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

A Thesis

Submitted to the Council of the College of Medicine, University of Babylon as a Partial Fulfillment of the Requirements for the Degree of Master of Science in Medical Microbiology

By

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[وَيَسْأَلُونَكَ عَنِ الرُّوحِ
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(الإسراء : الآية 85)

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Dedication

- To the person who stands by me and supports me with confidence, love and kindness ... my dear husband ... Mushtaq.
- To the person who stands my absence for long days ... my beloved son ... Moamel.
- To the invisible power which moves me ... my kind mother.
- To the person who shares me the days of patience ... my dear sister ... Bushra.
- To the most lovable people for me in life ... my sisters and brothers.

I dedicate my humble effort

Neran

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Bacterial infections was 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, and the results showed that:

1. All isolates of *Haemophilus influenzae* and *Neisseria gonorrhoeae* could not possess capsule, while all *Klebsiella pneumoniae* isolates possessed capsule, and it was found out that (42.1%) of *Staphylococcus aureus* isolates possessed capsule, whereas only (28.6%) of *Streptococcus pneumoniae* possessed capsule.

2. The results also showed that all isolates of *Haemophilus influenzae*, *Neisseria gonorrhoeae* and *Klebsiella pneumoniae* could not produce hemolysin, while all *Streptococcus pneumoniae* isolates were able to produce hemolysin, and only (68.4%) of *Staphylococcus aureus* isolates were able to produce hemolysin.
3. All isolates of *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Neisseria gonorrhoeae* were not able to produce siderophores, but all *Klebsiella pneumoniae* isolates were able to produce siderophores, and only (26.3%) of *Staphylococcus aureus* isolates were able to produce siderophores.
4. The results also showed that all isolates of *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Neisseria gonorrhoeae* were able to produce extracellular proteases, but all *Klebsiella pneumoniae* isolates were not able to produce extracellular proteases, whereas (42.1%) of *Staphylococcus aureus* isolates were able to produce extracellular proteases.
5. All isolates of *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Neisseria gonorrhoeae* and *Klebsiella pneumoniae* had adherence factors, whereas all *Staphylococcus aureus* isolates did not have the adherence factors.

Also, in vitro 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.

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CHAPTER ONE

Introduction

and

Literature Review

1. Introduction and

1.1 Introduction

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) (Smith, 2004).

Bacterial conjunctivitis is one of the most common causes of the red eye involving the mucous membrane of the conjunctiva, though the lysosomes and antibodies in the tear film along with the blinking mechanism keep their population in check (Idu and Odjimogho, 2003). 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* (Vaughan, *et. al*., 1996). 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 (Syed and Hyndink, 1992).

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 (Idu and Odjimogho, 2003). 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 (Brower, *et. al*., 1996).

There is no independent study on bacterial conjunctivitis conducted in Babylon governorate; this work therefore, aims to:

1. Study the isolation and identification of common bacterial pathogens associated with conjunctivitis.
2. Examine some virulence factors such as (Capsule, Hemolysin, Siderophores, Extracellular Proteases and Adherence Factors).
3. Show the effect of some antibiotics on bacterial isolates.

1.2 Literature Review

1.2.1 Anatomy of Conjunctiva

The conjunctiva is a thin mucous membrane that covers the posterior surface of the eyelids and the anterior surface of the eyeball itself (Chern, 2002). It is divided into three areas. The palpebral conjunctiva, the bulbar conjunctiva and the conjunctival fornix (Newell, 1986).

The palpebral conjunctiva begins at the eyelid margins and covers the entire surface of the inner eyelid to the fornix, where it is firmly attached to the underlying fibrous tissue, before being reflected back to cover the globe as the bulbar conjunctiva. At the point at which the conjunctiva is reflected back over sclera, the conjunctiva has numerous folds that allow the eye to move freely (Chern, 2002). As a mucous membrane, conjunctiva has an epithelium and submucosal lamina propria (Bron, *et. al.*, 1997).

The conjunctival epithelium contains many glands responsible for maintaining moisture and secreting the constituents of the precorneal tear film. The conjunctival epithelium contains numerous unicellular mucous glands (goblet cells) that secrete mucin, the wetting agent of the precorneal tear film. The glands which are most numerous in the conjunctival fornix, occur less frequently in the bulbar conjunctiva and are absent at the eyelid margins and corneosclera limbus (Newell, 1996).

The blood supply of the palpebral conjunctiva comes from the peripheral and marginal arterial arcades of the eyelid. The marginal arcade nourishes the margins of the eyelids and part of tarsal area of

palpebral conjunctiva. The bulbar and fornix conjunctiva are nourished by the peripheral arcade (Lang and Kageyama, 1990).

1.2.2 Conjunctivitis

Conjunctivitis refers to any inflammatory condition of the membrane that lines the eyelids and covers the exposed surface of the sclera. It is the most common cause of "red eye" (Miller, 2002), the clinical term red eye is applied to a variety of distinct infectious or inflammatory ocular disease processes that involve one or more tissue layers of the eye. Red eye is the most common ocular problem seen by primary care physicians. The term conjunctivitis encompasses a broad group of conditions presented as inflammation of the conjunctiva. The inflammation can be hyperacute, acute or chronic in presentation and infectious or noninfectious in origin (Morrow and Abbott, 1998).

Most frequently, conjunctivitis (and thus red eye) is commonly caused by bacteria and viruses. Sexually transmitted disease such as chlamydial infection and gonorrhoeae are less common cause conjunctivitis. However, these infections are becoming more prevalent and are important to recognize because of their significant associated systemic, ocular and social implications (Weir, 2002).

Ocular allergy in its many forms is one of the major causes of chronic conjunctivitis; Blepharitis (inflammation of the eyelid margin), dry eye and the prolonged use of ophthalmic medications, contact lenses and ophthalmic solutions are also relatively frequent causes of chronic conjunctival inflammation (Scott and Dhillon, 1998).

1.2.3 Signs and Symptoms

The different types of conjunctivitis can have different symptoms. In addition, symptoms may vary from person to person. One of the most common symptoms is discomfort or pain in the eye, which may feel like having sand in the eye (Jackson, 1993). Many patients have redness of the eye and inner eyelid as well; this redness led people to call conjunctivitis by its other common name, pink eye. The patients may also have swollen eyelids, hyperaemia, discharge, papillae, follicles, chemosis, itching and may be sensitive to bright light (photophobia) (Smith, 2004).

Discharge from the eyes may accompany the other symptoms. In bacterial conjunctivitis the discharge will be somewhat thick and colored white, yellow or green. Sometimes the discharge will cause the eyelids to stick together when the child wakens in the morning. Also, acute onset of redness, grittiness, itching, swelling of lids, burning or a gritty foreign-body sensation (Miller, 2002). In viral or allergic conjunctivitis, the discharge may be thinner and may be clear (Silverman and Bessman, 2003).

1.2.4 Diagnosis of Conjunctivitis

A detailed examination should be performed on patients presented with acute or chronic conjunctivitis. The patient should be examined in a well-lit room. Before performing the ocular examination, the physician should search for regional lymphadenopathy and should examine the face and eyelids carefully (Morrow and Abbott, 1998).

1.2.4.1 Patient History

The diversity of etiologies for conjunctivitis makes a detailed patients history the most important step in the differential diagnosis of conjunctivitis. The patient history includes the chief complaint, ocular history, general health history and review of systems, social history and family ocular and medical history (Miller, 2002).

1.2.4.2 Physical Examination

The physical examination includes investigation of the degree of redness (peripheral, whole conjunctiva, or whole conjunctiva and pericorneal), the presence of periorbital oedema, the kind of discharge (watery, mucous, or purulent), and bilateral involvement (yes or no) (Rietveld, *et. al*., 2004).

1.2.4.3 Bimicroscopy

Careful bimicroscopy should be performed on all patients with conjunctivitis (Quinn, *et. al.*, 1995).

1.2.4.4 Supplementary Testing

1.2.4.4.1 Cultures, Smears and Scrapings

Conjunctival cultures and smears or scraping should be obtained in cases of chronic, neonatal, hyperacute or recurrent conjunctivitis. Cultures also should be obtained in patients who do not respond to treatment and immunocompromised patients (Morrow and Abbott, 1998).

1.2.4.4.2 Immunoassay

Direct fluorescin-conjugated monoclonal antibody staining of smears, enzyme immunoassays tests are used to detect Chlamydia organisms, DNA hypridization assays and a polymerase chain reaction test are also used to identify chlamydial antigens in conjunctival scrapings (Schwab and Epstin, 1995).

1.2.4.4.3 Conjunctival Biopsy

Conjunctival biobsy is occasionally useful in refractory or atypical conjunctivitis and is mandatory in cases of suspected neoplasm (Quinn, *et. al.*, 1995).

1.2.5 Types of Conjunctivitis

1.2.5.1 Bacterial conjunctivitis

It is usually bilateral. It may start in one eye and later spread to the other. The spectrum of organisms causing bacterial conjunctivitis varies around the world. In most common cases, broad-spectrum topical antibiotics are the treatment of choice. Although most cases of bacterial conjunctivitis are self-limiting, treatment with antibiotics can lessen the patients' symptoms and the duration and chances of recurrence of the disease (Wood, 1999).

1.2.5.1.1 Hyperacute Bacterial conjunctivitis

Hyperacute bacterial conjunctivitis is a severe sight-threatening ocular infection that warrants immediate ophthalmic work-up and management. The infection has an abrupt onset and is characterized

by a copious yellow-green purulent discharge that reaccumulates after being wiped a way (Mannis, 1995). The symptoms of hyperacute conjunctivitis, which typically are rapidly progressive, also include redness, irritation and tenderness to palpation. Patients demonstrate marked conjunctival injection, conjunctival chemosis (excessive edema), lid swelling and tender preauricular lymph-adenopathy (Baum, 1997).

The most frequent causes of hyperacute purulent conjunctivitis are *Neisseria gonorrhoeae*, and *Neisseria meningitides*. These two infections have similar clinical presentations and they can be distinguished only by using laboratory tests (Morrow and Abbott, 1998).

Gonococcal ocular infection usually occurs in neonates (ophthalmia neonatorum) and sexually active young adults. Affected infants typically develop bilateral discharge three to five days after birth. Transmission of the *Neisseria* organism to infants occurs during vaginal delivery. In adults, the organism is usually transmitted from the genitalia to the hands and then to the eyes. If a gonococcal ocular infection is left untreated, rapid and severe corneal involvement is inevitable leading to blindness (Desenclos, *et. al*., 1992).

1.2.5.1.2 Acute Bacterial Conjunctivitis

Acute bacterial conjunctivitis typically occurs with burning, irritation, tearing and usually a mucopurulent or purulent discharge. Patients with this condition often report that their eyelids are matted together on a wakening. Conjunctival swelling and mild eyelid edema may be noted. The symptoms of acute bacterial conjunctivitis

are far less severe, less rapid in onset and progress at a much slower rate than those of hyperacute conjunctivitis (Jackson, 1993).

Acute conjunctivitis is the most common ocular infection in childhood, usually affecting children younger than 6 years old with a peak incidence between 12 to 36 months. Pediatric acute conjunctivitis is diagnosed by clinical signs of ocular purulent discharge or hyperemia of bulbar conjunctiva (Bodor, 1998).

The three most common pathogens in acute bacterial conjunctivitis are *Streptococcus pneumoniae, Haemophilus influenzae* and *Staphylococcus aureus*. In neonates or children *Streptococcus pneumoniae*, *Pseudomonas aeuroginosa* and *Staphylococcus aureus* may also cause acute conjunctivitis in neonates or children (Wald, 1997). In adult, the most common bacteria isolates from an acute conjunctivitis are *Streptococcus pneumoniae, Staphylococcus aureus* and *Staphylococcus epidermidis* (Prescott, *et. al*., 1999).

Although acute bacterial conjunctivitis is usually self-limited and does not cause any serious harm, there are several justifications for treatment. These include decreasing patient morbidity by shortening the course of the disease, reducing person-to-person spread, lowering the risk of sight-threatening complications such as corneal ulceration, and eliminating the risk of more widespread extraocular disease (Morrow and Abbott, 1998).

Cultures should be obtained in certain patients, including young children and debilitated persons. However, empiric treatment with a topical medication is a safe and cost-effective approach in

most patients with clinically mild acute bacterial conjunctivitis (Vaughan, *et. al*., 1996).

1.2.5.1.3 Chronic Bacterial Conjunctivitis and Blepharitis

Chronic bacterial conjunctivitis is most commonly caused by *Staphylococcus* species; however, other bacteria are occasionally involved. This type of conjunctivitis often develops in association with blepharitis, which is a common but often unrecognized inflammatory condition related to bacterial colonization of the eyelid margins. The symptoms of chronic bacterial conjunctivitis vary and can include itching, burning, a foreign-body sensation and morning eyelash crusting (Jackson, 1993).

The work-up of patients with chronic conjunctivitis and blepharo-conjunctivitis involves culturing the conjunctival and the eyelid margins swabs to identify the predominant bacterial pathogens (Mannis, 1995).

1.2.5.2 Ocular Chlamydial Infections

Ocular chlamydial trachomatis infections can occur in two distinct clinical forms; trachoma and inclusion conjunctivitis. Trachoma is a chronic, bilateral, cicatrizing keratoconjunctivitis caused by *Chlamydia trachomatis*. Inclusion conjunctivitis is acute ocular inflammation, primarily sexually transmitted disease that occurs in both newborns (ophthalmia neonatorum), and adults (adult inclusion conjunctivitis) (Dawson, 1995).

1.2.5.3 Ophthalmia Neonatorum

Ophthalmia neonatorum (neonatal conjunctivitis) is defined as a conjunctival inflammation that occurs during the first month of life. The most serious cause is *Neisseria gonorrhoeae*. The most common causes today are inclusion conjunctivitis (*Chlamydia trachomatis*) and bacteria, mainly *Staphylococcus* species and *Streptococcus pneumoniae* (O'Hara, 1993).

Most ophthalmic infections in the neonatal period are acquired during vaginal sexually transmitted diseases prevalent in the community. The transmission of disease from infected mother to her newborn infant is (30 - 50%). Ophthalmia neonatorum if left untreated can lead to blindness through corneal ulceration, perforation, scarring (Hammerschlag, 1993).

1.2.5.4 Viral Conjunctivitis

Most viral infections produce a mild, self-limiting conjunctivitis, but some have the potential to produce severe, disabling visual difficulties, and do not require antibiotic treatment unless a secondary bacterial infection occurs (Wood, 1999).

