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Original article

Bacterial Etiology and Antibiotic Susceptibility in Isolates from Conjunctivitis Patients in Tripoli, Libya

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ABSTRACT

Acute conjunctivitis is described as inflammation of the conjunctiva that lasts less than 3 to 4 weeks, showing cellular infiltration and exudation. Conjunctivitis can cause significant and lasting harm to the eye, resulting in severe inflammation in the corneal lid. This study seeks to isolate and identify the particular bacterial pathogens responsible for conjunctivitis in patients at Tripoli Eye Hospital. Collection of bacterial samples from patients with conjunctivitis was conducted at the ophthalmic consultative clinic within Tripoli Eye Hospital, Libya between September 2021 and January 2022. Tests were done to separate bacterial pathogens causing conjunctivitis and to test their resistance to antibiotics. Among 140 samples, bacterial infection was detected in 86% of cases while 14% yielded negative results. Males had a higher prevalence of bacterial infections at 63% in comparison to females. Both male and female individuals aged 60-79 years had the highest percentage of bacterial conjunctivitis cases at 51%. Segregation tests showed that the majority (82%) were Gram-positive, while a minority (18%) were Gram-negative, with Staphylococcus aureus being the most prevalent bacteria (35%), closely followed by Staphylococcus epidermidis (32%). Streptococcal pneumoniae and Enterobacter spp had rates of 2% which were lower. The majority of organisms showed sensitivity to levofloxacin (43%), while there was only a small amount of resistance to polymyxin B (4%). Professional attention and microbiological research are crucial for verifying clinical diagnoses and carrying out suitable and efficient treatments.

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INTRODUCTION

Conjunctivitis is the most widespread external ocular disease worldwide and a common cause of eye redness. It refers to the inflammation of the conjunctival tissue and engorgement of the blood vessels, classified as acute, hyperacute, and chronic conjunctivitis, infectious or noninfectious according to the mode of onset and the severity of the clinical response [1]. Ocular infection leads to conjunctivitis, ocular dermatitis, and endophthalmitis [2], resulting in corneal and lid involvement, which may cause complications [3]. It can lead to blindness, especially if appropriate treatment is not advised [4]. The inflammation is distinguished by dilation of conjunctival vessels leading to hyperemia and the

formation of edema of the conjunctiva, with discharge [5]. Therefore, it is widely known as one of the causes of "red eye" or pink eye [6].

Most bacterial pathogens can cause conjunctivitis, such as Staphylococcus aureus, Streptococcus pneumoniae, and Haemophilus influenzae in adults, but it is often caused by H. influenzae, S. pneumoniae, and Moraxella catarrhalis in children [7]. The duration of the disease usually lasts 7 to 10 days [8]. Frequently, it is treated with antibiotics such as ciprofloxacin, ofloxacin, chloramphenicol, tetracycline, erythromycin, and gentamycin before the result of the antibiotic sensitivity test [9]. Also, it may be caused by infectious agents like viral or noninfectious agents that involve toxic and allergic reactions. In addition, it can be primary or secondary to systemic diseases [10]. Some of these illnesses include chlamydia, gonorrhea, Graft-versus-host disease (GVHD), Stevens-Johnson syndrome, and nutritional deprivation (especially vitamin a deficiency), immune-related disorders, such as Reiter syndrome, congenital metabolic syndromes, such as porphyria and Richner-Hanhart syndrome [1]. These can lead to catastrophic systemic complications, and in this state systemic treatment is recommended [11].

Conjunctivitis can be transmitted from infected people directly or can be connected with abnormal proliferation of natural conjunctival flora [12]. Bacterial conjunctivitis occurs when the eye's surface tissues are colonized by normal flora. The epithelial covering of the conjunctiva is the primary defense mechanism against infection, and any disruption in this barrier can lead to infection [1]. The most common routes of transmission include contaminated fingers [13], and contaminated fomites [14]. Conjunctivitis may also affect one eye or may be transmitted to the other eye [3]. Additionally, the wide and random use of antibiotics can lead to the appearance of spontaneously mutated bacterial isolates with a high ability to resist antibiotics [15]. This study was undertaken to isolate and identify the specific bacterial pathogens causing conjunctivitis in patients attending Tripoli Eye Hospital.

METHODS

Study population and sample design

This cross-sectional study is designed to identify the specifics of bacterial pathogens causing conjunctivitis in Tripoli, Libya from September 2021 to January 2022. About 140 clinical samples were collected from patients who attended the ophthalmic consultative clinic at the eye hospital in Tripoli. The age range in this study was from 1 to 99 years. All samples collected according to age, gender, and antibiotic use were recorded.

