypersensitivity to natural chemicals. The severity of the infection depends on age, gender, and the individual's immunity in particular [2].

Gram-positive bacteria are among the main factors causing eye inflammation, and the most prominent genera are *Staphylococcus spp*. The most common species causing eye inflammation is *Staphylococcus aureus*, as these bacteria cause lysis of red blood cells because they produce the lytic toxin Hemolysin in addition to their ability to form biofilms avoided by the immune system and these characteristics increase their pathogenicity [3].

Other species belonging to the genus of *Staphylococcus spp* that cause eye infections (Conjunctivitis), *S.lentus*, *S.warneri*, *S.epidermidis*, *S.haemolytics*, *S.hominis*, *S.capaitis*, *S.simulant*, also appeared In addition, other species of several genera appeared from the samples taken from Patients with bacterial Conjunctivitis were as follows: *Kocuria spp*, *Streptococcus spp*, *Enterococcus faecium* [5].

Some of these types are opportunistic bacteria, and others are part of the microbiota found on the skin or all the body, but when any disturbance occurs to the surface of the eye due to the environment, the host's immunity and advanced age, bad habits and lack of personal hygiene, or a wound occurs in the eye tissue, it affects On the normal flora of the eye and infection occurs [6][7]. Recent studies have shown the possibility of microbes being transferred during the breathing process or from the lower parts, including the skin, to the surface of the eye through air flow, leading to eye inflammation [7].

Given the importance of the eye in human life the danger of conjunctivitis to the eye and the danger of microorganisms that cause it, which may lead to blindness, and the lack of studies related to this topic in the city of Samarra, therefore this research aimed to study the bacterial species that cause bacterial conjunctivitis and compare them with bacteria that were isolated from healthy people, Or those who do not show symptoms of the disease.

Materials and methods

`Method for isolation and collection

Samples were collected from patients with eye infections who arrived at Samarra General Hospital and specialized clinics and from the site of the eye infection after they were clinically diagnosed by the specialist doctor as suffering from bacterial conjunctivitis, as there were cases of dacryocystitis, but the specialist doctor refused to take samples from These patients, as there

were two samples of acute keratitis, but we did not include them in the results because they were few and so as not to affect the results statistically, Therefore, 130 samples of bacterial conjunctivitis were collected. Their ages ranged from 1-80 years and for both sexes, Samples were taken from the infected eye using a sterile cotton swab after disinfecting the area around the eye with70% ethyl alcohol, and under the supervision of a specialist doctor, and collected 20 samples from the eyes of healthy people as control, It was placed in carrier stearate medium and transferred to the microbiology laboratory at Samarra University.

Cultural Sample

It was then cultivated in the laboratory on Nutrient broth and incubated for several hours and then it is grown on other media such as: Nutrient agar medium, MacConkey medium, Mannitol and salt medium, and Blood agar medium, and incubated aerobically for 24 hours. The bacteria were then isolated, purified, and stained with the Gram stain. The samples were diagnosed by observing the cultural characteristics of the growing colonies in terms of the size of the colony, its height, the shape of its edge, and its ability to stain with the Gram stain, whether positive or negative. The cell shapes and arrangement were noted, and then catalase, oxidase, and clotting enzyme tests were performed on them. and the rest of the biochemical tests [8][9] After obtaining the bacterial species, the diagnosis was confirmed by sending them to the Vitek 2 device after activating the bacteria and cultivating them on nutrient agar medium or Mueller-Hinton medium and incubating them for 24 hours, and then sending them to the Vitek device, which conducted 64 analyzes on each type.

Isolation of bacteria

The 150 samples were collected, 20 samples from healthy people and 130 samples from patients visiting Samarra General Hospital and consulting clinics in the city, where 105 samples showed positive bacterial growth, which represents 80.78% of the number of samples, while the number of negative samples was 25 samples out of the total, i.e. 19.23%. The reason for not obtaining bacterial growth from patients with bacteria conjunctivitis may be that the infection may be either viral, fungal, or anaerobic bacteria, and this percentage was identical to what was reported in a study conducted in the United States of America, Which was done by [10] The percentage of positive growth samples was 82%, and it was higher than what was reported in a study conducted by Aladdin in Karbala in the year 2023, where the positive growth reached 45% out of a total of 169 swabs. As for the healthy samples, 15 samples gave positive growth and 5 samples were negative growth, the reason may be due to the presence of *Cornebacterium mastidius* bacteria, which is a microbiota of the eye and is found in various locations of the eye, which when present prevents or reduces the presence of other bacterial species such as *Staphylococcus spp*., *Streptococcus spp*., *Pseudomonas aeruginosa* [11].