The two most common self-limiting form of viral conjunctivitis are epidemic keratoconjunctivitis and pharyngo-conjunctival fever. Adenovirus is the most common cause of viral conjunctivitis, which may be sporadic or epidemic (Jackson, 1993). Some strains cause a conjunctivitis combined with a preauricular adenopathy, fever and pharyngitis. Other strains cause epidemic keratoconjunctivitis, which has been so widespread as to necessitate

temporary closure of major eye centers, although the condition can also be caused by other viruses (Vaughan, *et. al*., 1996).

Herps simplex virus type 1 and 2, Varicella-Zoster virus and Epstein-Barr virus can cause conjunctivitis. Primary herps simplex is responsible for the majority of cases of nonepidemic follicular conjunctivitis in young children. Herps simplex virus keratoconjunctivitis can closely mimic the presentation of ocular adenovirus infection. In such patients, topical corticosteroid therapy can lead to severe ocular complications as a result of uncontrolled virus proliferation (Darougar, *et. al*., 1989).

1.2.5.5 Allergic Conjunctivitis

Ocular allergy encompasses a spectrum of distinct clinical conditions usually characterized by itching. The most common of these conditions is seasonal allergic rhinoconjunctivitis, also called hay fever rhinoconjunctivitis (Wood, 1999).

Seasonal allergic rhinoconjunctivitis is an IgE-mediated hypersensitivity reaction precipitated by small airborne allergens. The condition is usually, although not invariably, seasonal. Patients typically experience intermittent bouts of itching, tearing, redness and mild eyelid swelling. The personal or family history is often positive for other atopic conditions, such as allergic rhinitis, asthma or eczema (Abelson, *et. al*., 1995).

1.2.5.6 Fungal Conjunctivitis

Primary fungal conjunctivitis is infrequently encountered clinically. Candida conjunctivitis is most often seen in patients

receiving long-term corticosteroid eye drops. Blastomycosis involving the eyelid may cause a granulomatous conjunctivitis (Slack, *et. al*., 1992).

Lid or conjunctival nodules are the usual form of ocular involvement with *Sporothrix schenckii. Rhinosporidium seeberi* may cause a characteristic granulomatous inflammatory mass in the conjunctiva, which may become pedunculated (Yetman and Coody, 1997).

1.2.5.7 Parasitic Conjunctivitis

A number of ocular parasites can cause conjunctivitis in human. Blepharoconjunctivitis may be caused by *Leishmania donovani* in association with nodules of post-kalaazar dermal Leishmaniasis, with infection of the conjunctiva by spread from contaminated fingers (Nady, *et. al*., 1991).

Parasites are being recovered in increasing frequency from the conjunctivitis of persons with the acquired immunodeficiency syndrome (AIDS). *Microspordia* are small, ubiquitous, obligate intracellular parasites that usually cause infections in animals and rarely humans. *Encephalitozoon* species may cause a mild conjunctivitis with punctate keratopathy in patients with (AIDS) (Friendberg, *et. al*., 1990).

1.2.6 Epidemiology of Bacterial Conjunctivitis

1.2.6.1 Prevalence and Incidence

Conjunctivitis has worldwide distribution, affecting persons of all ages, races, social strata and both genders.

In the United States, its prevalence in the population ages (1 - 74) years was 13 in 1,000 according to the National Health Survey conducted in 1971 - 1972 (Ganley and Roberts, 1983). The prevalence of conjunctivitis is increased to 30 in 1,000, in 2002 – 2003 (Silverman and Bessman, 2003).

1.2.6.2 Risk Factors

Most cases of infection-associated conjunctivitis are sporadic or related to epidemic outbreaks. The specific risk factors for its development are well-defined and related to the underlying etiology (Miller, 2002).

Patients need to take steps to limit the spread of infections forms of conjunctivitis to others. Following good hygiene practices and limiting direct personal contact can reduce the potential for transmission of the infection (Smith, 2004).

1.2.7 Causes of Bacterial Conjunctivitis

1.2.7.1 *Haemophilus influenzae*

Haemophilus influenzae is a small, pleomorphic, gram-negative coccobacilli. It is non-motile, non-spore-forming, fastidious and facultative anaerobe. Some strains of *Haemophilus influenzae* possess a polysaccharide capsule (Hacker, *et. al*., 1997).

Haemophilus influenzae strains are classified as either capsulated (typeable) strains (based on the antigenic structure of the capsular polysaccharide), and uncapsulated (nontypeable) strains.

There are six structurally and antigenically distinct capsular types (serotypes) designated serotypes A to F (Joseph and St Geme, 2002).

Also, eight biotypes of *Haemophilus influenzae* have been identified. Biotyping and serotyping have been used to investigate patterns of colonization of *Haemophilus influenzae*, as well as to identify strains of the bacterium that appear to be associated with more severe infection (Al-Rawi, *et. al.*, 2002). Biotype I, serotype b, for instance, is often associated with severe meningitis in children. In contrast, non-serotypeable strains of *Haemophilus influenzae*, particularly biotypes II and III, are frequently commensal to the upper respiratory tract. While colonization with biotypes II and III usually does not progress to disease, these same biotypes have been implicated in the pathogensis of sinusitis, otitis media, acute and chronic exacerbations of lower respiratory tract infection, and acute and chronic conjunctivitis (Murphy, 1992).

Haemophilus "Loves heme" more specifically requires a precursor of heme in order to grow. Nutritionally, *Haemophilus influenzae* prefers a complex medium and requires preformed growth factors that are present in blood, specifically X factor (i.e. hemin) and V factor (NAD or NADPH). In the laboratory, it is usually grown on chocolate agar which contains both X and V factors (Chang, *et. al*., 2000).

The bacterium grows best at (35 - 37) C^o and has an optimal pH of 7.6. *Haemophilus influenzae* generally grows in the laboratory under aerobic conditions or under slight CO_2, although it is capable of glycolytic growth and of respiratory growth using nitrate as a final electron acceptor (Moxon and Murphy, 2000).

1.2.7.1.1 Pathogenicity

A major virulence factor of *Haemophilus influenzae* associated with it's pathogenicity, is the presence of capsule. Nontypeable strains (noncapsulated strains) are less invasive from typeable strains (capsulated strains), but they are apparently able to induce an inflammatory response that causes disease.

However, *Haemophilus influenzae* is predominant pathogens of conjunctivitis, it recovered in some cases of conjunctivitis with acute otitis media (Foxwell, *et. al.*, 1998). Strains of *Haemophilus influenzae* recovered from acute bacterial conjunctivitis have been shown to be identical to those in acute otitis media (Block, *et. al.*, 2000).

1.2.7.1.2 Virulence Factors

1.2.7.1.2.1 Capsule

Production of capsule and factors that mediate bacterial attachment to human epithelial cells are the primary virulence factors associated with *Haemophilus influenzae. Haemophilus influenzae* strains are typed into: typeable and nontypeable (based on capsular characteristic) (Foxwell, *et. al*., 1998).

Polysaccharide capsule, which plays a central rule pathogenesis and the immune response, is composed of a sugar-alcohol phosphate (i.e. polyribitol phosphate) complex. Differences in this complex are the basis for separating in encapsulated strains into one of six groups: type a, b, c, d, e, and f. Type b *Haemophilus influenzae* is most commonly encountered in serious infections in

humans, and nontypeable strains do not produce a capsule (St Geme, 2000).

Some *Haemophilus influenzae* infections are caused by the type b serotype, and its capsular polysaccharide containing ribose, ribitol and phosphate, is the proven determinant of virulence. Polyribosyl ribitol phosphate (PRP) is the most important virulence factor because it renders type b *Haemophilus influenzae* resistant to phagocytosis by polymorphonuclear leukocytes in the absence of specific anticapsular antibody, and it reduces the bacterium susceptibility to the bactericidal effect of serum (Musher, *et. al*., 1986).

1.2.7.1.2.2 Siderophores

Like other organisms, bacteria require iron as a co-factor for redox-dependent enzymes. Iron is an oxidant as well as a nutrient for invading microbial and neoplastic cells (Weinberg, 1997). Several researchers evaluate the role of iron acquisition as a virulence factor for both typeable and nontypeable *Haemophilus influenzae* (Jarosik, *et. al*., 1994).

A common and well defined mechanism for iron uptake in many bacteria involves synthesis of low molecular weight high-affinity iron chelators called siderophores. Once a bacterium secrets its siderophore into external milieu, a ferric iron- siderophore complex is formed and the iron is internalized via a specific set of protein in the outer membrane, periplasmic space and inner membrane. *Haemophilus influenzae*, however, doesn't synthesis its own siderophore (Smoot, *et. al*., 1998). Instead, it binds iron-loaded

transferring directly and transports the iron into the cell. Once the transferring is bound, the microorganism is able to extract the iron from the host glycoprotein and transport the iron into the bacterial cell via cascade of other transport proteins located in the periplasmic space and inner membrane. The expression of genes that encode the component of iron acquisition systems is regulated by the iron concentration of the growth medium (Morton and Williams, 1990).

1.2.7.1.2.3 Extracellular Proteases

Protease represents a class of enzymes that are involved in essential biological processes like blood clotting, controlled cell death, and tissue differentiation. Proteases assist the hydrolysis of large polypeptides chain (Beynom and Bond, 1989).

Haemophilus influenzae has distinct immunoglobulin A1(IgA1) proteases that may be involved as a virulence factor by inter defenses. *Haemophilus influenzae* strains produce three types of IgA1 protease, all of which cleave the heavy chain of IgA1in the hinge region at one of several postproline sites (Kilian and Poulsen, 1992).

The IgA1 proteases derived from *Haemophilus influenzae* are antigenically quite diverse, with more than 30 antigenic types having been recognized. The polymorphism is more pronounced in nontypeable *Haemophilus influenzae* than in capsulated *Haemophilus influenzae* (Lomholt, *et. al*., 1993).

1.2.7.1.2.4 Adherence Factors

Haemophilus influenzae infections, including otitis media, sinusitis, pneumoniae and persistent infections in chronic bronchitis patients, are preceded by airway colonization, which is a process facilitated by fimbriae (Cerquetti, *et. al*., 2000).

Also, *Haemophilus influenzae* biogroup aegyptius is an important cause of conjunctivitis, which expresses surface structures (pili), which facilitate attachment to conjunctival cell (St Geme, *et. al*., 1991). Pili or Fimbriae seem to be multifunctional in the pathogenesis of *Haemophilus influenzae* infections. It is not known, however, whether fimbriae are involved in the pathogenesis of persistent infections, and if so, whether its function is at the onset or during persistence of infection (van Schilfgaarde, *et. al*., 2000).

1.2.7.1.2 *Staphylococcus aureus*

Staphylococcus aureus is a gram-positive, spherical in shape, it is single or in clusters, and occasionally in short chains. It is a non-motile, non-spore-forming, facultative anaerobe; it is catalase-positive, oxidase-negative, and coagulase-positive. In common with other facultative anaerobes, *Staphylococcus aureus* can grow in the absence of oxygen by has both fermentation and oxidative pathways (Masalha, *et. al*., 2001).

Several studies suggest that oxygen plays a role in the pathogenesis of *Staphylococcus aureus* in both its capacity to produce virulence factors and its ability to persist and grow in different and often hostile environmental niches (Yarwood and Schlievert, 2000).

1.2.7.2.1 Pathogenicity

Staphylococcus aureus express many potential virulence factors such as: surface proteins that promote colonization of host tissues, factors that probably inhibit phagocytosis (capsule, immunoglobulin binding protein A), toxins that damage host tissues and cause disease symptoms (Archer, 1998).

Staphylococcus aureus the major causative agent in surgical wound infections and epidermal skin diseases in newborn infants. Also, it can cause septicaemia, folliculitis, boil, furnculosis, scalded skin syndrome, paronchia and mastitis (Chigbu and Ezeronye, 2003). *Staphylococcus aureus* is the most common cause of bacterial conjunctivitis worldwide. This ubiquitous-aerobic gram-positive organism which colonizes humans within afew days after birth is not normally part of the ocular flora. It is seen both singly and in pairs and less commonly in clusters in conjunctival smears. The ability of this organism to elaborate biologically active substances including toxins accounts for the multiple manifestations. It produces blepharitis, marginal ulcers, epithelial; keratitis and phlyctenulosis in the eye in addition to conjunctivitis (Schneewind, *et. al.*, 1995).

1.2.7.2.2 Virulence Factors

1.2.7.2.2.1 Capsular Polysaccharide

Staphylococcus aureus isolates can produce one of 11 different capsular serotypes. Serotypes 5 and 8 are the predominant which account for about 80% of isolates (Arbeit, *et. al*., 1984).

Staphylococcus aureus isolates producing capsular polysaccharide serotypes 1 and 2 have been shown to be virulent in

animal models; mutants deficient in capsule production are less virulent. Clinical isolates of serotype 1 and 2 are rare. Serotypes 5 and 8 have been shown to be anti-phagocytic, some in vivo studies suggest that these capsular polysaccharides do not affect virulence (Xu, *et. al*., 1992).

1.2.7.2.2.2 Siderophores

Staphylococcus aureus possesses both siderophore-mediated and nonsiderophore iron uptake systems. Each system plays a role during pathogenesis. Several *Staphylococcus aureus* strains produce siderophores. Two of these siderophores, staphyloferrin A and staphyloferrin B are of the polycarboxylate class, while the third aureochelin is chemically uncharacterized (Dale, *et. al*., 2004).

Staphylococcus aureus may be able to scavenge various different source of host iron (e.g., heme and hemoglobin) during the establishment of an infection, and indeed, *Staphylococcus aureus* does possess the ability to bind heme and hemoglobin involved in the transport of staphylobactin (Mazmanian, *et. al*., 2003).

1.2.7.2.2.3 Extracellular Proteases

Several *Staphylococcus aureus* strains have distinct immunoglobulin A1(IgA1) proteases that can cleave and degrade a number of important host proteins including the heavy chains of all human immunoglobulin classes, plasma proteinase inhibitor and elastin (Prokesova, *et. al*., 1992), indicating that they are important virulence factors. Recent reports have suggested that proteases also play a role in the transition of *Staphylococcus aureus* cells from an

adhesive to an invasive phenotype by degrading bacterial cell surface proteins, such as fibronectin binding protein and protein A (Karlsson, *et. al*., 2001).

Staphylococcus aureus produces four major extracellular proteases: serine protease, a cysteine protease, metalloprotease and a second cysteine protease (also named staphopain) (Karlsson and Arvidson, 2002).

1.2.7.2.2.4 Adherence Factors

Some of the most well-characterized colonization factors in gram-positive bacteria, the polypeptides of the antigen I/II family. Surface proteins of the antigen I/II family contain alanine-rich repeats, which adopt an α-helical coiled-coil structure, proline rich repeats and a carboxy-terminal region that includes the gram-positive cell wall anchor motif LPXTG (Wizemann, *et. al*., 1999).