Specimens collected and sensitivity test

Specimens were collected using sterile cotton swabs, then immediately transferred to the hospital's laboratory for culturing on the blood agar, chocolate agar, and MacConkey agar. They were incubated for 24 hours at 37°C. The antibiotic sensitivity test for bacterial isolates was done using 12 antibiotic discs, sterile swabs of bacterial suspensions, and plated on Mueller-Hinton agar sensitivity plates. Several antibiotic sensitivity discs were placed on each plate, then incubated at 37°C for 24 hours. The bacterial strains were tested against 15 antibiotic discs including Amoxicillin (10µg), Fusidic acid (10µg), Levofloxacin (5µg), Erythromycin (10µg), Tetracycline (30µg), Augmentin (30µg), Neomycin (10µg), Chloramphenicol (30µg), Tobramycin (30µg), Vancomycin (30µg), Polymyxin-B (300µg), Gentamycin (10µg), Ciprofloxacin (5µg), Amikacin (30µg), Ceftriaxone (30µg). After 24 hours of incubation, they were measured for the growth inhibition area around the antibiotic disc to determine the sensitivity strains.

Statistical analysis

In this study, the Statistical Package for Social Sciences (SPSS) software was used to analyze the data.

RESULTS

Evaluation of the frequency of conjunctivitis infection

140 samples were obtained from patients diagnosed with conjunctivitis at the Tripoli Eye Hospital. There were 120 samples (86%) tested positive for bacterial infection, with 20 samples (14%) testing negative. Bacterial infections were more common in men at a rate of 62.50% compared to women at 37.50%.

The findings presented in Table 1 shows the ages of the patients who have been infected, within the age range of 1-99 years. The findings showed a greater number of people with bacterial conjunctivitis in the (60-79) age group, with a 51% infection rate in both men and women. Similarly, in the (80-99) age range, females had a little higher percentage of infection at 13%, compared to males at 12%, resulting in an overall prevalence of 13%. This is then succeeded by a notable rise in the number of infections among individuals aged 20 to 39, with a higher percentage increase of 22% in females compared to 20% in males, resulting in an overall prevalence of 21%. During the age of 10-19, there were only a small number of infections found in young children and teenagers under 20 years old, with a prevalence of 9% in

females and 8% in males, resulting in a total prevalence of 8%. Individuals between the ages of 40 and 59 had a lower infection rate compared to other age groups, with females at 4% and males at 9%, resulting in an overall prevalence of 8%.

Table 1. Displays the prevalence rate of conjunctivitis within the study population.

Age groups	Female (n=45)		Male (n=75)		Total (n=120)	
	N	%	N	%	N	%
1-19	4	9%	6	8%	10	8%
20-39	10	22%	15	20%	25	21%
40-59	2	4%	7	9%	9	8%
60-79	23	51%	38	51%	61	51%
80-99	6	13%	9	12%	15	13%
Total (Growth & No growth)	45+12 = 57		75+8 = 83		140	100%

In this study, segregation was identified using gram staining and biochemical tests (catalase, coagulase, oxidase) as outlined in table 2. Data from 120 samples revealed that 98 samples (82%) were Gram-positive bacteria, while only 22 samples (18%) were gram-negative.

Table 2. Identification of bacterial pathogens among individuals in the study.

Isolated	Number	Percentage (%)
Gram-positive	98	82%
Gram-negative	22	18%
Total	120	100%

According to table 3, Staphylococcus aureus was the most prevalent microorganism, making up 35% of all isolates, with Staphylococcus epidermidis following closely at 32%, and Pseudomonas aeruginosa, Staphylococcus hemolytic, Streptococcus, and Klebsiella species accounting for 13%, 8%, 6%, and 3% respectively. Streptococcal pneumoniae and Enterobacter spp. have the lowest rates, at 2% each, according to Table. 3.

Table 3. Bacterial etiology of conjunctivitis

Bacteria isolated	Number of isolates	Percentage (%)
Staphylococcus aureus	42	35%
Staphylococcus epidermidis	38	32%
Pseudomonas aeruginosa	16	13%
Staphylococcus heamolytic	9	8%
Streptococcal species	7	6 %
Klebsiella species	4	3%
Streptococcal pneumonia	2	2%
Enterobacter species	2	2%
Total	120	100%

As shown in table 4, the findings from the sensitivity test on bacterial strains to antibiotic discs revealed that numerous bacteria were responsive to antimicrobial substances, with most organisms being susceptible to levofloxacin at a rate of 43%, trailed by tobramycin at 28%, chloramphenicol at 26%, and vancomycin at 25%. Next, tetracycline accounted for 23%, amikacin for 14%, fusidic acid for 12%, ceftriaxone and neomycin both at 10%, as well as amoxicillin, Augmentin, and ciprofloxacin all at 9%. On the other hand, bacteria show minimal resistance to gentamicin (7%), erythromycin (6%), and polymyxin B (4%).

