Isolation and Characterization of Bacteria from Patients with Conjunctivitis in Hilla Province

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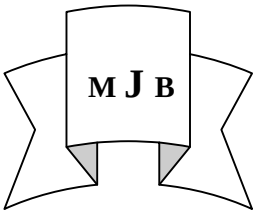

Abstract

This work therefore, aims to study the isolation and identification of common bacterial pathogens associated with conjunctivitis and examine some virulence factors such as (Capsule, Hemolysin, Siderophores, Extracellular Proteases and Adherence Factor and Show the effect of some antibiotics on bacterial isolates.

In our study, 120 conjunctival swabs were subjected to isolate and identify the bacterial strains associated with conjunctivitis. These swabs were obtained from patients suffering from conjunctival inflammation of both sexes who were attended to the Hilla Teaching Hospital/ Ophthalmic Unit in Hilla province.

Bacterial infections were detected in 82 (68.3%) samples and no growth resulting in 38 (31.7%) samples. It was indicated that *Haemophilus influenzae* was the first colonizer of the conjunctival sac (49 isolates) among the children and adults, followed by *Staphylococcus aureus* (19 isolates), *Streptococcus pneumoniae* (7 isolates) and a few number (4 isolates) of *Neisseria gonorrhoeae* on the first week of life were isolated, an act, which confirmed the predominance of Gram-negative bacteria early in life.

Also (3 isolates) of *Klebsiella pneumoniae* were isolated from children below one year old and from adults. Some virulence factors of these bacterial isolates were investigated. Also, invitro effect of some antibiotics on these bacterial isolates was studied, and the results showed that Ciprofloxacin and Chloramphenicol were the most sensitive among the other drugs; hence they should be the first choice for the treatment of bacterial conjunctivitis.

عزل وتشخيص البكتريا من المرضى المصابين بالتهاب ملتحمة العين في مدينة الحلة

الخلاصة

تهدف هذه الدراسة الى عزل وتشخيص البكتريا المرضية السائدة والمسببة لالتهاب ملتحمة العين ، دراسة بعض عوامل الضراوة مثل (الكبسولة ، الهيمولايسين ، انزيمات البروتيز الخارجية ، وعوامل الاستيطان)، وكذلك تمّ دراسة بعض المضادات الحياتية على العزلات البكتيرية .

تمّ في هذه الدراسة ، إخضاع (120) مسحة من ملتحمة العين ، لعزل وتشخيص السلالات البكتيرية المسببة لالتهاب الملتحمة . استحصلت هذه العزلات من المرضى الذين يعانون من التهاب الملتحمة ومن كلا الجنسين ، المراجعون إلى مستشفى الحلة التعليمي العام / وحدة العيون ، للفترة من تشرين الثاني 2003 ولغاية أيار 2004 في محافظة بابل .

تمّ التحري عن الإصابات البكتيرية في (82) عينة، وعدم وجود نمو في (38) عينة، وقد لُوحظ بان (*Haemophilus influenzae*) هي المستوطن الأول للملتحمة (49) عزلة عند الأطفال والبالغين ، ثم تليها (*Staphylococcus aureus*) (19) عزلة ، (*Streptococcus pneumoniae*) (7) عزلات ، وعدد قليل (4) عزلات من نوع بكتيري آخر تمّ عزله عند الأسبوع الأول من العمر ، إذ يُعد النوع السائد من البكتريا السالبة لصبغة كرام في بداية العمر ، وهذا النوع هو (*Neisseriae gonorrhoeae*) .

كما تمّ عزل (3) عزلات من (*Klebsiella pneumoniae*) من الأطفال الذين أعمارهم أقل من سنة واحدة ، وكذلك من البالغين .

كما تمّ الكشف عن بعض عوامل الضراوة لهذه العزلات البكتيرية .

تمّ دراسة تأثير بعض المضادات الحياتية على هذه العزلات البكتيرية ، وقد أشارت النتائج بأن سيبروفلوكساسين وكلورامفنيكول أكثر حساسية من المضادات الحياتية الأخرى ، إذن يجب أن يتم اختيارهما أولاً في علاج (Bacterial conjunctivitis) .