Staphylococcus aureus expresses fibronection-binding adhesions. Two genes encoding for fibronection-binding proteins have been identified in *Staphylococcus aureus-fnbA* and *fnbB*. Fibronection binding activity is critical in pathogenesis because it allows the bacteria to adhere to extracellular matrix components including fibronection and collagen. This can result in cutaneous infections and in life-threatening bacteremia and endocarditis (Schennings, *et. al.*, 1993).

1.2.7.3 *Streptococcus pneumoniae*

Streptococcus pneumoniae is a gram-positive, Lancet-shaped cocci, it is seen as pairs, diplococci, but it also occurs singly and in short chains. It is alpha hemolytic when cultured on blood agar, but

can cause Beta hemolysis during anaerobic conditions. It is a non-motile, non-spore-forming, fastidious, fermentative aerotolerant anaerobe (Facklam and Elliott, 1995).

Streptococcus pneumoniae can be divided into more than 80 serotypes (based on the differences in capsular polysaccharide structure). The distribution of types isolated from adults differs substantially from that isolated from children. *Streptococcus pneumoniae* exists in two forms; capsulated and uncapsulated strains, only capsulated strains have been isolated from clinical material, and found to be at least 10^5 times more virulent than strains lacking the capsule (Watson and Musher, 1990). Recently, pneumococcal mutants that apparently differ only in the type of capsular polysaccharide have been produced (Kelly, *et. al*., 1994).

1.2.7.3.1 Pathogenicity

The pathogenicity of *Streptococcus pneumoniae* has been attributed to various structures, most of which are situated on its surface. The best defined virulence factor which plays an important role in pathogenesis is the polysaccharide capsule which provides resistance to phagocytosis and thus promotes the escape of pneumococci from the host immune defense. Other factors, including cell wall components and the intracellular toxin pneumolysin, are involved mainly in the inflammation caused by infection (Musher, 1992).

Streptococcus pneumoniae is now the most common cause of meningitis in children and older adults, and can cause otitis media, sinusitis, bactermia, pericarditis and arthritis. Nontypeable,

Streptococcus pneumoniae is a common cause of epidemic bacterial conjunctivitis. A previous molecular fingerprinting study identified a clone of nontypeable *pneumococcus* which lacks the exterior polysaccharide capsule that was responsible for a recent outbreak of conjunctivitis (Barker, *et. al*., 1999).

1.2.7.3.2 Virulence Factors

1.2.7.3.2.1 Capsule

The capsule has long been recognized as the major virulence factor of *Streptococcus pneumoniae*. It is composed of polysaccharide completely envelopes the pneumococcal cells. During invasion, the capsule is an essential determinant of virulence. The capsule interferes with phagocytosis by preventing C_3b opsonization of the bacterial cells. Ninety different capsule types of pneumococci have been identified and form the basis of antigenic serotypes of the organism (Alonsodevelasco, *et. al*., 1995).

Geographical, temporal and age differences in the distribution of the 90 different serotypes, and the ability of *Streptococcus pneumoniae* to transfer cassettes of capsular genes from one strain to another leading to a change in capsule specifity, all have potential implications for vaccine strategy (Tuomanen, *et. al*., 1995).

1.2.7.3.2.2 Siderophores

Streptococcus pneumoniae is a causative agent for bacterial pneumoniae, otitismedia, meningitis, bacteremia and pneumocuccal conjunctivitis. Mechanisms for acquisition of iron by this organism under low-iron conditions were investigated. Siderophores

production was not detected by either chemical or biological methods. Its utilization of iron-containing compounds found in human hosts was tested. Both hemin and hemoglobin supported the full growth of *Streptococcus pneumoniae* in a culture lacking other iron source, while lactoferrin and transferrin failed to do so (Tai, *et. al*., 1993).

1.2.7.3.2.3 Extracellular Proteases

All strains of *Streptococcus pneumoniae* tested by immnnoelectrophoretic techniques have been shown to produce extracellular protease that cleaves immunoglobulin A1(IgA1) into Fab and Fc fragments. Proteases studied so far have been from human isolates of *Streptococcus pneumoniae* and have been shown to cleave only human IgA1 (Kilian and Reinhold, 1986).

IgA proteases have been implicated as potentially important virulence factors produced by a variety of human bacterial pathogens. Most of these organisms are known to be pathogenic for only human beings and do not cause significant naturally occurring disease in other animal species. *Streptococcus pneumoniae* is an exception because this organism resides as unapparent infection in the respiratory tract of several animal species (Proctor and Manning, 1990).

1.2.7.3.2.4 Adherence Factors

Streptococcus pneumoniae expresses two antigens I/II family. Binding to fibronection is essential for the attachment of *Streptococcus pneumoniae* to respiratory endothelial cells. This binding activity is mediated by several fibronectin binding proteins.

Included in this group are *PsaA* from *Streptococcus pneumoniae* (Wizemann, *et. al*., 1999). These membrane-bound lipoproteins are part of a larger family of ATP-binding cassette metal permease, involved in the acquisition of manganese.

The *Streptococcus pneumoniae* homologue of *FimA*, *PsaA*, also has a role in pathogenesis. While it commonly colonizes the nasopharynx of healthy persons, *Streptococcus pneumoniae* is a common pathogen in children and older adults and a leading cause of otitis media, pneumonia sepsis and meningitis (Novak, *et. al*., 1998).

1.2.7.4 *Neisseria gonorrhoeae*

Neisseria gonorrhoeae is a gram-negative cocci, usually seen in pairs with adjacent flattened sides. It is non-motile diplococcus, non-spore-forming, and grows best under aerobic conditions, but some can grow in an anaerobic environment. Gonococcus can ferment only glucose and differ antigenically from the other *Neisseria* (Katz, *et. al*., 1998).

Neisseria gonorrhoeae is antigenically hetrogencous and capable of changing its surface structures in vitro and presumably invivo to avoid host defenses. Surface structures include the Pili (Fimbriae), Por (Protein I) Opa (Protein II), Rmp (Protein III), Lipooligosacchride (LOS), and other proteins (Handsfield and Sparling, 1995).

Neisseria gonorrhoeae is more fastidious and requires an enriched chocolate agar for growth on primary culture, Thayer-Martin medium which contains colistin (to inhibit gram-negative

bacilli), nystatin (to inhibit yeast), and vancomycin (to inhibit gram-positive bacteria) (Matters, *et. al*., 1998).

1.2.7.4.1 Pathogenicity

Gonorrhoeae in adults is almost transmitted by sexual intercourse. The bacteria adhere to columnar epithelial cells, penetrate them, and multiply on the basement membrane. Adherence is mediated through fimbriae and Opa (Protein II). *Neisseria gonorrhoeae* has a strong affinity for columnar and transitional tissues of the mucosal cells beings to produce substances like proteases, elastases which play an important role in pathogenicity (Katz, *et. al*., 1998)

Neisseria gonorrhoeae is the major cause of neonatal conjunctivitis (Ophthalmia Neonatorum) which is lead to childhood blindness through corneal ulceration, perforation and scarification (O'Hara, 1993).

1.2.7.4.2 Virulence Factors

1.2.7.4.2.1 Capsule

Neisseria gonorrhoeae strains don't have polysaccharide capsules, but it is capable of causing diseases by expressing other virulence factors such as pili (fimbriae), which play an important role in their pathogenecity (Katz, *et. al*., 1998).

1.2.7.4.2.2 Siderophores

Heme is the most abundant source of iron in the body. Many pathogenic microrganisms posses specific heme uptake systems that

harness heme iron for metabolic needs. *Neisseria gonorrhoeae* does not produce siderophores (Weinberg, 1997), but it possess multiple heme uptake pathways to scavenge iron, through the use of TonB-dependent transferrinn, lactoferrin and hemoglobin receptors (Cornelissen, *et. al.*, 1992).

Protection against heme toxicity must be based on the destruction of heme and not on inability of heme to reach the cell's interior (Schryvers and Stojiljkovic, 1999).

1.2.7.4.2.3 Extracellular Proteases

The neisserial IgA1 proteases are derived from precursor molecules which are secreted through an autocatalytic process. Strains of *Neisseria gonorrhoeae* produce two distinct extracellular IgA1 proteases, which cleave the heavy chain of human immunoglobulin A1 (IgA1) at different points within the hings region (Pohlner, *et. al*., 1992).

The role of IgA1 protease in neisserial pathogenesis comes from it's ability to inhibit TNF-α-mediated opoptosis in human monocytic tumor cells; it has exclusive association with human pathogens (Wolff and Stern, 1995).

1.2.7.4.2.4 Adherence Factors

Neisseria gonorrhoeae strains have numerous pili extensions from the cell surface. Piliated gonococci are better able to attach to human mucosal surfaces than nonpiliated organisms. Pili also contribute to killing by neutrophils (Katz, *et. al*., 1998).

Neisseria gonorrhoeae first attaches to epithelial cells by means of its fimbriae, specifically N-methylphenylalanine (type 4) pili, the main subunit of which is pilE (major fimbrial protein) which initiates binding to epithelial cells, and the pilus associated adhesion pilc (Meyer, *et. al*., 1994).

1.2.7.5 *Klebsiella pneumoniae*

Klebsiella pneumoniae is a gram-negative, usually capsulated rod-shaped, facultative anaerobic. It is non-motile, non-spore-forming flagellated, produces large sticky colonies when plated on nutrient media (Podschun and Ulmann, 1998).

It is a member of the family *Enterobacteriaceae*, which produce lysine decarboxylase but not ornithine decarboxylase and it is generally positive in the voges-proskauer test. *Klebsiella pneumoniae* usually have well-developed polysaccharide capsules, which give their colonies their characteristic mucoid appearance (Williams and Tomas, 1996).

1.2.7.5.1 Pathogenicity

Klebsiella's pathogenicity can be attributed to its production of a heat stable enterotoxin. The major virulence factor of *Klebsiella pneumoniae* which play essential role in pathogenesis, is the thick capsule (it’s antiphagocytic), and fimbriae (pili) (Padschun, *et. al*., 2001).

Klebsiella infections are encountered more often now than in past. This is probably due to bacteriums antibiotic resistance properties. In general, *Klebsiella pneumoniae* can cause pneumoniae,

urinary tract infection, and bacterial conjunctivitis. *Klebsiella pneumoniae* is one of the etiologic agents of conjunctivitis in early infancy; this type of bacteria was transmitted to the infants' eyes after birth and not from the birth canal (Krohn, *et. al*., 1993).

1.2.7.5.2 Virulence Factors

1.2.7.5.2.1 Capsule

Thick capsule is a major virulence factor of *Klebsiella pneumoniae*, which is composed of complex acidic polysaccharides. The capsular repeating subunits consisting of four to six sugars and very often, uronic acids (as negatively charged components). The capsular material forms thick bundles of fibrillous structures covering the bacterial surface in massive layers (Amako, *et. al*., 1988).

The capsule protects the bacterium from phagocytosis by polymorphonuclear granulocytes on the one hand, and prevents killing of the bacteria by bactericidal serum factors on the other (Podschun, *et. al*., 1992).

1.2.7.5.2.2 Siderophores

Klebsiella pneumoniae produces two types of siderophores that are capable of competitively taking up iron bound to host proteins. The production of both enterobactin (a cyclic trimer of 2, 3-dihydroxy-benzoyl-serine), and aerobaction (consists of hydroxymatic acid). In the genus *Klebsiella pneumoniae* has been demonstrated. However, enterobactin is synthesized by almost all strains (Podschun, *et. al*., 1992).

1.2.7.5.2.3 Extracellular Proteases

Proteases enzymes are important enzymes that play essential roles in pathogenesis. *Klebsiella pneumoniae* strains can not produce extracellular proteases but can depend on other mechanisms for uptake materials as a need to their growth, such as production of siderophores (Al-Saedi, 2000).

1.2.7.5.2.4 Adherence Factors

The adhesive properties in the *Enterobactriaceae* are generally mediated by different types of pili. Pili are nonflagellar, filamentous projections on the bacterial surface. There are different types of pili described in *Enterobactriaceae*, but there are two predominant types in *Klebsiella spp.* Type 1 (common) pili and type 3 pili are the best investigated of the bacterial adhesive. This fimbrial type (Type 3) is synthesized only by *Klebsiella*. Later studies demonstrated that type 3 pili occur in many enteric genera (Podschun and Ullmann, 1998).

1.2.8 Effect of Antibiotics on Bacterial Conjunctivitis

Many antibiotics can be used as first-line therapy in bacterial conjunctivitis. Most cases of routine bacterial conjunctivitis respond to the commercially available combination of antibiotics. Bacterial infection is more common in the affected eye and is usually treated with antibiotic eye drops or ointments that cover a broad range of bacteria (Wald, 1997).

The clinical response to the antibiotic should be assessed after the patient has completed a short course of therapy. If the inflammation has resolved, the antibiotic should be discontinued. In

such patients, laboratory test results can be used to direct changes in therapy. In general, in patients with acute bacterial conjunctivitis, most remit spontaneously, but antibiotic treatment further increases clinical and microbiological remission rates (Sheikh and Hurwitz, 2001).

Topical antibiotics are prescribed in an attempt to shorten the illness, reduce complications and re-infection. Also, topical antibiotics may improve the clinical remission rate at (2 - 5) days by 30%. Examples of currently available topical broad-spectrum antibiotics include erythromycin ointment and bacitracin-polymyxin B ointment (e.g. polysporin ophthalmic ointment), as well as combination solutions such as trimethoprim-polymyxin B (e.g. polytrim). These medications are well tolerated and they provide excellent coverage for most conjunctival pathogens in both children and adults (Wanger, 1995).

Most patients should be treated with systemic antibiotics supplemented by topical ocular antibiotics and saline irrigation. Because of the increasing prevalence of penicillin resistance *Neisseria gonorrhoeae* in the United States, ceftriaxone, a third-generation cephalosporin, currently the new drug of choice for treatment of such cases. Spectinomycin or oral ciprofloxacin can be used for patients who are allergic to penicillin (Haimovici and Roussel, 1989). Over 30% of patients with gonococcal conjunctivitis have concurrent chlamydial venereal disease. For this reason, it is advisable to treat patients with supplementary oral antibiotics that are effective against *Chlamydia spp* (Morrow and Abbott, 1998).

In addition to that, aminoglycosides, such as Gentamycin, Tobramycin and Neomycin are inexpensive choices for the treatment of acute bacterial conjunctivitis. However, Aminoglycosides are associated with a relatively high incidence of toxicity to the corneal epithelium (primarily with prolonged use). Neomycin, in particular, can cause local oculocutaneous allergic reactions. For this reason, topical ophthalmic preparations containing neomycin probably should be avoided as first-line therapy (Abelson and Welch, 1994).