Table 4. Presents sensitivity tests for antimicrobials on bacterial strains in the study sample.

Antibiotics	Bacterial isolates								Total = 120
	S.aureus N=42	*Staphylococcus epidermidis 38*	*Ps.aeruginosa N=16*	*S.heamolytic N=9*	*Streptococcal species N=7*	*Klepsiella species N=4*	*S.pneumonia N=2*	*Enterobacter species N=2*	
Amoxycillin (30µg) AMC	2 (5%)	3 (8%|)	2 (13%)	1 (11%)	2 (29%)	0 (0%)	1 (50%)	0 (0%)	11 (9%)
Tobramycin (30µg) TOB	12 (29%)	7 (18%)	6 (38%)	4 (44%)	1 (14%)	1 (25%)	2 (100%)	1 (50%)	34 (28%)
Chloramphenicol (15µg) C	18 (43%)	2 (5%)	1 (6%)	4 (44%)	3 (43%)	1 (25%)	1 (50%)	1 (50%)	31 (26%)
Tetracyclin (15µg) TE	12 (29%)	8 (21%)	2 (13%)	2 (22%)	2 (29%)	0 (0%)	1 (50%)	1 (50%)	28 (23%)
Fusidic acid (10µg) FA	6 (14%)	4 (11%)	0 (0%)	0 (0%)	2 (29%)	0 (0%)	2 (100%)	0 (0%)	14 (12%)
Vancomycin (30µg) VA	14 (33%)	3 (8%)	0 (0%)	6 (67%)	4 (57%)	0 (0%)	2 (100%)	1 (50%)	30 (25%)
Levofloxacin (5µg) LEV	16 (38%)	11 (29%)	8 (50%)	6 (67%)	5 (71%)	2 (50%)	2 (100%)	2 (100%)	52 (43%)
Ceftriaxone (30µg) CRO	3 (7%)	1 (3%)	3 (19%)	3 (33%)	1 (14%)	1 (25%)	0 (0%)	0 (0%)	12 (10%)
Erythromycin (15µg) E	2 (5%)	2 (5%)	0 (0%)	1 (11%)	1 (14%)	0 (0%)	0 (0%)	1 (50%)	7 (6%)
Augmentin (30µg) AUG	5 (12%)	(0%)0	0 (0%)	2 (22%)	3 (43%)	0 (0%)	1 (50%)	0 (0%)	11 (9%)
Gentamycin (10µg) GN	0 (0%)	2 (5%)	4 (25%)	0 (0%)	0 (0%)	1 (25%)	0 (0%)	1 (50%)	8 (7%)
Neomycin (10µg) N	7 (17%)	1 (3%)	0 (0%)	3 (33%)	1 (14%)	0 (0%)	0 (0%)	0 (0%)	12 (10%)
Amikacin (30µg) AK	1 (2%)	3 (8%)	11 (69%)	0 (0%)	0 (0%)	1 (25%)	0 (0%)	1 (50%)	17 (14%)
Polymyxin-B (300µg) P-B	0 (0%)	2 (5%)	3 (19%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (4%)
Ciprofloxacin (5µg) CIP	3 (7%)	0 (0%)	6 (38%)	0 (0%)	0 (0%)	2 (50%)	0 (0%)	0 (0%)	11 (9%)

DISCUSSION

Frequent outbreaks of conjunctivitis occur globally, affecting individuals of all ages and socio-economic backgrounds, it is a common cause of eye redness and a common complaint in the emergency department, urgent care clinics [16]. The current investigation looked into how common Conjunctivitis infection is among individuals attending at the ophthalmic consultative clinic at the eye hospital in Tripoli. The present research shows a high prevalence of bacterial eye infections in 86% of the participants in the study. Males have a higher prevalence rate of 62.50% compared to females at 37.50%, consistent with a study in Iraq [3]. But contrasting with study in Saudi Arabia [12] where females have a higher percentage at 64.1%.

Our result research indicating mainly on Gram-positive bacteria with (82%), while Gram-negative bacteria made up only 18% of our Findings. This agreed with recent study in Libya [9], that found that Gram-positive organisms were isolated in 77.57% of cases while Gram-negative organisms were isolated in 14.02% of cases. A comparable result in the UK [17], with 75.2% of the bacteria being Gram-positive and 24.8% being Gram-negative. Also, similarly to findings in Ethiopia [18], which reported that (68.2%) of Isolates was Gram-positive and (31.8%) Gram-negative bacteria. As the previously research in Northwest Ethiopia [4] which found that (74.2%) of the bacteria were gram-positive.