Introduction

The conjunctiva is a thin mucous membrane that covers the posterior surface of the eyelids and the anterior surface of the eyeball itself [1]. It is divided into three areas. The palpebral conjunctiva, the bulbar conjunctiva and the conjunctival fornix [2].

Conjunctivitis, commonly known as “red eye” or “pink eye” is an inflammation of the conjunctiva of the eye (the thin protective membrane, that lines the inside of eyelids and covers the outer part of eyeball) [3].

Bacterial conjunctivitis is one of the differential diagnoses of the red eye involving the mucous membrane of the conjunctiva. There are two types of bacterial conjunctivitis; acute and chronic bacterial conjunctivitis; however, the majority of bacterial conjunctivitis are self limiting, without needing any medical intervention. The commonest types of bacteria implicated in bacterial conjunctivitis include *Staphylococcus aureus*, *Neisseria gonorrhoeae*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella lacunata*, *Klebsiella pneumoniae*, *Corynebacterium diphteriae* and *Chlamydia trachomatis* [4]. The pathogenic bacteria have the ability to produce several types of virulence factors associated with its pathogenecity and play a major role in the causation of infection in eye [5].

Mostly, primary eye care providers start the treatment of external ocular infection before the causative microorganisms have been identified, or submitted to antibiotic susceptibility tests. Consequently broad-spectrum antibiotics are routinely used in the treatment of bacterial conjunctivitis. Of the many antibiotics in use is the group of fluoroquinolones, particularly Ciprofloxacin, Ofloxacin and Norfloxacin [6]. Fluoroquinolones groups were more effective in the treatment of ocular infections than some other broad-spectrum antibiotics e.g. Gentamycin, Chloramphenicol, Tobramycin, Erythromycin and Tetracycline [7].

There is no independent study on bacterial conjunctivitis conducted in Babylon governorate.

Materials and Methods

Patients

Swabs of conjunctiva were obtained from one hundred and twenty patients with conjunctivitis of age groups ranging from four days to seventy four years from both sexes of the attendants of Ophthalmic Unit in Hilla Teaching Hospital; during six months
for the interval between November 2003 and May 2004, by questionnaire papers including information about:

1. Age of patients.
2. Sex of patients.
3. Internal genital infection and birth circumstances.
4. Clinical features, like ocular pain, redness, swollen eyelids, discharge.

5. Antibiotic treatment, the patients must not have any antibiotics to avoid the negative false results.

Specimens Collection

Swabs of bacterial cultures were collected from patients suffering from conjunctival inflammation. Also, specimens were obtained from patients who did not receive any antibiotics. One sample of the conjunctiva of each eye was taken by rolling a cotton swab over the conjunctiva of the lower fornix, and the swabs were put into transport medium in the sterilized swab tube, then they were sent to the investigating laboratory within two hours of collection. In the laboratory, they were placed and inoculated onto medium.

Inoculating the conjunctival swabs onto Blood agar enriched with 5% human blood, MacConky agar and Chocolate agar (was made by heating blood agar to $70C^{o}$ - $80C^{o}$ until it became chocolate brown in colour). After standard inoculation, the Blood agar, MacConky agar and Chocolate agar plates were incubated at $37C^{o}$ for 24 hours.

Laboratory Diagnosis

According to the diagnostic procedures recommended by Bergy's manual for determinative bacteriology [8-10], the isolation and identification of bacteria were performed as follows:

1. Colonial Morphology and Microscopy Examination

A single colony was taken from each primary positive culture and its identification depended on the morphology properties (Colony size, shape, color, borders and texture), and then some colonies were selected and investigated by gram-stain to observe a specific shape, color, aggregation and specific intracellular compounds. After staining the bacteria by gram stains, specific biochemical tests were done to reach the final identification such as (Catalase test, Oxidase test, Coagulase test, Indole test, Methyl red test, Voges-Proshauer test, Citrate test and Urease test). Also, Satellite test should be done for identification of *Haemophilus influenzae* strains and some virulence factors tests were done such as Capsule stain test (Hiss's Method) [11], Hemolysin production test, Siderophores production test, Extracellular protease production test and Haemagglutination test (HA).