In topical form, Chloramphenicol and Tetracycline are commonly used to treat bacterial conjunctivitis. Chloramphenicol which is available in both drop and ointment forms, has a broad-spectrum of antimicrobial activity. Although chloramphenicol is generally well tolerated, topical application of this agent has been associated with afew cases of aplastic anemia. Tetracycline is available only in an ointment form (Rayner and Buckley, 1996).

Also, Lorriane-Steefl, (2003) have found that the treatment in the newborn infant consists of administration of tetracycline ointment to the conjunctiva and erythromycin orally or through intravenous therapy for fourteen days. In adults, tetracycline ointment or drops should be applied to the conjunctiva and oral tetracycline, erythromycin should be taken for three weeks. Oral tetracycline should not be administered to children whose permanent teeth have not erupted (Lohr, 1991).

Furthermore, the fluoroquinolones, which include Ciprofloxacin, Ofloxacin and Norfloxacin are a new class of potent topical antimicrobials. Agents from this class are commonly used to treat bacterial conjunctivitis (Morrow and Abbotts, 1998).

On the other hand, Idu and Odjimogho, (2003) have found that Ciprofloxacin is the most sensitive of the three drugs and hence should be the first choice of the fluoroquinolones for the treatment of bacterial conjunctivitis.

CHAPTER TWO

Materials

and

Methods

2. Materials and

Part One: Materials

2.1 Instruments and Chemical Materials

Table (2.1)

Instruments used in the study

No.	Instruments	Company
1	Autoclave	Stermite , Japan
2	Incubator	Memmert , Germany
3	Oven	Memmert , Germany
4	Water bath	Memmert , Germany
5	Distillator	GFL-Germany
6	Sensitive electronic balance	A & D, Japan
7	Refrigerator	Concord , Italy
8	Light microscope	Olympus , Japan
9	Benson burner	Germany
10	Inoculating loop	Japan
11	Inoculating needle	Japan
12	Micropipette	Oxford , USA
13	Millipore filter	Sartorius membrane filter GmbH, W. Germany
14	PH meter	Hoeleze & Cheluis, KG, Germany
15	Centrifuge	Harmle, Japan

Table (2. 2)

Chemical materials used in the study

No.	Chemical Materials	Company
1.	Na_2HPO_4, NaCl, NH_4Cl, $CaCl_2$, KH_2PO_4, K_2HPO_4, KOH, $MgSO_4$.	Merk - Darmstadt.
2.	H_2O_2, Kovac's reagent, Urea solution, Glucose, dipyridyl, 99% alcohol.	Fluka chemika-Switzerland.
3.	Tetra-Methyl-P-Phenylene-Diamine-Dihydrochloride, α-naphthol, Trichloroacetic acid.	B.D.H.

2.2 Culture Media

2.2.1 Agar-Agar Media (MAST)

It was prepared as described by the manufacturing company (MAST).

2.2.2 Blood Agar Base (MAST)

It was prepared as described by the manufacturing company (MAST), and it was used for primary plating for culturing of colonies and was especially useful for detecting hemolytic activity of bacteria. It was also used for the demonstration of the "Satellite Phenomenon" by *Haemophilus influenzae*.

2.2.3 Brain Heart Infusion Agar (MAST)

It was used for the cultivation of fastidious bacteria and as a storage medium after the addition of 5% glycerol, prepared as showed by Cruickshank, *et. al*., (1975).

2.2.4 MacConky Agar (MAST)

It was used for the differentiation of the gram-negative bacteria from gram-positive bacteria, and was prepared as described by manufacturer company (MAST).

2.2.5 (MR-VP) Medium (MAST)

It was used for the differentiation of the streptococci, and was prepared as described by the manufacturing company (MAST).

2.2.6 Muller-Hinton Agar (OXIOD)

It was used as antimicrobial susceptibility testing medium, and was prepared as recommended by Cruickshank, *et. al*., (1975).

2.2.7 M9 Media

6 gm of Na_2HPO_4, 3 gm of KH_2PO_4, 0.5 gm of NaCl, and 1 gm of NH_4Cl; were dissolved in 950 ml of distill water with 2% agar, and then sterilized by autoclave. After cooling, 2 ml of 1M of $MgSO_4$, 10 ml of 20% glucose and 0.1 ml of 1M of $CaCl_2$ (sterilized separately by filtration) were added, then the volume was completed to 1000 ml. This media was used for the detection of the Siderophores and Extracellular Proteases production (Miniatis, *et. al.*, 1982).

2.2.8 Nutrient Broth (DIFCO)

It was prepared as in Difco Manual, (1953).

2.2.9 Peptone Water Media (MAST)

This medium was prepared as described by manufacturing company (MAST), and it was used to detect the ability of bacteria to produce Indole.

2.2.10 Simon Citrate Agar (DIFCO)

It was prepared as in Difco Manual, (1953), and it was used to discover the bacterial ability to exhaust citrate as the only source of carbon.

2.2.11 Urea Base Agar (DIFCO)

It was prepared as in Difco Manual, (1953), and it was used to detect the production of Urease by some bacteria.

2.3 Reagents and Solutions

2.3.1 Methyl red reagent

0.1 gm of methyl red was dissolved in 300 ml of 99% alcohol and then the volume was completed to 500 ml by distilled water; it was used to detect glucose hydrolysis (MacFaddin, 2000).

2.3.2 Voges-Proskauer reagent

A- 5 gm of α-naphthol was dissolved in 100 ml of 99% alcohol.

B- 40 gm of KOH was dissolved in 100 ml of distilled water; it was used to detect glucose hydrolysis (Collee, *et. al*., 1996).

2.3.3 Oxidase reagent

It was prepared by dissolving 1 gm of Tetra-Methyl-P-Phenylene-Diamine- Dihydrochloride in 100 ml of distill water and it was stored in dark container (Baron, *et. al.*, 1995).

2.3.4 Catalase reagent

Catalase reagent was prepared by adding 3 gm of H_2O_2 to 100 ml of distill water and storing it in dark container, it was used to study bacterial ability to produce catalase (Baron, *et. al.*, 1995).

2.3.5 Phosphate buffer saline solution

80 gm of NaCl, 0.34 gm of KH_2PO_4, and 1.12 gm of K_2HPO_4; were dissolved in 1000 ml of distill water. The PH was 7.3 then it was sterilized in autoclave (Baron, *et. al*., 1995).

2.3.6 Gram-stain solutions

The solutions were prepared according to the required microbiological methods (Carsen, 1990).

2.4 Antibiotics Discs

They are shown in Table (2.3).

Table (2.3)

Antibiotic Discs Potency

Discs potency (μg)						
CL	**CP**	**GN**	**PN**	**TE**	**CTX**	**ER**
30	30	30	10	30	25	25

CL: Chloramphenicol; CP: Ciprofloxacin; GN: Gentamycin;

PN: Penicillin; TE: Tetracycline; CTX: Cefotaxime; ER: Erythromycin

Part Two: Patients and Methods

2.5 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. Clinical features, like: ocular pain, redness, swollen eyelids, discharge.
4. Antibiotic treatment, the patients must not have any antibiotics to avoid the negative false results.

2.6 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.

2.7 Laboratory Diagnosis

According to the diagnostic procedures recommended by Bergy's manual for determinative bacteriology (Holt, *et. al.*, 1994); (Collee, *et. al.*, 1996); (Baron and Finegold, 1990), the isolation and identification of bacteria were performed as follows:

2.7.1 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 colonies suspected to be pathogens 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.

2.7.2 Biochemical Tests

2.7.2.1 Catalase Test

A small amount of bacterial growth which grew on a medium in an age of 24 hours was transferred by sterile wooden stick onto the surface of a clean, dry glass slide, and one drop of 3% H_2O_2 was

added to it. The formation of gas bubbles indicated the positive results (Collee, *et. al.*, 1996).

2.7.2.2 Oxidase Test

A small portion of the colony to be tested was transferred by sterile wooden stick to filter paper saturated with indicator prepared soon. When the smear turned to violet, the oxidase test was positive (Collee, *et. al.*, 1996).

2.7.2.3 Coagulase Test

Coagulase test was an important method for differentiating between pathogenic and non-pathogenic strains of *Staphylococcus*. Several colonies of bacteria were transferred by a loop to a tube containing 0.5 ml of plasma; the tube was covered to prevent evaporation and was incubated at 37C^{o} overnight. A positive test was denoted by a clot formation in the test tube after the allotted time. A negative test resulted in the plasma remaining free-flowing with no evidence of a clote (MacFaddin, 2000).

2.7.2.4 Indole Test

Indole is a component of the amino acid tryptophan. Some bacteria have the ability to break down tryptophan for nutritional needs using the enzyme tryptophanase, with the production of indole, pyruvic acid and ammonia. Peptone water media was prepared in tubes then it was sterilized by the autoclave. After that the broth was inoculated with bacterial colonies and it was incubated for (48 - 72) hours at 37C^{o}. Testing for indole production

was fulfilled by adding (6-8) drops of Kovac's reagent (p-dimethylaminobenzaldehyde in amyl alcohol). Kovac's reagent reacted with indole and produced a red color as ring on the surface of the broth which was a positive reaction. A yellow color of ring was a negative reaction (MacFaddin, 2000).

2.7.2.5 Methyl Red Test

The methyl red test was performed on 1 ml of MR-VP broth cultured by the organism and then it was incubated for 24 hours at 37C^{o}. After that, the (6 - 8) drops of methyl red reagent were added to culture. The change of color to orange-red was a positive reaction; the change of color to yellow (pH over 6.0) was a negative reaction (MacFaddin, 2000).

2.7.2.6 Voges-Proskauer Test

Some bacteria have the ability to produce Acetyl methyl carbinol in the presence of KOH and O_2 for oxidation it to diacetyl.

The voges-proskauer test was performed by using MR-VP broth. The inoculated media was incubated for 24 hours at 37C^{o}, afterward, 15 drops of 5% α-naphthol (reagent A) were added and followed by 10 drops of 40% KOH (reagent B). The mixture was shaken well and was allowed to stand for up to 15 minutes before calling a reaction negative. If positive, the culture turned red at the surface of the liquid, and the color spread gradually throughout the tube. The positive result indicates a partial analysis of glucose which produces (Acetyl methyl carbinol) (Collee, *et. al.*, 1996).

2.7.2.7 Citrate Test

The citrate test was used to determine the ability of a bacterium to utilize citrate as its only source of carbon. After the sterilization of Simon citrate slants by autoclave, the bacterial culture was inoculated and incubated for (24 - 48) hours at 37C^{o}. The positive result was a change of the color of media from green to blue. The unchanging of the color was a negative reaction (Collee, *et. al.*, 1996).

2.7.2.8 Urease Test

Urease is an enzyme that breaks the carbon-nitrogen bond of amides to form carbon dioxide, ammonia and water. The urea base agar was sterilized by autoclave, after that it was cooled to 50 C^{o} and urea substrate was added to it and it was poured in sterile tubes; then it was inoculated by bacterial cultures, which were incubated for (24 - 48) hours at 37C^{o}. When urea was broken down, ammonia was released and the pH of the medium increased. This pH change was detected by a pH indicator that turned pink in a basic environment. A pink medium indicated a positive test for urease. Failure of deep pink color to develop was a negative reaction (MacFaddin, 2000).

2.7.2.9 Satellite Test for *Haemophilus influenzae*

Inoculate the surface of a blood agar plate containing 5% sheep blood with a lawn of the test organism (*Haemophilus influenzae*) by streaking or surface spreading. Then place single streak of the beta-hemolytic *Staphylococcus aureus* down the center

of the plate, and then incubate the plate for (24 - 48) hours at 37C^{o}. The positive result is the organism requiring X and V factors which will grow in the area adjacent to the *Staphylococcus* streak due to the availability of the growth factors. The negative result is the organism not requiring X and/or V factors which will grow uniformly on the plate wherever the lawn of the growth is streaked (Washington, 1981).

2.8 Virulence Factors Tests

2.8.1 Capsule Stain Test (Hiss's Method)

A thin smear of organism is prepared on the slide and allow to air dry. Treat thins freshly prepared smear with hot 1% crystal violet for about four minutes.

The smear is washed with 20% solution of copper sulfate and allowed to dry in air, and then it is examined under microscope, capsule appear as a light blue and the body of the bacteria stain a deep purple (Cruickshank, *et. al.*, 1975).

2.8.2. Hemolysin Production Test

Hemolysin production was shown on blood agar media. The result were obtained after the incubation of the non-cultured plates for 24 hours at 37C^{o} to exclude any contamination of blood, then the organism was inoculated at this blood agar plates and was incubated again for 24 hours at 37C^{o}. The presence of hemolysis (either α or β) should be detected around the colonies (de Boy, *et. al.*, 1980).

2.8.3 Siderophores Production Test

M9 Media was prepared and then supplemented with 2% agar. After sterilization in autoclave and cooling to 50C^{o}, 0.25 mg/L glucose (sterilized by filtration) and 200 µmol/L of dipyridyl were added. Then the organisms were inoculated into this media and it was incubated for 24 hours at 37C^{o}. The results were seen when the growth of organism was present or not (Nassif, *et. al*., 1989).

2.8.4 Extracellular Protease Production Test

This method was carried out by using M9 media supplemented with 2% agar. After sterilization in autoclave and cooling at 50C^{o}, 0.25 mg/L glucose (sterilized by filtration) was added, and then, the media was supported by 1% gelatin. After the inoculation of this media with bacterial strain and incubation for (24 - 48) hours at 37C^{o}, 3 ml of Trichloroacetic acid (5%) was added to precipitate the protein. The positive result was read by observing a transparent area around the colony (Piret, *et. al.*, 1983).

2.8.5 Haemagglutination Test (HA)

It was performed to show the ability of bacterial group to produce adherence factors.

RBC suspension was prepared from the human blood (group A). Human blood washed with phosphate buffer saline (repeated 3 times), 3% suspension from RBC (V/V) was then prepared and at the same time, brain heart infusion agar was prepared and supplied by blood, then it was inoculated with bacteria and

incubated at 37C^{o} for 24 hours, after that, place 1 drop of human red blood cells with loopful onto a clean slide, to this drop added 1 drop of bacterial culture by using a flamed loop and bacterial culture were mixed with human red blood cells on clean slide. The blood agglutination with bacteria was detected in room temperature during (1 - 5) minutes. Agglutinated red blood cells in suspension (positive reaction) had a clumped appearance distinct from non-agglutinated red blood cells (negative reaction) (Francis, *et. at*., 2002)

2.9 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 using a caliper. Zone size was compared to standard zones to determine the susceptibility or resistance of the organism to each antibiotic (MacFaddin, 2000).