Distribution of bacterial eye infections according to age

The results of the current study, in Table 1, showed that the highest percentage of bacterial isolation was in the age groups 40-59 years, where it constituted 33.33% of the isolates, followed by the infection rate in the age group 19-39 years, amounting to 25 samples (23.81%), and then after that a percentage The incidence of infection in the age group between 1-5 years, which

amounted to 18 isolates, or a rate of 17.14%, followed by the age group 60-80, where the infection rate reached 15.23, and the lowest percentages were in the age group 6-18, where the infection rate reached 10.47%. It is believed that the high rate of infection in the third and fourth categories is due to their exposure to external pollutants due to their involvement in the labor market, frequent exposure to dust, wearing contact lenses, and lack of attention to eye hygiene, as direct contact with the eyes with contaminated fingers causes the transfer of pollution to the eye. This study was consistent with a study reported by [12], who confirmed that the average age for bacterial eye infections was 47 years, and this study was also similar to a local study conducted by [13], through which they showed that eye infections occur largely in the age groups between 20-49 years, As for the first age group, eye infections occurred relatively frequently, which is believed to be due to excessive playing and frequent exposure to pollutants, with the immune system not developing sufficiently, which leads to conjunctivitis.

As for the age group between 6-18, the rates of infection with eye infections were lowest, and it was inconsistent with the study conducted by Muluye *et al*, which they conducted in [14] on patients with eye infections, who concluded that the average age of infected patients was 8.5 years.

Table 1: Bacterial infection according the age.

Age	**Infected**	**%**
5-1	18	17.14
18-6	11	10.47
39-19	25	23.81
59-40	35	33.33
80-60	16	15.23
Total	105	

Distribution of bacterial conjunctivitis according to gender

The current study showed that the prevalence of conjunctive in males is higher than in females, in Table 2, as the number of infected males reached 64, or 61%, while the number of females reached 41 (39%). This result was similar to what was stated in a study conducted in Basra by Mahdi *et al*., [15], which confirmed that bacterial eye infections are more common in males, amounting to 60.1%. and it also agreed with what was obtained by DheyAuldeen [16], where the percentage of infected males was 60.53%, and the reason for the high infection rate in males is due to the type of work they do and their frequent presence outside with sources of pollution in the environment, in addition to the presence of hereditary factors, and immunological factors, represented by primary immunodeficiency cases, which make infected males more susceptible to bacterial infections than others, in addition to hormonal factors represented by the level of steroid hormones, as their increase, especially the androgen hormone, makes males more frequently and strongly affected by bacterial infections [17].

Table 2: Bacterial infection according the gender.

Groups	Number of infected	%
Males	64	٪61
Females	41	٪39
Total	105	٪100

Distribution of conjunctivitis according to place of residence

The results showed that there is a clear difference in the distribution of patients according to their place of residence, whether in the city or the countryside, as the number of patients residing in the city was 80, i.e. 76.19%, while the number of infected people in rural areas was 25, i.e. 23.81%, as shown in Table 3, This study was consistent with a local study conducted in Kirkuk by [18], which confirmed that the infection rate in the city is higher than in the countryside. The reason behind this could be attributed to various factors such as urban pollution, dense population density, poor personal hygiene practices, sleep deprivation, and excessive use of electronic gadgets, which can cause eye fatigue and irritation, ultimately leading to eye infections, Furthermore, inadequate nutrition impairs immunity, making it harder for the body to naturally combat bacterial infections; however, this is not the case with the research done in Babylon by Kareem *et al* in [13], which discovered that the rate of bacterial infection in the countryside is higher than in the city.

Table 3: Number of bacterial infection according to place of residence.

Groups	Number of infected	%
City	80	٪76.19
Countryside	25	٪23.81
Total	105	٪100

Identification

The samples were diagnosed based on what was stated in Diagnostic Microbiology (2007) regarding the phenotypic characteristics of the bacterial colonies growing on culture media, specifically the differentiation media, as well as staining with the Gram stain, and various diagnostic tests, where all bacterial isolates were positive for the Gram stain. This percentage was contrary to what was reported in some studies that dealt with this subject, in that negative bacterial isolates were not obtained. The increase in the percentage of Gram-positive bacteria may be because some of them form part of microbiota of the mucous membranes and skin [18], as well as the chemical composition of the wall of Gram-positive bacteria, which consists of a greater percentage of peptidoglycan, which may protect them from the antibiotics as much as possible and help them invade and necrotize ocular tissues, thereby inhibiting the phagocytosis process [19].

Some studies supported the result of the high percentage of Gram-positive bacteria causing eye infections, including what was stated in the study of Bourcier *et al* [20], where the percentage of Gram-positive isolates was 83.3% of the total number of isolated bacteria.