2. Antibiotics Sensitivity Test (Kirby-Bauer Method)

It was performed by using a pure culture of previously identified bacterial organism. The inoculum to be used in this test was prepared by adding growth from 5 isolated colonies grown on blood agar plate to 5 ml of Nutrient broth. This culture was then incubated for 2 hours to produce bacterial suspension of moderate turbidity. A sterile swab was used to obtain an inoculum from the standardized culture. This inoculum then was streaked on a Muller-Hinton plate. The antibiotic discs were placed on the surface of the medium at evenly spaced intervals with flamed forceps or a disc applicator. Incubation was usually overnight with an optimal time of 14 hours at $37C^{o}$. Antibiotic inhibition zones were measured by using a caliper. Zone size was compared to standard zones to determine the susceptibility or resistance of the organism to each antibiotic [12].

Results and Discussion

1. Age and Sex

In this study, 120 conjunctival swabs from patients (male and female) suffering from conjunctivitis of age groups ranging from four days to seventy four years were taken. The results are shown in Table (1).

Table 1 Distribution of patients with conjunctivitis according to the age groups and sex

Age groups (year)	No. of patients %	Sex	
		Male	Female
< 1	28 (23.3%)	15	13
1 – 9	24 (20%)	13	11
10 – 19	19 (15.8%)	9	10
20 – 29	13 (10.8%)	7	6
30 – 39	12 (10%)	4	8
40 – 49	11 (9.2%)	6	5
50 – 59	8 (6.7%)	5	3
≥ 60	5 (4.2%)	3	2
Total	**120**	**62 (51.7%)**	**58 (48.3%)**

It is clear that conjunctivitis occurs in all age groups with both sexes, there is also decrease in the percentage of conjunctivitis among patients with increasing age.

Table 2 Conjunctivitis among children and adults

Age group (year)	Sex		Total	%
	Male	Female		
Children (≤ 1 - 15)	29	40	69	57.5
Adults ≥ 16	33	18	51	42.5

The results showed that conjunctivitis is common in children (57.5%), whose age groups are (≤ 1 - 15) years, but it is less common in adults (42.5%), whose age groups are (≥ 16), Table (2).

This is probably due to the fact that children with conjunctivitis are in schools which help to spread the infection to other healthy children through contact with discharge from the eyes or upper respiratory tract of infected children and it can spread by contaminated fingers or vomits (contaminated objects).

These results are identical with those obtained by [13] have pointed out that the incidence of conjunctivitis in children is (66%).

2 . Isolation and Characterization

In our study, all conjunctival swabs were subjected for culturing on Blood agar base, MacConky agar and Chocolate agar, and it was found out that the total of 120 samples, only 82 samples showed positive cultures (bacterial isolates), no growth was seen in the other samples (38 samples) which could indicate the presence of microorganisms that might be cultured with difficulty such as fungi, viruses, Chlamydia, or other agents. Table (3) .

Table 3 Distribution of conjunctival cases acording to the type of results

Type of result	No. of cases	%
Positive culture	82	68.3
Negative culture	38	31.7
Total	120	100

Bacterial isolates are found in unhealthy conjunctiva and it reveals that these bacteria are more predominant than other agents such as viruses.

The most common types of the bacteria isolated in this study is shown in Table (4).

Table 4 Distribution of patients with conjunctivitis according to the type of bacteria

Age group (year)	*Haemophilus influenzae*	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Neisseriae gonorrhoeae*	*Klebsiella pneumoniae*	No growth	Total %
	No. %	No. %	No. %	No. %	No. %	No. %	
< 1	15 (30.6)	0	0	4(100)	2 (66.7)	7 (18.4)	28 (23.3)
1 – 9	13 (26.5)	2 (10.5)	5 (71.4)	0	0	4 (10.5)	24 (20)
10 – 19	10 (20.4)	2 (10.5)	2 (28.6)	0	0	5 (13.2)	19 (15.8)
20 – 29	4 (8.1)	3 (15.8)	0	0	0	6 (15.8)	13 (10.8)
30 – 39	3 (6.1)	4 (21.05)	0	0	1 (33.3)	4 (10.5)	12 (10)
40 – 49	2 (4.1)	4 (21.05)	0	0	0	5 (13.2)	11 (9.2)
50 – 59	1 (2.1)	3 (15.8)	0	0	0	4 (10.5)	8 (6.7)
³ 60	1 (2.1)	1 (5.3)	0	0	0	3 (7.9)	5 (4.2)
Total	49 (40.8)	19 (15.8)	7 (5.8)	4 (3.3)	3 (2.5)	38 (31.8)	120 (100)