CHAPTER THREE

Results

and

Discussion

3. Results and

3.1 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 (3.1).

Table (3.1)

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%)**

From the results in Table (3.1), it is clear that conjunctivitis occurs in all age groups, there is also decrease in the percentage of conjunctivitis among patients with increasing age.

Also, 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 (3.2).

Table (3.2)
Distribution of conjunctivitis among children and adults

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

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 Stenson, *et. al.*, (1981) have showed that a high incidence of conjunctivitis occurs in children.

Also, Modarres, *et. al.*, (1998) have pointed out that the incidence of conjunctivitis in children is (66%).

Furthermore, in this study, it was found out that both sexes had been affected with conjunctivitis, without any preference regarding sex, Table (3.1).

This result is in agreement with the results obtained by Silverman and Bessman, (2003) who have observed that both sexes can be affected with conjunctivitis, and no sex predilection exists.

3.2 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 (68.3%) showed positive cultures (bacterial isolates), no growth was seen in the other samples 38 samples (31.7%) which could indicate the presence of microorganisms that might be cultured with difficulty such as fungi viruses, Chlamydia, or other agents, Table(3.3)

Table (3.3)

Distribution of conjunctival cases according to the type of result

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 are shown in Table (3.4).

Table (3.4)

Distribution of patients with conjunctivitis according to the type of bacteria

Age groups (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)
≥ 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**	**(31.7)**	**120 (100)**

In Table (3.4), it is seen that the most common causative micro-organism implicated in bacterial conjunctivitis in all age groups is *Haemophilus influenzae*, (49) isolates, followed by *Staphylococcus aureus* (19) isolates, *Streptococcus pneumoniae* (7) isolates, *Neisseriae gonorrhoeae* (4) isolates, and *Klebsiella pneumoniae* (3) isolates.

This study showed that *Haemophilus influenzae* was isolated from children and adults with conjunctivitis at the high rate (40.8%).

This result is correlated with the result obtained by Trottier, *et. al.*, (1991) who found out that the prevalence rate of *Haemophilus influenzae* which caused conjunctivitis was (40%), and it was responsible for bacterial conjunctivitis in children more than adults, and they pointed out that the same strain was recovered from the eyes and nasopharynx of the symptomatic children, suggesting that the *Haemophilus influenzae* in the eyes originated from the nasopharynx.

Nineteen isolates of *Staphylococcus aureus* have also been isolated in this study at a rate of (15.8%) in children and adults.

This result is identical with the result obtained by Spindel, *et. al.*, (1995) who have isolated this bacteria from children and adults at a rate of (15%) from 120 cases, but this result is contrary to the result obtained by Rao, *et. al.*, (1992) and Idu and Odjimogho, (2003), in India, who have isolated this bacteria from patients at a rate of (37.4%) from 117 cases, and at a arate of (34%) from 100 cases respectively.

On the other hand, seven isolates of *Streptococcus pneumoniae* have been isolated in this study at a rate of (5.8%), and this bacteria is mostly isolated from children but not from adults.

In Riyadh, Saudi Arabia, Tabbara, *et. al.*, (2000), isolated this bacteria from patients with conjunctivitis at a rate of (9%) from 190 cases.

However, this result is in contrast with the result obtained by Medeiros, *et. al.*, (1999), in Brazil, who have proved that the rate of isolation of *Streptococcus pneumoniae* from conjunctival swabs is (40%) from 92 cases. Besides, Block, *et. al.*, (2000), have isolated it from patients at a rate of (30%) from 250 cases.

Streptococcal infections are self limiting and may occur in epidemic pattern. They are more frequent in temperate climates and winter months, and are associated with sub-conjunctival haemorrhages (Baum, 1997).

Also, four isolates of *Neisseria gonorrhoeae* have been isolated in this study from cases of conjunctivitis in neonates (less than one month of age) at a rate of (3.3%), and this type of bacteria was not isolated from other age groups.

This result is in agreement with the result of Nsanze, (1996), in the United Arab Emirates, who have isolated this bacteria from neonates with conjunctivitis (Ophthalmia Neonatorum) at a rate of (5%). However, this result is contrary to the result obtained by Schaller and Klauss, (2001), who have found out that the prevalence of *Neisseria gonorrhoeae* is (20.2%). Also, the prevalence of *Neisseria gonorrhoeae* have been isolated in this study much higher than the prevalence of *Neisseria gonorrhoeae* which

have been isolated in Iran by Modarres, *et. al.*, (1998) who have isolated this bacteria from neonates with Ophthalmia Neonatorum at a rate of (0.8%) from 251 cases.

Gonococcal infection occurs due to the transmission of *Neisseria gonorrhoeae* from mother to her newborn infant through the infected birth cannel (Iyamu and Enabulele, 2003).

Three isolates of *Klebsiella pneumoniae* have also been isolated in this study at a rate of (2.5%) from children and adults with bacterial conjunctivitis.

This result is in contrast with the result obtained by Iroha, *et. al.*, (1999) and Idu and Odjimogho, (2003) who have isolated this bacteria from patients with conjunctivitis at a rate of (12.9%) from 155 cases and (12%) from 100 cases respectively.

3.2.1 Identification and Characterization of Bacterial Isolates

The results of tests used for identification of the bacterial isolates from conjunctival swabs are shown in Table (3.5).

It is noticed that all the bacterial isolates which belong to the same species are similar in their characteristics, especially in staining (Gram stain) , cultural behaviour and biochemical test.

Table (3.5)

Diagnostic Tests of Bacterial Isolates

Type of bacteria	Culture media	Gram stain	Catalase	Oxidase	Coagulase	Indole	Methyl Red	Voges-Proskauer	Citrate	Urease	Satellite phenomenon
H. influenzae	**Chocolate agar (also use Blood agar)**	-	-	-	-	+	-	-	-	+	+
Staph. aureus	**Blood agar and Chocolate agar**	+	+	-	+	-	+	-	-	-	+
St. pneumoniae	**Blood agar**	+	-	-	-	-	-	-	-	-	-
N. gonorrhoeae	**Chocolate agar**	-	+	+	-	-	-	-	-	-	-
K. pneumoniae	**MacConky agar**	-	+	-	-	-	+	-	+	+	-

3.3 Investigation of Virulence Factors of Bacterial Isolates

3.3.1 *Haemophilus influenzae*

In our study, the ability of *Haemophilus influenzae* , 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 (3.6).

It was found out that all isolates of *Haemophilus influenzae* strains could not possess capsule (uncapsulated strains).

Truk, (1984) have stated that non-capsulated or non-typeable *Haemophilus influenzae* strains were a common cause of bacterial conjunctivitis.

Al-Rawi, *et. al.*, (2002) reported that all strains of *Haemophilus influenzae* isolated from patients with conjunctivitis were non-capsulated or non-typeable strains and belonged to biotypes II and III.

Furthermore, Joseph and St Geme, (2002) have showed that non-typeable or non-capsulated strains of *Haemophilus influenzae* are a common cause of human disease such as otitis media, bacterial conjunctivitis, and a number of adhesive factors exist, each has recognized a distinct host cell structure and influenced cellular binding specificity.

Table (3.6)

Virulence factors of *Haemophilus influenzae* strains from patients with bacterial conjunctivitis

Isolate No.	Capsule	Hemolysin	Siderophores	Extracellular proteases	Adherence factors
1	-	-	-	+	+
2	-	-	-	+	+
3	-	-	-	+	+
4	-	-	-	+	+
5	-	-	-	+	+
6	-	-	-	+	+
7	-	-	-	+	+
8	-	-	-	+	+
9	-	-	-	+	+
10	-	-	-	+	+
11	-	-	-	+	+
12	-	-	-	+	+
13	-	-	-	+	+
14	-	-	-	+	+
15	-	-	-	+	+
16	-	-	-	+	+
17	-	-	-	+	+
18	-	-	-	+	+
19	-	-	-	+	+
20	-	-	-	+	+
21	-	-	-	+	+
22	-	-	-	+	+
23	-	-	-	+	+
24	-	-	-	+	+
25	-	-	-	+	+
26	-	-	-	+	+
27	-	-	-	+	+
28	-	-	-	+	+
29	-	-	-	+	+
30	-	-	-	+	+
31	-	-	-	+	+
32	-	-	-	+	+
33	-	-	-	+	+
34	-	-	-	+	+
35	-	-	-	+	+
36	-	-	-	+	+
37	-	-	-	+	+
38	-	-	-	+	+
39	-	-	-	+	+
40	-	-	-	+	+
41	-	-	-	+	+
42	-	-	-	+	+
43	-	-	-	+	+
44	-	-	-	+	+
45	-	-	-	+	+
46	-	-	-	+	+
47	-	-	-	+	+
48	-	-	-	+	+
49	-	-	-	+	+
Total	**0**	**0**	**0**	**49**	**49**
%	**0**	**0**	**0**	**100**	**100**

Also, in this study, the detection of hemolysin production was studied, it was found that all *Haemophilus influenzae* could not produce hemolysin extracellularly and the type of hemolysis was gamma-hemolysis because there was no hemolysis present on Blood agar base.

Haemophilus influenzae was also tested for it's ability to siderophores synthesis when grown on M9 media containing dipyridyl. The results were recorded according to the ability of the isolates to grow or not, and the results showed that all isolates of *Haemophilus influenzae* could not produce siderophores, because non of the isolates has the ability to grow on M9 media which contain dipyridyl, Table (3.6).

These results are identical with those obtained by Morton and Williams, (1990) and Otto, *et. al.*, (1992) who have demonstrated that *Haemophilus influenzae* strains can not synthesize its own siderophore. Instead, it binds iron-loaded transferrin directly and transports the iron into the cell.

Crosa, (1989) has pointed out that *Haemophilus influenzae* initially invades the conjunctiva and then disseminates to the blood stream, and during this disease progression, the microorganism must acquire this essential nutrient.

On the other hand, Smoot, *et. al.*, (1998) have found out that the high affinity transferrin receptors in the outer membrane are crucial components for the acquisition of iron by *Haemophilus influenzae*.

The ability of *Haemophilus influenzae* to produce extracellular proteases by using M9 media (supported by 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, (1976), 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.

Delacroix, *et. al.*, (1982) have pointed out that one potentially important strategy used by this invader is the subversion of the host immune defense by specific cleavage of immunoglobulin A1(IgA1). This notion is supported by the fact that the microbial port of entry i.e. human conjunctiva, is protected primarily by antibodies of the isotype.

Lomholt and Kilian, (1995) have found out that all *Haemophilus influenzae* isolated from patients with conjunctivitis produce type 1 immunoglobulin A1(IgA1) proteases, were hemin and NAD dependent, and these isolates had the characteristics of *Haemophilus influenzae* biotype II and 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 or colonization Factors due to the positive reaction with human blood cells (group A).

These factors may be considered primary factors which cause adhesion of bacteria to the target cell of the hosts, and their presence indicates that the bacteria contain cell surface fimbrial antigens.

This result is similar to those obtained by St Geme, *et. al.*, (1991) who observed that all *Haemophilus influenzae* isolates biogroup aegyputius were able to express long peritrichous pili, these pili correlated with colony binding of human erythrocytes, and all strains possessed short, thin, surface fibers distinct from long pili and demonstrated efficient attachment to cultured human conjunctival cells.

St Geme and Falkow, (1991) have showed that non-typeable *Haemophilus influenzae* isolated from the conjunctiva can express pili, and the most important roles of pili in the pathogenic pathway of *Haemophilus influenzae* infection appear to be their mediation of bacterial adherence to mucosal surfaces and their facilitation of respiratory tract colonization.

Furthermore, St Geme, (1993) has pointed out that the attachment to conjunctival cells occurred independently of long pili or a capacity for haemagglutination.

3.3.2 *Staphylococcus aureus*

The ability of *Staphylococcus aureus* isolates to produce some virulence factors was investigated and the results of virulence factors are shown in Table (3.7).

Table (3.7)

Virulence factors of *Staphylococcus aureus* strains from patients with bacterial conjunctivitis

Isolate No.	Capsule	Hemolysin	Siderophores	Extracellular proteases	Adherence Factors
1	-	+	-	+	-
2	-	+	-	-	-
3	+	+	-	-	-
4	+	+	-	-	-
5	-	-	+	+	-
6	-	+	-	-	-
7	-	-	+	+	-
8	-	-	+	+	-
9	-	-	+	+	-
10	+	+	-	+	-
11	+	+	-	+	-
12	-	+	-	-	-
13	-	+	-	-	-
14	-	+	-	-	-
15	+	-	+	+	-
16	+	+	-	-	-
17	+	+	-	-	-
18	+	+	-	-	-
19	-	-	-	-	-
Total	**8**	**13**	**5**	**8**	**0**
%	**42.1**	**68.4**	**26.3**	**42.1**	**0**

In Table (3.7), it is noticed that 8 isolates at a rate of (42.1%) from 19 isolates of *Staphylococcus aureus* possessed the polysaccharide capsule which surrounded the bacterial cell.

This result is correlated with the result obtained by Nair, *et. al.,* (2000) who have found out that some *Staphylococcus aureus* strains produced capsular polysaccharide, which formed slime layer on the outer surface of the peptidoglycan.

Riordan and Lee, (2004) have pointed out that *Staphylococcus aureus* capsules is an important component in the pathogenesis, and enhances bacterial virulence by impeding phagocytosis, resulting in

bacterial persistence in the bloodstream of infected hosts. Although the capsule has been shown to modulate *Staphylococcus aureus* adherence to endothelial surface in vitro, animal studies suggest that it also promotes bacterial colonization and persistence on mucosal surfaces.

On the other hand, the hemolysin production was also investigated and it was found that 13 isolates at a rate of (68.4%) of *Staphylococcus aureus* had the ability to produce the extracellular hemolysin, and the type of hemolysis was Beta-hemolysis due to the presence of a clear zone of hemolysis (lyses of red blood cells) around the bacterial colonies formed on human blood agar.

The zone of hemolysis is usually narrow. The role of beta-hemolysin which is produced by *Staphylococcus aureus* strains in disease is not clearly understood (Dinges, *et. al.*, 2000).

Staphylococcus aureus was also tested for it's ability to siderophores synthesis, and the results showed that some isolates of *Staphylococcus aureus* 5 isolates at a rate of (26.3%) were found able to produce siderophores.

These results are identical with the result obtained by Heinrichs, (2004) who has found out that *Staphylococcus aureus* possesses both siderophore-mediated as well as non-siderophore-mediated iron transport systems, indicating that this bacterium is highly versatile insofar as iron acquisition is concerned.