The results showed that most of the bacterial isolates belong to the genus *Staphylococcus*, which are staphylococci whose cells are clustered in the form of grape clusters. This result was identical to what was stated by Miller and Iovieno [21], and most of the isolates belonging to this genus were bacteria that were negative for the coagulation enzyme Coagulase_Negative Staphylococci (CoNS). The number of isolates of this type reached 67 isolates from the eyes of patients and healthy people, meaning their percentage of growth-positive isolates was 55.8%. The number of isolates of coagulation enzyme-negative bacteria in the eyes of patients was 53 isolates out of the total growth-positive isolates, meaning a percentage of 50.47%. As for the samples of healthy people, they were the vast majority belong to coagulase-negative staphylococci. This percentage was close to what was reported by Khudair and Saleh [22] (2013) and Lin [23].

The percentage of Coagulase_Negative Staphylococci bacteria out of the total samples was 43% and 45.2%, respectively, and agrees with what was stated by both [24] and [25], as it was the largest growth percentage in the isolates taken from the eyes of healthy people, coagulase-negative staphylococci were present.

Diagnostic results for patient samples also showed that the most common type of coagulase-negative bacteria is *Staphylococcus lentus*, with an infection rate of 11.42% (12 isolates), followed by *Staphylococcus warneri* and *Staphylococcus epidermidis*, with an infection rate of 7.61% for each of them, meaning 8 isolates. 6 isolates were obtained. Isolates of S. *haemolytics* (5.71), and 5 isolates of each of *S*. *hominis* and *S*. *capitis* (4.76%) were obtained.

As for bacteria positive for the coagulation enzyme, 30 samples of *Staphylococcus aureus* bacteria were obtained, i.e. 28.57% of the total positive samples, i.e. the highest of all genera. The results of the study were consistent with what was reported in a study held in Erbil Agha in (2020), which showed that *staphylococcus aureus* bacteria is one of the most common causes of eye inflammation. Our study did not agree with a study conducted in the United States of America, which stated that the most common species causing conjunctivitis is *Hemophilus influenzae* bacteria [26].

The high presence of *Staphylococcus aureus* bacteria in eye inflammation is due to its presence within the natural microbiota in the nose, and because of the connection between the eye and the nose through the nasolacrimal duct, the bacteria may be transmitted from the nose to the eye, causing conjunctivitis [27]. In addition, Therefore, these bacteria can produce toxins, proteins, and enzymes that can cause damage to tissues.

It also can change the permeability of the host membranes through its toxin production, which gives it the ability to resist immune cells and makes it able to bypass epithelial barriers, thus enhancing the growth and spread of bacteria [28].In healthy people who carry these bacteria in normal flora in the frontal openings, about 15%. If a person is exposed to systemic diseases or

immunodeficiency, these bacteria may become pathogenic, and bacterial strains with resistance to many antibiotics will appear, which increases the rate of infection [27].

Also, 3 samples of *Staphylococcus pseudintermedius* bacteria were obtained, i.e. 2.85%, which were classified as coagulation enzyme-positive bacteria. Table 4 and table 5 shows the number of infections.

Table 4: The number of bacterial infections belonging to the genus *Staphylococcus* and their percentages out of the total number of isolates.

Genus of Staphylococcus isolates	Number of isolated samples	%
S. aureus	30	28.57
S. lentus	12	11.42
S. warneri	8	7.61
S. epidermidis	8	7.61
S. haemolytics	6	5.71
S. hominis	5	4.76
S. capitis	5	4.76
S. pseudintermedius	3	2.85
S.simulant	3	2.85
S.saprophyticus	3	2.85
Total	83	٪79.04

Table 5: Number of isolates from patients for the different species in this study and their percentages.

Genus of bacteria	Number of isolated samples	%
Kocuria kristinae	8	7.61
Kocuria varians	5	4.76
Streptococcus agalactiae	3	2.85
Dermococcus nishinomiynesis	2	1.90
Kytococcus sedentarius	2	1.90
Enterococcus faecium	2	1.90
Total	22	٪20.92

The isolation results for the control group, which numbered 20 people, also showed that all the isolates belonged to coagulase-negative staphylococci, as 5 isolates were for *S. epidermidis* bacteria, 3 isolates were for *S.haemolyticus* bacteria, 2 isolates were for each of *S.hominis*, *S.lenutus*, and *S. warneri*, so that the isolates were 15 isolates, and this result was consistent with the study conducted by Grzybowski *et al* [9], who confirmed that the largest growth rate in the isolates taken from the eyes of healthy people was for coagulase-negative staphylococcus bacteria, as for the bacterial isolates taken from the control group that No bacterial growth

appeared, which may be due to blinking, which automatically washes the eye, including bacteria. Tears also contain antimicrobial compounds such as lysozyme, lactoferrin, and immunoglobulin [29].