It is seen that the most common causative microorganism implicated in bacterial conjunctivitis in all age groups is *Haemophilus influenzae*, being responsible for (49) isolates, followed by *Staphylococcus aureus* (19) isolates, *Streptococcus pneumoniae* (7) isolates, *Neisseriae gonorrhoeae* (4) isolates, and *Klebsiella pneumoniae* (3) isolates.

3. Investigation of Virulence Factors of Bacterial Isolates

In our study, the ability of bacterial isolates, which have been isolated from patients with bacterial conjunctivitis to produce some virulence factors, was studied. The results of virulence factors are shown in Table (5).

Table 5 Virulence factors of bacterial isolates from patients with bacterial conjunctivitis

Type of bacteria	No. of isolates	Capsule	Hemolysin	Siderophores	Extracellular proteases	Adherence Factors
H. influenzae	49	0 0	0 0	0 0	49 100%	49 100%
Staph. aureus	19	8 42.1 %	13 68.4%	5 26.3%	8 42.1%	0 0
St. pneumoniae	7	2 28.6 %	7 100%	0 0	7 100%	7 100%
N. gonorrhoeae	4	0 0	0 0	0 0	4 100%	4 100%
K. pneumoniae	3	3 100%	0 0	3 100%	0 0	3 100%
Total	82	13	20	8	68	63
%	68.3	15.9	24.4	9.8	82.9	76.8

It was found out that the capsule was not seen in all isolates of *Haemophilus influenzae* strains (uncapsulated strains), hemolysin could not produce extracellularly and the type of hemolysis was gamma-hemolysis because there was no hemolysis present on blood agar base, and all isolates could not produce siderophores because non of the isolates has the ability to grow on M9 media which contain dipyridyl.

The ability of *Haemophilus influenzae* to produce extracellular proteases by using M9 media (supported by 0.2%glucoseand 1% gelatin) was investigated and it was found out that all isolates were able to produce extracellular proteases after 24 hours and there was a transparent area which formed around the colony after the addition of 3 ml (5%) of trichloroacetic acid.

The same result was reported by(Kilian) [14], In Tunisia, who isolated *Haemophilus influenzae* in the early 1970s from cases of conjunctivitis, and found out that this bacteria was able to produce type 1 (IgA1) proteases and had the characteristics of *Haemophilus influenzae* biotype III.

Also, the isolates were tested for their abilities to produce adherence factors by Haemmaglutination Test (HA). The results showed that all *Haemophilus influenzae* isolates were able to produce adherence factors due to the positive reaction with human blood cells (group A).

[15] Have showed that non-typeable *Haemophilus influenzae* isolated from the conjunctiva can express pili.

It was found that nombers of ***Staphylococcus aureus*** strains could produce polysaccaride capsule Hemolysin, Siderophores, and Extracellular proteases but all strains could not produce adherence factors by Haemmaglutination Test (HA) due to the negative reaction with human blood.

Also, it was found that all ***Streptococcus pneumoniae*** strains could produce hemolysin, extracellular

proteases and adherence factors but could not produce siderophores and only2 isolate by arate (28.6%) from 7 isolates of *Streptococcus pneumoniae* were able to possess the polysaccharide capsule which surrounded the bacterial cell.

The results in Table (5), reveal that all isolates of *Neisseriae gonorrhoeae* which are isolated from patients with bacterial conjunctivitis could not possess the polysaccharide capsule, hemolysin and siderophors. On the other hand, it was found that all *Neisseriae gonorrhoeae* isolates were able to produce an extracellular proteases and adherence factors.

From the results in Table (5), it can be noticed that all *Klebsiella pneumoniae* isolates were able to possess the polysaccharide capsule which surrounded the bacterial cell, siderophors and adherence factors, but all *Klebsiella pneumoniae* isolates could not produce hemolysin and extracellular proteases.