Mazmanian, *et. al.*, (2003) have pointed out that siderophore production may not be required for the establishment of an infection but most certainly appears to be required for prolonged survival of *Staphylococcus aureus* in vivo.

Also, the ability of *Staphylococcus aureus* to produce extracellular proteases was investigated, and it was found that 8 isolates of *Staphylococcus aureus* at a rate of (42.1%) were able to produce extracellular proteases, but other bacterial isolates, 11 isolates at a rate of (57.9%) could not produce this enzyme.

Karlsson and Arvidson, (2002) have pointed out that the production of extracellular protease varied considerably among clinical isolates of *Staphylococcus aureus*, and the presence of the major protease genes, in all the protease-negative strains analyzed suggested that the lack of protease production was due to some regulating host cell factors.

Furthermore, The isolates were tested for their abilities to produce adherence factors by Haemmaglutination Test (HA), and it was noticed that all bacterial isolates could not produce adherence factors due to the negative reaction with human blood cells (groupA).

There are no previous studies about the ability of *Staphylococcus aureus* strains to produce adherence factors. However, teichoic acid has a major role in the adherence *Staphylococcus aureus* in various tissues (Nolt, 1992).

3.3.3 *Streptococcus pneumoniae*

The ability of *Streptococcus pneumoniae* isolates to produce of some virulence factors are shown in Table (3.8).

Table (3.8)

Virulence factors of *Streptococcus pneumoniae* strains from patients with bacterial conjunctivitis

Isolate No.	Capsule	Hemolysin	Siderophores	Extracellular proteases	Adherence Factors
1	+	+	-	+	+
2	-	+	-	+	+
3	-	+	-	+	+
4	-	+	-	+	+
5	+	+	-	+	+
6	-	+	-	+	+
7	-	+	-	+	+
Total	**2**	**7**	**0**	**7**	**7**
%	**28.6**	**100%**	**0**	**100**	**100**

It was found that only 2 isolates by a rate (28.6%) from 7 isolates of *Streptococcus pneumoniae* were able to possess the polysaccharide capsule which surrounded the bacterial cell.

This result is in agreement with the result obtained by Barker, *et. al.*, (1999) who have pointed out that not all strains of *Streptococcus pneumoniae*, which were isolated from patients with bacterial conjunctivitis possessed polysaccharide capsule, but some isolates, 8 isolates at a rate of (44%) from 18 isolates possessed polysaccharide capsule (nontypeable).

Ertugrul, *et. al.*, (1997) have showed that nontypeable pneumococci rarely cause invasive infection, but for unclear reasons, they are frequently implicated in cases of pneumococcal conjunctivitis. While it is still uncertain whether the nontyeable strains are always uncapsulated, recent studies of phase variation in

pneumococci suggest that decreased capsule production may facilitate bacterial persistence in certain tissues (Barker, *et. al.*, 1999).

Kim and Weiser, (1998) have pointed out that colonies with decreased amounts of polysaccharide capsule are adept at nasopharyngeal colonization, but that variant with greater amounts of polysaccharide capsule are adept at invasion. However, there is no evidence that the conjunctival strains undergo phase changes, these studies suggest possible tissue-specific advantages of decreased or absent capsule production.

Additionally, the detection of hemolysin production was studied, it was found that all *Streptococcus pneumoniae* isolates produced hemolysin,and the type of hemolysis was Alpha-hemolysis due to the presence of a greenish zone of hemolysis that surrounded the bacterial colonies formed on blood agar.

The function of hemolysin is to provide the micro-organism with iron and it makes the bacteria unable to produce any factor for obtaining the iron from environment (Valvano, *et. al.*, 1986).

The same results were obtained by Facklam and Pigott, (1994) who have found out that all strains of *Streptococcus pneumoniae* produced an Alpha-hemolysin that reduced hemoglobin (red) to methemoglobin (green), caused a greenish zone to surround the colony.

Moreover, the ability of *Streptococcus pneumoniae* to siderophores synthesis was studied, and it was found that all isolates of *Streptococcus pneumoniae* could not produce siderophores.

This result correlates with the result obtained by Tai, *et. al.*, (1993) who indicated that *Streptococcus pneumoniae* strains

did not produce extracellular siderphores or iron-chelating factors under low-iron conditions and could use either hemin or hemoglobin as a sole source for the require iron.

Virulent *Streptococcus* that neither bind to siderophilins nor produces siderophores can invade and replicate in many tissues and in diverse host species. The cellulytic activities of these pathogens enable them to access such intracellular sources of host iron as hemoglobin, myoglobin, catalase and ferritin (Eichenbaum, *et. al.*, 1996).

Furthermore, it is known that bacteria which are able to produce hemolysin have no ability to produce siderphores, so bacteria need only one mechanism for obtaining iron (Al-Saeed, 1997).

The ability of *Streptococcus pneumoniae* to produce extracellular proteases was investigated and it was found that all *Streptococcus pneumoniae* isolates were able to produce extracellular proteases.

These results are similar to those obtained by Poulsen, *et. al.*, (1996) have showed that *Streptococcus pneumoniae* can produce IgA1 proteases, and these proteases are of the serine type which enables *Streptococcus pneumoniae* to evade the protective functions of the principal immunoglobulin isotype of the upper respiratory tract.

Reinholdt and Kilian, (1997) have indicated that all *Streptococcus pneumoniae* strains which were isolated from patients with bacterial conjunctivitis produced type 1 immunoglobulin A1(IgA1) proteases, these proteases degrade human immunoglobulin A1, and the role of IgA1 proteases may depend on the quantity of

IgA1 protease generated as well as on he balance between secreted and cell-associated forms of the enzyme.

Furthermore,the isolates were tested for their abilities to produce adherence factors by Haemmaglutination Test (HA), it was found that all isolates were able to produce adherence factors due to the positive reaction with human blood cells (group A).

Bacteria adhere to tissue by having pili. Pili or fimbriae are rod-shaped structures that consist primarly of an orderd array of a single protein subunit called pilin. An important function of pilus replacements, at least for some bacteria, is to provide a way for the bacterium to evade the hosts immune response (Salyers and Whitt, 1994).

3.3.4 *Neisseria gonorrhoeae*

In our study, the ability of *Neisseria gonorrhoeae* to produce some virulence factors was studied. The results are shown in Table (3.9).

Table (3.9)

Virulence factors of *Neisseria gonorrhoeae* strains from patients with bacterial conjunctivitis

Isolate No.	Capsule	Hemolysin	Siderophores	Extracellular proteases	Adherence Factors
1	-	-	-	+	+
2	-	-	-	+	+
3	-	-	-	+	+
4	-	-	-	+	+
Total	**0**	**0**	**0**	**4**	**4**
%	**0**	**0**	**0**	**100**	**100**

The results in Table (3.9), reveal that all isolates of *Neisseria gonorrhoeae* which are isolated from patients with bacterial conjunctivitis could not possess the polysaccharide capsule.

This result is similar to the result observed by Richardson and Sadoff, (1977) who have pointed out that *Neisseria gonorrhoeae* could not possess the polysaccharide capsule but can be induced to form a large capsule in the presence of *Streptococcus viridan*s (SV) that can be visualized with Indian ink, and capsulated gonococci appeared to be resistant to non-antibody-mediated phagocytosis compared with uncapsulated gonococci of the same strain and colony type.

Also, this result is indicated by Ram, *et. al.*, (1999) who have found out that *Neisseria gonorrhoeae* had not the polysaccharide capsule.

Also, the hemolysin production was detected, and it was found that all *Neisseria gonorrhoeae* isolates could not produce the extracellular hemolysin, and the type of hemolysis was gamma hemolysis (no hemolysis).

Neisseria gonorrhoeae was also tested for it's ability to siderophores synthesis. The results showed that all isolates of *Neisseria gonorrhoeae* could not produce siderophores

These results are also obtained by Biswas and Sparling, (1995) who have found out that *Neisseria gonorrhoeae* could not produce siderophores, but uses various other mechanisms to scavenge iron, through the use of hemoglobin receptors.

Beucher and Sparling, (1995) have pointed out that gonococci do not produce their own siderophores, but they are able to scavenge

siderophores made by other bacteria, including the *Escherichia coli* hydroxamate siderophore aerobactin.

Besides, Rutz, *et. al.*, (1991) have showed that *Neisseria* species could also transported the *E. coli* phenolate siderophore ferric enterobactin, although it was previously shown that *Neisseria* species transport iron from ferric enterobactin, this is the first demonstration that any neisserial spp. can utilize ferric enterobactin as an iron source for growth and that *FetA* may act as classic siderophore receptor.

On the other hand, the ability of *Neisseria gonorrhoeae* to produce extracellular proteases was investigated and it was found that all *Neisseria gonorrhoeae* isolates were able to produce extracellular proteases.

These results are correlated with the results obtained by Reinholdt and Kilian, (1997) who have found out that all strains of *Neisseria gonorrhoeae* can produce proteases, which can cleave human IgA1 in the hing region.

Also, Lorenzen, *et. al.*, (1999) have showed that pathogenic *Neisseria* species produce IgA1 proteases, and are of the serine type that cleave preferentially human IgA1 and other target proteins.

The isolates were tested for their abilities to produce adherence factors. The results showed that all isolates were able to produce adherence factors.

van Putten and Duensing, (1997) have pointed out that Adherence is mediated through fimbriae and Opa (P.II) protein. Bacteria attach only to microvilli of nonciliated columnar epithelial cells. Attachment to ciliated cells does not occur. *Neisseria gonorrhoeae* first attaches to epithelial cells by means of its

fimbriae, specifically (Type 4) pili. After initial attachment, the bacteria enter a second stage of binding mediated by the outer membrane protein P.II .

Furthermore, Katz, *et. al.*, (1998) showed that *Neisseria gonorrhoeae* have numerous pili. Pili are the hair-like appendages that extend up to several micrometers from the gonococcal surface. They enhance attachment to host cells and resistance to phagocytosis.

3.3.5 *Klebsiella pneumoniae*

The ability of *Klebsiella pneumoniae* isolates to produce some virulence factors was investigated and the results are shown in Table (3.10).

Table (3.10)

Virulence factors of *Klebsiella pneumoniae* strains from patients with bacterial conjunctivitis

Isolate No.	Capsule	Hemolysin	Siderophores	Extracellular proteases	Adherence Factors
1	+	-	+	-	+
2	+	-	+	-	+
3	+	-	+	-	+
Total	**3**	**0**	**3**	**0**	**3**
%	**100**	**0**	**100**	**0**	**100**

From the results in Table (3.10), it can be noticed that all *Klebsiella pneumoniae* isolates were able to possess the polysaccharide capsule which surrounded the bacterial cell.

This result agrees with the result obtained by Podschun and Ulmann, (1998) who have pointed that *Klebsiella pneumoniae* usually develop prominent capsules composed of complex acidic polysaccharides, and it's essential virulence factor for *Klebsiella pneumoniae*.

Yokochi, *et. al.,* (1979) had reported obout the ability of capsule to inhibit the differentiation and functional capacity of macrophages in vitro.

Podschun, *et. al.*, (1992) showed that the capsule of *Klebsiella pneumoniae* protects the bacterium from phagocytosis by polymorphonuclear granulocytes .

Also, the hemolysin production was studied. The results showed that all *Klebsiella pneumoniae* isolates from patients with bacterial conjunctivitis could not produce hemolysin extracellulary when cultured on blood agar, and the type of hemolysis was gamma hemolysis because there was no hemolysis present on blood agar.

Klebsiella pneumoniae isolates were also tested for their abilities to siderophores synthesis.The results showed that all isolates of *Klebsiella pneumoniae* were able to produce siderophores.

These results are in agreement with those obtained by Podschun, *et. al.*, (1992) who have shown that all *Klebsiella* strains have the ability to possess siderophores system (enterobactin and aerobactin), and *Klebsiella pneumoniae* depend on this system for the uptake of iron which may be considered an essential factor in bacterial growth. Thus, production of these two siderophores may give access to both sources of iron, resulting in enhanced growth in the host.

Podschun and Ulmann, (1998) have demonstratee that aerobactin gene was located on the plasmids of *Klebsiella pneumoniae* strains of serotypes K1 and K2, and they found out that when the aerobactin gene was cloned from the plasmids of these *Klebsiella pneumoniae* strains and was transferred to a nonvirulent (siderophore-negative) strain, the transformant then exhibited markedly enhanced virulence in a mouse peritonitis model.

Also, the ability of *Klebsiella pneumoniae* isolates to produce extracellular proteases was investigated and it was found that all isolates of *Klebsiella pneumoniae* could not produce extracellular proteases.From this result, it was noticed that *Klebsiella pneumoniae* could not produce extracellular proteases, and it is may be could not analyze the proteins compounds which were present in the blood such as transferrein, and it was necessary to find another mechanisms for the uptake of materials which were needed, such as the production of siderophores.

These results are identical with those obtained by Al-Saedi, (2000) who have found out that all isolates of *Klebsiella pneumoniae* from patients with eye infections could not produce extracellular proteases.

Also, the isolates were tested for their abilities to produce adherence factors , and it was found that all *Klebsiella pneumoniae* isolates were able to produce adherence factors due to the positive reaction with human blood cells (group A).

These results are correlated with the results obtained by Venegas, *et. al.*, (1995) who found that *Klebsiella pneumoniae* possess type 1 fimbriae, and the relevance of these pili to bacterial

virulence was thought to arise mainly from binding of the bacteria to mucous or to epithelial cells of the urogenital, respiratory, and intestinal tracts.

Martino, *et. al.*, (1996) have noticed that *Klebsiella pneumoniae* strains had the ability to possess type 3 pili, which was an important factor in adherence of this type of bacteria to host tissues.

On the other hand Hornick, *et. al.*, (1999) have demonstrated that strains of *Klebsiella pneumoniae* expressing type 3 pili adhere to endothelial cells, epithelial cells of the respiratory tract, and uroepithelial cells.

3.4 Antibacterial Sensitivity Assay

3.4.1 Effect of Some Antibiotics on *H. influenzae*

In this study, some antibiotics were used to show their effect on *Haemophilus influenzae* isolates (Chloramphenicol, Ciprofloxacin, Gentamycin, Penicillin, Tetacycline, Cefotaxime and Erythromycin). The results of sensitivity test are shown in Table (3.11).

It was found that bacterial isolates were sensitive (100%) to cefotaxime (Third-generation cephalosporins), and most isolates were sensitive (95.9%, 91.8%) to ciprofloxacin and tetracycline respectively. On the other hand, (89.8%) of isolates were sensitive to chloramphenicol and (42.9%) were sensitive to gentamycin, whereas some isolates were sensitive (18.4%) to erythromycin and only (6.1%) were sensitive to pencillin.