The above isolates were diagnosed according to the traditional diagnostic methods mentioned in the book Prescott's Microbiology [30], based on the phenotypic characteristics of the various culture media, and were stained with Gram stain. The shapes of the bacterial colonies were identified and biochemical tests were performed as shown in Table 4-6, after which the final diagnosis was made. For bacterial isolates using the Vitech device, where (GP-GN) cards containing 64 specialized biochemical tests were used.

The probability of diagnosis reached 99%, and the diagnostic results in this system confirmed the results we obtained from the biochemical tests. Table 6 shows the biochemical tests for the isolated bacteria under study.

Table 6: Biochemical tests for the isolated bacteria under study.

Novobiocin	Urease	Hemolysine	Coagulase	Oxidase	Catalase	Bacteria
S	+	+	+	-	+	*S.aureus*
S	-	-	-	-	+	*S. lentus*
S	+	V	-	-	+	*S. warneri*
S	+	-	-	-	+	*S.epidermidis*
S	-	+	-	-	+	*S.haemolytics*
R	-	-	-	-	+	*S.hominis*
S	-	-	-	-	+	*S. capitis*
S	+	+	+	-	+	*S.pseudintermedius*
S	-	V	-	-	+	*S.simulant*
R	+	-	-	-	+	*S.saprophyticus*
S	+	-	-	-	+	*K.kristinae*
S	+	-	+	-	+	*K.varians*
R	-	+	+	+	-	*Streptococcus agalactiae*
S	-	V	+	+	+	*Dermococcus nishinomiynesis*
S	-	+	+	+	+	*Kytococcus sedentarius*

R:resistant, V:variable, S:sensitive, +:positive, -:negative

Conclusions

The most common species causing bacterial conjunctivitis are Gram-positive bacteria, specifically bacteria belonging to the genus *Staphylococcus*, with the presence of other genera and other species.

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عزل وتشخيص البكتيريا المسببة لألتهاب الملتحمة البكتيري

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البحث مستل من أطروحة دكتوراه الباحث الأول

معلومات البحث:
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البكتيريا الإيجابية
المكورات العنقودية سلبية التخثر
معلومات المؤلف

الخلاصة:
تتعرض عين الانسان الى أنواع متعددة من الكائنات الحية المجهرية وان لعدوى العين عواقب وخيمة اذا تم اهمالها، حيث يمكن ان تؤدي الى العمى لذا تم اجراء الدراسة الحالية بهدف التحري عن اهم الأحياء المجهرية البكتيرية المسببة لالتهاب الملتحمة البكتيري Conjunctive في مدينة سامراء، إذ جمعت لهذا الغرض 150 عينة (120من المرضى المصابين بالتهاب الملتحمة البكتيري بعد ان شخصوا سريرياً من قبل الطبيب المختص و 20 عينة أصحاء) وتراوحت أعمارهم بين 1-80 سنة ولكلا الجنسيين، وللمدة من نيسان 2022 ولغاية كانون الثاني 2023 .وقد أظهرت النتائج الخاصة بزرع البكتيريا ان جميع العينات موجبة لصبغة كَرام، وكانت بكتريا *Staphylococcus aureus* هي السائدة حيث بلغت نسبة وجودها 28.57%، تلتها بكتيريا *Staphylococcus lentus* حيث كانت نسبتها 11.42%، وجاءت بعدها باقي العزلات البكتيرية، كما أعطت عينات السيطرة 15 عينة نموا موجباً، وكانت جميع العزلات تابعة للمكورات العنقودية السالبة لأنزيم التخثر، إذ كانت 5 عزلات لبكتيريا *Staphylococcus epidermidis* و3 عزلات لبكتيريا *Staphylococcus haemolyticus*، وعزلتين لكل من بكتيريا *Staphylococcus hominis*, *Staphylococcus lenutus*, *Staphylococcus warneri* .
ووجد في هذه الدراسة هناك فروق واضحة في الإصابة بين الفئات العمرية حيث كانت الفئة العمرية من 40-59 سنة هي الأعلى في الإصابة فقد بلغت نسبة الإصابة لديهم 33.33%، كما كان للجنس ومكان الإقامة علاقة في توزيع الإصابات البكتيرية حيث كانت نسبة الإصابة في الذكور هي الأعلى مما في الاناث حيث بلغت 61% وكذلك الحال الساكنين في المدينة فقد كان نصيبهم من الإصابة بالتهاب العيون الأعلى حيث بلغت نسبة الإصابة لديهم 76.19%.