These results are supported by(AL-Saedi) [16] who have found out that all isolates of *Klebsiella pneumoniae* from patients with eye infections could not produce extracellular proteases.

4. Antibacterial Sensitivity Assay

In our study, some antibiotics were used to show their effect on bacterial isolates which were isolated from patients with bacterial conjunctivitis such as (Chloramphenicol, Ciprofloxacin, Gentamycin, Penicillin, Tetacycline, Cefotaxime and Erythromycin). The results of sensitivity test are shown in Table (6).

Table 6 The sensitivity of bacterial isolates from patients with bacterial conjunctivitis to some antibiotics

Type of bacteria	No. of isolates	CL	CP	GN	PN	TE	CTX	ER
H. influenzae	49	44 (89.8%)	47 (95.9%)	21 (42.9%)	3 (6.1%)	45 (91.8%)	49 (100%)	9 (18.4%)
Staph. aureus	19	17 (89.5%)	18 (94.7%)	19 (100%)	0	17 (89.5%)	14 (73.7%)	13 (68.4%)
St. pneumoniae	7	6 (85.7%)	6 (85.7%)	0	6 (85.7%)	6 (85.7%)	5 (71.4%)	7 (100)
N. gonorrhoeae	4	3 (75%)	3 (75%)	2 (50%)	1 (25%)	4 (100)	3 (75%)	4 (100%)
K. pneumoniae	3	3 (100%)	3 (100%)	2 (66.7%)	0	0	1 (33.3%)	0
Total	82	73	77	44	10	72	72	33
%	68.3	89	93.9	53.7	12.2	87.8	87.8	40.2

CL: Chloramphenicol; CP: Ciprofloxacin; GN: Gentamycin; PN: Penicillin; TE: Tetracycline; CTX: Cefotaxime; ER: Erythromycin.

From the result in table (6), it was seen that Ciprofloxacin and Chloramphenicol have major effect on all bacterial isolates from patients with

bacterial cnjunctivitis; hence they should be the first choice for the treatment of bacterial conjunctivitis. Also, the results showed that (87.8%) of bacterial isolates were sensitive to Tetracycline and Cefotaxime, (53.7%) of isolates were sensitive to Gentamycin, whereas (40.2%) of isolates were sensitive to Erythromycin. In contrast, most of the bacterial isolates were resistant to pencillin.

(St Geme and Falkow) [17] have shown that resistance of *Staphylococcus aureus* strains to penicillin is attributed to their production of beta-lactamase enzymes that break down the beta lactam ring and render it inactive and these enzymes are encoded by genes located on plasma. Plasmid-mediated Beta-lactamases are produced constitutively and have a high propensity to move from one species of bacteria to another.

Conclusions

The results of this study can yield to the following conclusions:

1. The prevalence bacterial infection in (120) patients (male and female) suffering from conjunctivitis is 82 (68.3%) bacterial isolates. This result refers to that bacterial infection is higher than other infections.
2. Conjunctivitis is more frequent among children with ages ranging between (1 - 15) years, and less frequent among adults and elderly patients with age ranging between (15 - 74) years.
3. The common type of bacteria which cause bacterial conjunctivitis in this study is *Haemophilus influenzae*.
4. There is a non significant difference between male and female patients with conjunctivitis.
5. All bacterial isolates in this study have the ability to possess more than one virulence factor such as Capsule, Haemolysin, Siderophores and others.
6. All bacterial isolates have shown that Ciprofloxacin and Cholramphenicol are the most sensitive antibiotics for the treatment of bacterial conjunctivitis.

Recommendations

1. Medical staff working in neonatal care unit should wear gloves with frequent hand washing which is necessary to reduce transmission of infection.
2. Schools and childcare facilities with children with conjunctivitis are advised to thoroughly clean and disinfect shared items “toys and desks”.
3. The patients should be encourage to wash their hands frequently and thoroughly using soap and water or using alcohol based hand sauitizer especially after touching the eyes or face.
4. We should encourage other researchers to be done for the detection of other pathogens such as viruses and *Chlamydia trachomatis* which caused conjunctivitis.

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