Table (3.11)
Sensitivity of *Haemophilus influenzae* isolates to some antibiotics

Isolate No.	CL	CP	GN	PN	TE	CTX	ER
1	-	-	+	+	-	-	+
2	-	-	-	+	-	-	+
3	-	-	+	+	-	-	+
4	-	-	+	+	-	-	+
5	-	-	-	+	-	-	+
6	-	-	-	+	-	-	+
7	+	-	-	-	+	-	-
8	-	-	+	+	-	-	+
9	-	-	+	+	-	-	+
10	-	-	+	+	-	-	+
11	-	+	-	+	+	-	-
12	-	-	-	+	-	-	+
13	-	-	-	+	-	-	+
14	+	-	-	-	-	-	-
15	-	-	+	+	-	-	+
16	-	-	+	+	-	-	+
17	-	-	-	+	-	-	+
18	-	-	-	+	-	-	+
19	-	-	+	+	-	-	+
20	-	-	+	+	-	-	+
21	-	-	-	+	-	-	+
22	+	-	-	+	-	-	-
23	-	-	+	+	-	-	+
24	-	-	+	+	-	-	+
25	-	-	+	+	-	-	+
26	-	-	+	+	-	-	+
27	-	-	-	+	-	-	+
28	-	-	-	+	-	-	+
29	-	-	+	+	-	-	-
30	-	-	+	+	-	-	-
31	-	-	-	+	-	-	+
32	-	-	+	+	-	-	+
33	+	-	-	+	+	-	-
34	-	-	+	+	-	-	+
35	-	-	+	+	-	-	+
36	-	-	+	+	-	-	+
37	-	-	+	+	-	-	+
38	+	-	-	+	-	-	-
39	-	-	+	+	-	-	+
40	-	-	+	+	-	-	+
41	-	-	-	+	-	-	+
42	-	-	-	+	-	-	+
43	-	+	-	-	+	-	-
44	-	-	+	+	-	-	+
45	-	-	-	+	-	-	+
46	-	-	+	+	-	-	+
47	-	-	+	+	-	-	+
48	-	-	+	+	-	-	+
49	-	-	+	+	-	-	+
Total	**44**	**47**	**21**	**3**	**45**	**49**	**9**
%	**89.8**	**95.9**	**42.9**	**6.1**	**91.8**	**100**	**18.4**

- + Resistance, - Sensitive.
- CL: chloramphenicol, CP: ciprofloxacim, GN: gentamycin, PN: penicillin, TE: tetracycline, CTX: cefotaxime, ER: erythromycin.

Schleiss, (2002) has shown the high sensitivity rate of this bacteria to cefotaxime (Third-generation cephalosporins) because of their potent bacteriocidal activity and penetration into the subarachnoid space.

This data was correlated with previous observation Tamargol, *et. al.*, (2003) and Jones *et. al.*, (2004) who reported that all strains of *Haemophilus influenzae* were sensitive to cefotaxime, and no strain of this bacterium was resistant to cefotaxime.

Farajzadeh-Sheikh, *et. al.*, (2004) have shown the same results about the high sensitivity rate of this bacteria to some antibiotics (chloramphenicol, tetracycline and ciprofloxin,) because of all these bacterial isolates from patients with bacterial conjunctivitis are uncapsulated (non-typeable) and these uncapsulated strains have more sensitivity to these antibiotics than capsulated strains (typeable), and they have showed that the sensitivity of *Haemophilus influenzae* strains to chloramphenicol at a rate of (95%).

Furthermore, Schleiss, (2002) has demonstrated that chloramphenicol is effective against *Haemophilus influenzae* strains by binding to 50s bacterial subunits and inhibiting bacterial growth by inhibiting protein synthesis.

However, these results are contrary to those obtained by Matthews *et. al.*, (1988) who found that all isolates of *Haemophilus influenzae* are resistant to chloramphenicol, due to the production of chloramphenicol acetyltransferase, that is under plasmid control which destroy a drug activity.

On the other hand, Gold and Moellering, (1996) have demonstrated that tetracycline is effective against *Haemophilus*

influenzae strains by inhibiting protein synthesis in bacteria by blocking the attachment of charged aminoacyl-tRNA. Thus, it prevents the introduction of new amino acids to the nascent peptide chain.

The same result was observed by Martinez, *et. al.*, (2004) who found that (97.5%) of *Haemophilus influenzae* strains which were isolated from patients with bacterial conjunctivitis were sensitive to tetracycline.

Block, *et. al.*, (2000) showed that tetracycline was notably active (93%) against most strains of *Haemophilus influenzae* which caused conjunctivitis.

The results also showed that most *Haemophilus influenzae* isolates were sensitive to ciprofloxacin.

This result is consistent with those carried out by Brower, *et. al.*, (1996) who have pointed out the high efficacy of ciprofloxacin in the treatment of bacterial keratitis, ciprofloxacin has exhibited it's highest sensitivity on *Haemophilus influenzae* with a zone of inhibition of 18 mm.

Also, this result is in agreement with those obtained by Dhara, *et. al.*, (2001) who have found that all *Haemophilus influenzae* strains are sensitive to ciprofloxacin.

Idu and Odjimogho, (2003) have shown that *Haemophilus influenzae* strains are the most sensitive to ciprofloxacin, and suggested that ciprofloxacin should be the first choice of the fluoroquinolenes for the treatment of bacterial conjunctivitis, and it has been more effective in the treatment of ocular infection than some other antibiotics e.g. gentamycin and chloramphenicol.

Also, the results showed that some isolates were resistant to gentamycin, erythromycin and penicillin.

Edson and Terrell, (1991) have stated that gram-negative bacteria is resistant to aminoglycosides e.g., Gentamycin by plasmid-dependent resistance to aminoglycosides produce adenylylating, phosphorylating or acetylating enzymes that destroy the drugs.

Also, Nathwani and Wood, (1993) have showed that the resistance to penicillin may be attributed to the production of Beta-lactamases enzymes and these enzymes are encoded by genes located on plasmid. Plasmid-mediated Beta-lactamases are produced constitutively and have a high propensity to move from one species of bacteria to another.

These results are similar to the results obtained by Modarres, *et. al.*, (1998) who have observed that *Haemophilus influenzae* strains isolated from patients with bacterial conjunctivitis are sensitive to gentamycin and erythromycin by a rate (39%) and (16%) respectively, and only (2%) of bacterial isolates are sensitive to penicillin.

Block, *et. al.*, (2000) have pointed out that all *Haemophilus influenzae* isolates from patients with conjunctivitis are intermediately active to gentamycin and erythromycin.

3.4.2 Effect of Some Antibiotics on *Staph. aureus*

Some antibiotics were used to show their effect on *Staphylococcus aureus* isolates (Chloramphenicol, Ciprofloxacin,

Gentamycin, Penicillin, Tetacycline, Cefotaxime and Erythromycin) The results of sensitivity test are shown in Table (3.12).

Table (3.12)

Sensitivity of *Staphylococcus aureus* isolates to some antibiotics

IsolateNo.	CL	CP	GN	PN	TE	CTX	ER
1	-	-	-	+	-	-	-
2	-	-	-	+	-	-	-
3	-	-	-	+	-	-	-
4	-	-	-	+	-	-	-
5	+	+	-	+	+	-	-
6	-	-	-	+	-	-	-
7	-	-	-	+	-	-	-
8	-	-	-	+	-	+	+
9	-	-	-	+	-	+	+
10	-	-	-	+	-	-	-
11	-	-	-	+	-	-	-
12	+	-	-	+	+	-	-
13	-	-	-	+	-	-	+
14	-	-	-	+	-	+	+
15	-	-	-	+	-	-	-
16	-	-	-	+	-	+	+
17	-	-	-	+	-	-	-
18	-	-	-	+	-	-	-
19	-	-	-	+	-	+	+
Total	17	18	19	0	17	14	13
%	89.5	94.7	100	0	89.5	73.7	68.4

- + Resistance, - Sensitive
- CL: chloramphenicol, CP: ciprofloxacim, GN: gentamycin, PN: penicillin, TE: tetracycline, CTX: cefotaxime, ER: erythromycin.

It has been found that all isolates were sensitive (100%) to gentamycin and most isolates were sensitive (94.7%) to ciprofloxacin, and (89.5%) of isolates were sensitive to tetracycline and chloramphenicol, whereas some isolates revealed sensitivity in a lesser degree to cefotaxime (73.7%) and erythromycin (68.4%), and all isolates have shown resistance to penicillin .

Barid, (1996) has 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, this is mediated by extra-chromosomal piece of DNA (plasmid).

Also, all isolates have shown sensitivity to gentamycin, and most isolates were sensitive to ciprofloxacin and tetracycline.

Chigbu and Ezeronye, (2003) have shown that the high rate of sensitivity to ciprofloxacin may be this antibiotic is relatively new and not in common use by the population as compared to penicillin.

These results are identical with those obtained by Martinez, *et. al.*, (2004) who have found that all isolates of *Staphylococcus aureus* which are isolated from patients with bacterial conjunctivitis are sensitive to ciprofloxacin and gentamycin, and (94.7%) of the isolates are sensitive to tetracycline.

Silverman and Bessman, (2003) have shown that gentamycin is effective against *Staphylococcus aureus* strains, and it is used as an ointment for treatment of bacterial conjunctivitis.

Marlin, (2003) has stated that ciprofloxacin is effective against *Staphylococcus aureus* strains, and this type of antibiotic is a bactericidal antibiotic that inhibits bacterial growth by inhibiting

DNA gyrase, therefore, *Staphylococcus aureus* strains are sensitive to this type of antibiotics.

Also, the isolates were sensitive to chloramphenicol and erythromycin at a rate of (89.5%) and (68.4%) respectively.

The mechanism of chloramphenicol sensitivity in bacteria includes blocking the attachment of amino acids to the nascent peptide chain on the 50s unit of ribosomes by interfering with the action of peptidyl transferase (Gold and Moellering, 1996).

These results are correlated with those obtained by Modarres, *et. al.*, (1998) who have found that *Staphylococcus aureus* strains which have been isolated from patients with bacterial conjunctivitis are sensitive to chloramphenicol and erthromycin at a rate of (83%, 66%) respectively, and all *Staphylococcus aureus* isolates are resistant to penicillin.

Chigbu and Ezeronye, (2003) have found that (68.4%) of *Staphylococcus aureus* isolates are sensitive to erythromycin.

Also, Ruiz, *et. al.*, (1993) have pointed that chloramphenicol and erthromycin are the most agents active against *Staphylococcus aureus* strains isolated from patients with conjunctivitis, except penicillin. It has been found that all strains of *Staphylococcus aureus* are resistant to penicillin.

Furthermore, Ako-Nai, *et. al.*, (1991) have found that nearly all (99%) of the strains isolated from eye infections are resistant to penicillin, (91%) of the isolates are sensitive to chloramphenicol.

Also, in our study, the results showed that (73.7%) of isolates were sensitive to cefotaxime.

This result is similar to the result observed by Jones, *et. al.*, (2004) who have found that (74.8%) of the isolates are sensitive to cefotaxime.

3.4.3 Effect of Some Antibiotics on *St. pneumoniae*

Some antibiotics (seven antibiotics) were used to show their effect on *Streptococcus pneumoniae* isolates, and the results are shown in Table (3.13).

Table (3.13)

Sensitivity of *Streptococcus pneumoniae* isolates to some antibiotics

IsolateNo.	CL	CP	GN	PN	TE	CTX	ER
1	-	-	+	-	-	-	-
2	-	-	+	-	-	-	-
3	-	-	+	-	-	-	-
4	-	-	+	-	-	+	-
5	-	-	+	-	-	-	-
6	-	-	+	-	-	+	-
7	+	+	+	+	+	-	-
Total	**6**	**6**	**0**	**6**	**6**	**5**	**7**
%	**85.7**	**85.7**	**0**	**85.7**	**85.7**	**71.4**	**100**

- + Resistance, - Sensitive
- CL: chloramphenicol, CP: ciprofloxacim, GN: gentamycin, PN: penicillin, TE: tetracycline, CTX: cefotaxime, ER: erythromycin.

The results showed that all isolates were sensitive (100%) to erythromycin and (85.7%) were sensitive to chloramphenicol,

ciprofloxacin, penicillin and tetracycline, whereas (71.4%) were sensitive to cefotaxime, and all the isolates showed resistance (100%) to gentamycin.

Edson and Terrell, (1991) have showed that the resistance to some aminoglycosides e.g., Gentamycin may depend mainly on the lack of permeability to the drug, which apparently occurs due to an outer membrane change that impairs active transport into the cell. So the drug cannot reach the ribosome.

These results are consistent with those observed by Modarres, *et. al.*, (1998) who have found that all *Streptococcus pneumoniae* strains isolated from patients with bacterial conjunctivitis are resistant to gentamycin.

Also, all the isolates have shown sensitivity to erythromycin.This result is in agreement with the result obtained by Chin, *et. al.*, (1999) who have found that (95%) of the isolates are sensitive to erythromycin.

The mechanisms of erythromycin sensitivity in *Streptococcus pneumoniae* includes inhibiting protein synthesis by interfering with translocation reactions and the formation of initiation complexes (Bahal and Nahata, 1992).

Also, Chivez, *et. al.*, (2002) have found that (95.8%) of *Streptococcus pneumoniae* isolates are sensitive to erythromycin. But these results are contrary to those obtained by Leighton, (2003) who has demonstrated that all *Streptococcus pneumoniae* strains isolated from patients with pneumococcal conjunctivitis are resistant to erythromycin.

The results also showed that most bacterial isolates were sensitive to ciprofloxacin, chloramphenicol and tetracycline.

These results are similar to the results obtained by Martinez, *et. al.*, (2004) who have found that (93.5%) of *Streptococcus pneumoniae* strains isolated from patients with bacterial conjunctivitis are sensitive to ciprofloxacin, and all of the isolates (100%) are sensitive to chloramphenicol, whereas (84.1%) are sensitive to tetracycline.

Nichol, *et. al.*, (2003) have shown that the susceptibility of *Streptococcus pneumoniae* isolates to ciprofloxacin, in fact, has demonstrated less genetic variability than isolates with reduced susceptibility to ciprofloxacin. So these results suggest that the emergence of ciprofloxacin-resistant *Streptococcus pneumoniae* is currently not attributable to clonal dissemination.

Voils, *et. al.*, (2004) have shown that tetracycline is very beneficial in the management of conjunctivitis and blepharitis.