Staphylococcus aureus (SA) was the most commonly found bacteria in the study, representing 35% of the samples, while Staphylococcus epidermidis (SE) accounted of 32%. This consistent with recent study in Libya [9] found that Staph. aureus was the most common organism isolated at 26.17%, while Staph. epidermidis had the lowest prevalence at 6.54%. This compatible with findings of research conducted in Ethiopia [18], which reported of the most common bacterial isolate was S. aureus at a rate of 37%. Similar to the results of India [11], where (SA) was the majority repetition organism identified all year at a rate of 32.1%, while the plurality frequent single organism isolates were (SE) at 26.1%. Similar findings, found in a prior Nigerian study [19] that Staphylococcus aureus was the implicated pathogen. As study in New Delhi-India [20] also identified Staphylococcus aureus and Staphylococcus epidemidis as prevalent isolates, consistent with research from Nepal, and other areas highlighting these bacteria as primary culprits of bacterial conjunctivitis. In contrast, in Iraq [7] that Pseudomonas aeruginosa was the most common species, with Staphylococcus aureus and Staphylococcus epidermises following closely behind, possibly because of geographic variations. According to the recent study conducted in Libya [9], Staph. aureus was the most commonly found organism at a rate of 26.17%, while Staph. epidermidis was the least prevalent at 6.54%.

The most effective antibiotics were found to be Levofloxacin (43%), Tobramycin (28%), and Chloramphenicol (26%), according to this research. On the other hand, Erythromycin (6%) and Polymyxin-B (4%) exhibit reduced effectiveness. This finding aligns with the results of recently study [9] was conducted in country, showing that (85.4%) of gram-positive isolates responded well to Levofloxacin, a Fluoroquinolone. Contrary to results of found in Ethiopia [21] the majority of gram-negative and positive cocci were susceptible to ciprofloxacin. Furthermore, the majority of bacterial isolates were found to be resistant to tetracycline in another study also conducted in Ethiopia [18] and most of them showed susceptibility to ciprofloxacin.

CONCLUSION

All age groups can be affected by conjunctivitis, with the elderly being particularly vulnerable. For that, it is important to focus on patients in this age bracket to avoid any potential complications. Educating the patient on minimizing infection spread is crucial in bacterial conjunctivitis treatment. As not touching or rubbing the impacted eye, cleansing eye secretions multiple times daily, and refraining from sharing personal items when infected are crucial.

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Conflict of interest

Authors declared no inflict of interest.

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المسببات البكتيرية وحساسية المضادات الحيوية في عينات معزولة من مرضى التهاب الملتحمة في طرابلس، ليبيا

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المستلخص

التهاب الملتحمة الحاد يُعرف بأنه التهاب الملتحمة الذي يستمر من 3 إلى 4 أسابيع، مع ظهور إفرازات قيحية أو مخاطية. قد يسبب التهاب الملتحمة أضرارًا خطيرة ودائمة للعين، مما يؤدي إلى التهاب شديد في غشاء القرنية. تهدف هذه الدراسة إلى عزل وتحديد المسببات البكتيرية لالتهاب الملتحمة لدى المرضى الذين يترددون على مستشفى طرابلس للعيون، ليبيا. جمعت العينات المعزولة من مرضى التهاب الملتحمة في العيادات الاستشارية لطب العيون داخل المستشفى خلال الفترة ما بين سبتمبر 2021 ويناير 2022. ثم إجراء اختبارات العزل لتشخيص المسببات البكتيرية لالتهاب الملتحمة، وكذلك اختبار حساسية السلالات البكتيرية للمضادات الحيوية. من بين 140 عينة، تم الكشف عن العدوى البكتيرية في 86٪ من الحالات بينما أسفرت 14٪ من الحالات عن نتائج سلبية. وكانت نسبة انتشار التهاب الملتحمة البكتيري أعلى عند الرجال منها عند النساء بنسبة (63%). في حين كان لدى كل من الذكور والإناث في الفئة العمرية (60-79) أعلى نسبة انتشار لالتهاب الملتحمة الجرثومي بنسبة 51٪. أظهر اختبار العزل أن (82%) من العينات المعزولة كانت (موجبة الجرام)، مقابل (18%) منها كانت (سلبية جرام)، حيث كانت المكورات العنقودية الذهبية هي الأكثر شيوعا بنسبة (35%) تليها المكورات العنقودية الجلدية بنسبة (32%)، بينما كانت أقل المعدلات هي المكورات العقدية الرئوية والأمعائية بنسبة (2%). أظهرت معظم العزلات البكترية حساسية لليفوفلوكساسين بنسبة (43٪)، في المقابل كانت محدودة الحساسية للمضاد الحيوي بوليميكسين (4٪). تعد الدراسات الميكروبيولوجية ضرورية لتحقيق اختيار العلاج المناسب والفعال.

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