Also, Jones, *et. al.*, (2004) have demonstrated that (96%) of the isolates are sensitive to chloramphenicol.

In our study, the level of sensitivity to penicillin was (85.7%) and seems to be much higher than the study of Soh, *et. al.*, (2000) who have found that the sensitivity to penicillin was (30%). But the same results are recorded by Watase, *et. al.*, (1998) who demonstrated that the susceptibility of *Streptococcus pneumoniae* isolates to penicillin was (75.8%).

Also, these results are in agreement with the results observed by Donna, *et. al.*, (2002) who have found that (83%) of the isolates are sensitive to penicillin.

The results also showed that (71.4%) of *Streptococcus pneumoniae* isolates were sensitive to cefotaxime.

This result is concurrent with the findings of Gums and Gainesville, (2000) who have found that (70.7%) of the isolates are sensitive to cefotaxime, and they have suggested that for *Streptococcus pneumoniae*, cefotaxime may not be therapeutically equivalent. This information may be clinically important given the new recommended breaking points for cefotaxime (Third generation cephalosporins).

3.4.4 Effect of Some Antibiotics on *N. gonorrhoeae*

Some antibiotics (seven antibiotics) were used to show their effect on *Neisseria gonorrhoeae* isolates, and the results are shown in Table (3.14).

Table (3.14)

Sensitivity of *Neisseria gonorrhoeae* isolates to some antibiotics

Isolate No.	CL	CP	GN	PN	TE	CTX	ER
1	-	-	-	-	-	+	-
2	-	-	-	+	-	-	-
3	-	-	+	+	-	-	-
4	+	+	+	+	-	-	-
Total	3	3	2	1	4	3	4
%	75	75	50	25	100	75	100

- + Resistance, - Sensitive.
- CL: chloramphenicol, CP: ciprofloxacim, GN: gentamycin, PN: penicillin, TE: tetracycline, CTX: cefotaxime, ER: erythromycin.

The results showed that all isolates of *Neisseria gonorrhoeae* were sensitive (100%) to tetracycline and erythromycin, (75%) of the isolates were sensitive to chloramphenicol, ciprofloxacin and cefotaxime, and (50%) were sensitive to gentamycin.However, only (25%) of the isolates were sensitive to penicillin.

This result is correlated with the result obtained by Ebong, *et. al.*, (1992) who have found that (31.2%) of the isolates are sensitive to penicillin, and this resistance is due to the production of penicillinase enzyme and chromosomally-mediated resistance, but the result is not correlated with the result obtained by Modarres, *et. al.*, (1998) who have found that (99%) of the isolates from patients with bacterial conjunctivitis are sensitive to penicillin.

Zhao and Enzenauer, (2004) have suggested that penicillin G is the choice for penicillin-susceptible *Neisseria gonorrhoeae* infection, which interferes with the synthesis of cell wall mucopeptide during active multiplication, resulting in bactericidal activity against susceptible microorganisms.

Also, all isolates were sensitive to tetracycline and erythromycin. These results are similar to a previous observation by Ebong, *et. al.*, (1992) who have pointed that all *Neisseria gonorrhoeae* isolates from neonates with Ophthalmia Neonatorum are sensitive to erythromycin.

Also, Bell, *et. al.*, (1993) have found that nearly all isolates of *Neisseria gonorrhoeae* are sensitive to tetracycline and erythromycin.

Iyamu and Enabulele, (2003) have shown that erythromycin would prevent Ophthalmia Neonatorum when applied to the neonates' eyes some hours after birth.

Beside, WHO, (2001) has reported that tetracycline can be used as eye ointment 1%, instill in both neonates eyes immediately after birth.

On the other hand, the results showed that (75%) of *Neisseria gonorrhoeae* isolates were sensitive to chloramphenicol.

This result is in agreement with the result obtained by Normann, *et. al.*, (2002) who have found that (87%) of *Neisseria gonorrhoeae* isolated from patients with bacterial conjunctivitis are sensitive to chloramphenicol, so chloramphenicol is mostly administrated in the form of eye drops for the treatment of acute neonatal bacterial conjunctivitis.

Besamusca and Bastiaensen, (1986) have pointed out that the use of chloramphenicol in neonates may cause aplastic anaemia and give rise to "gray baby syndrome", which describes the clinical signs of toxicity "cyanosis and vascular collapse" in the neonatal period. While rarely, aplastic anaemia is caused by chloramphenicol when it is used as eye drops.

The results also showed that (75%) of *Neisseria gonorrhoeae* isolates were sensitive to ciprofloxacin.

This result is correlated with the result obtained by Schwarcz, *et. al.*, (1990) who have demonstrated that most isolates of *Neisseria gonorrhoeae* are sensitive to ciprofloxacin, and they have suggested that ciprofloxacin must be administrated orally, and can be used in patients who are allergic to penicillin.

Furthermore, Brower, *et. al.*, (1996) have showed that antibiotic of the fluoroquinolone group (ciprofloxacin) is more effective in the treatment of bacterial conjunctivitis than other broad spectrum antibiotics e.g. gentamycin.

The results also showed that *Neisseria gonorrhoeae* isolates were intermediately sensitive (50%) to gentamycin.

The same result was obtained by Modarres, *et. al.*, (1998) who found that (49%) of the isolates were sensitive to gentamycin.

Zhao and Enzenauer, (2004) have stated that systemic gentamycin is an alternative drug for penicillinase producing *Neisseria gonorrhoeae*. Topical gentamycin is also used for other gram-negative bacterial infections when applied to each conjunctival sac.

Also, the effect of cefotaxime on *Neisseriae gonorrhoeae* isolates was detected and it was found that (75%) of the isolates were sensitive to cefotaxime (Third-generation cephalosporins).

Zhao and Enzenauer, (2004) have shown that cefotaxime is another alternative drug for treatment of *Neisseria gonorrhoeae*.

3.4.6 Effect of Some Antibiotics on *K. pneumoniae*

In our study, some antibiotics (seven antibiotics) were used to show their effect on *Klebsiella pneumoniae* isolates (Chloramphenicol, Ciprofloxacin, Gentamycin, Penicillin, Tetracycline, Cefotaxime and Erythromycin). The results of sensitivity test are shown in Table(3.15).

Table (3.15)

Sensitivity of *Klebsiella pneumoniae* isolates to some antibiotics

IsolateNo.	CL	CP	GN	PN	TE	CTX	ER
1	-	-	-	+	+	-	+
2	-	-	-	+	+	+	+
3	-	-	+	+	+	+	+
Total	3	3	2	0	0	1	0
%	100	100	66.7	0	0	33.3	0

- + Resistance, - Sensitive.
- CL: chloramphenicol, CP: ciprofloxacim, GN: gentamycin, PN: penicillin, TE: tetracycline, CTX: cefotaxime, ER: erythromycin.

According to Table (3.15), it was found that all isolates of *Klebsiella pneumoniae* were sensitive (100%) to chloramphenicol and ciprofloxacin, whereas some isolates showed sensitivity in a lesser degree to gentamycin (66.7%) and then to cefotaxime (33.3%).

On the other hand, all isolates were resistant (100%) to penicillin, tetracycline and erythromycin.

The resistance of *Klebsiella pneumoniae* isolates to penicillin is attributed to that most clinical isolates of *Klebsiella pneumoniae* normally produce enzymes that destroy the penicillin. These enzymes are classified as penicillinase enzymes (Alles, *et. al.*, 2000).

This result is similar to the result observed by Modarres, *et. al.*, (1998) who have found that all the isolates are resistant to penicillin.

Also, the resistance to erythromycin results from an alteration (methylation) of the rRNA receptor. This is under control of a transmissible plasmid (Bahal and Nahata, 1992).

This result is identical with the result observed by Modarres, *et. al.*, (1998) who pointed out that all of the isolates are resistant to erythromycin.

Furthermore, the results showed that all the isolates were resistant to tetracycline.

Siegel, (1978) has shown that resistance to tetracycline results from changes in permeability of the bacterial cell envelope. Also, this drug is not actively transported into the cell or leaves it so rapidly that inhibitory concentration is not maintained.

This result is similar to the result observed by Malik, *et. al.*, (2003) who have found that all strains of *Klebsiella pneumoniae* are resistant to tetracycline, but this result is in contrast with the result reported by Alles, *et. al.*, (2000) who have observed that all strains of *Klebsiella pneumoniae* are sensitive to tetracycline.

The results also showed that only one isolate of *Klebsiella pneumoniae* was sensitive to cefotaxime and two isolates were resistant.

Masters, (1997) has shown that the resistance of *Klebsiella pneumoniae* to cefotaxime is probably attributed to the fact that most clinical isolates of *Klebsiella pneumoniae* produce sixty different plasmid and/or chromosomal mediated beta-lactamase enzymes. As plasmid mediated enzyme production is much more easily transferred to cells, there has been increase in beta-lactamase resistance to third generation cephalosporins (i.e. cefotaxime). These

enzymes are called “Extended Spectrum Beta-lactamses” and these enzymes are currently inhibited by clavulanic acid.

Thus, this result is in agreement with those obtained by Martinez, *et. al.*, (1996) who have shown that increase of resistance to cefotaxime can be attributed to the fact that these strains have lacked two major porins simultaneously and these results indicate that beta-lactam resistance in *Klebsiella pneumoniae* increases significantly when its both porins are lost simultaneously.

But the result is in contrast with the result obtained by Malik, *et. al.*, (2003) and Jones, *et. al.*, (2004) who have found that (100%) and (87.2%) of the isolates are sensitive to cefotaxime respectively.

On the other hand, the results also showed that (66.7%) of the isolates were sensitive to gentamycin.

This result was identical to the result observed by Modarres, *et. al.,* (1998) who have found that (73%) of *Klebsiella pneumoniae* isolates were sensitive to gentamycin.

Also, all *Klebsiella pneumoniae* isolates have been found to be sensitive (100%) to chloramphenicol.

This result is correlated with those obtained by Alles, *et. al.*, (2000) who have observed that most *Klebsiella pneumoniae* strains are sensitive to chloramphenicol, and they have suggested that the sensitivity to this drug does not change after capsule, antigen O loss or porin mutation, even when expression of both major porins is lost.

Furthermore, all isolates of *Klebsiella pneumoniae* were sensitive to ciprofloxacin which was classified within the fourth generation of fluoroquinolones.

This result is in agreement with the result obtained by Lima, *et. al.*, (2004) who have pointed out that all *Klebsiella pneumoniae* strains isolated from patients with bacterial conjunctivitis are sensitive to ciprofloxacin, and have suggested that the fourth generation of fluoroquinolones seem to be more effective than previous generations of fluoroquinolones against *Klebsiella pneumoniae* strains and other organisms isolated from patients with bacterial conjunctivitis.

Conclusions

and

Recommendations

Conclusions and

1. Conclusions

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

1. The prevalence of 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 ages ranging between (15 - 74) years.
3. The common type of bacteria which cause bacterial conjunctivitis in this study is *Haemophilus influenzae* followed by *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Neisseria gonorrhoeae*, and *Klebsiella pneumoniae*.
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.

2. Recommendations

According to the results obtained in the present study, we may recommend the following:

1. Patients' education about personal hygiene to reduce the risk of infection can be achieved through the following:
    - 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.
    - House hold member and other contacts of case patients also are encouraged to practice proper hand hygiene and to avoid sharing such items as towels, contact lens solutions, eye make-up, eye drops and eye medications.
2. Medical staff working in neonatal care unit should wear gloves with frequent hand washing which is necessary to reduce transmission of infection.
3. Schools and child care facilities with children with conjunctivitis are advised to thoroughly clean and disinfect shared items "toys and desks".
4. New antibiotics should be used but not for long time to avoid creating new generations of bacterial resistant to these antibiotics.
5. We should encourage other researchers to be done for the detection of other pathogens such as viruses and *Chlamydia trachomatis* which caused conjunctivitis.

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

رسالة

مقدمة إلى مجلس كلية الطب / جامعة بابل

كجزء من متطلبات

نيل شهادة الماجستير في الأحياء المجهرية الطبية

من قبل

نيران كاظم فرهود الربيعي

1426 جمادى الثاني

2005 تموز

الخلاصة

أجريت هذه الدراسة باخضاع (120) مسحة من ملتحمة العين من المرضى الذين يعانون من خمج ملتحمة العين لعزل وتشخيص السلالات البكتيرية المسببة للخمج. تراوحت أعمار المرضى من (4) أيام إلى (74) عاماً ، من كلا الجنسين ، والمراجعون إلى مستشفى الحلة التعليمي العام / وحدة العيون ، للفترة من تشرين الثاني 2003 ولغاية أيار 2004 في مدينة الحلة.

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

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

كما تم الكشف عن بعض عوامل الضراوة لهذه العزلات البكتيرية ، وقد أظهرت النتائج بأن :

1. جميع عزلات (*Haemophilus influenzae*) و(*Neisseria gonorrhoeae*) غير حاوية على المحفظة ، في حين جميع عزلات (*Klebsiella pneumoniae*) كانت حاوية على المحفظة . وكذلك وجد بأن (42.1%) من عزلات (*Staphylococcus aureus*) كانت حاوية على المحفظة ، و (28.6) فقط من عزلات (*Streptococcus pneumoniae*) حاوية على المحفظة .
2. جميع عزلات (*Haemophilus influenzae*) و(*Neisseria gonorrhoeae*) و (*Klebsiella pneumoniae*) لم تظهر القابلية على إنتاج الهيمولايسين ، في حين جميع عزلات (*Streptococcus pneumoniae*) تمتلك القدرة لإنتاج الهيمولايسين . وكذلك وجد أن (68.4%) فقط من عزلات (*Staphylococcus aureus*) تمتلك القدرة لإنتاج الهيمولايسين .
3. جميع عزلات (*Haemophilus influenzae*) و (*Streptococcus pneumoniae*) و (*Neisseria gonorrhoeae*) غير قادرة على إنتاج السايديروفورات ، ولكن جميع عزلات (*Klebsiella pneumoniae*) كانت قادرة على إنتاج السايديروفورات ، و (26.3%) فقط من عزلات (*Staphylococcus aureus*) كانت قادرة على إنتاج السايديروفورات .

4. جميـع عـزلات (*Haemophilus influenzae*) و (*Streptococcus pneumoniae*) و (*Neisseria gonorrhoeae*) تمتلك القابلية علـى إنتـاج أنزيمـات البروتييـز الخارجيـة ، ولكـن وجـد إن جميـع عـزلات (*Klebsiella pneumoniae*) ليس لها القابلية على إنتاج أنزيمات البروتييـز الخارجيـة ، فـي حـين وجـد أن (42.1%) مـن عـزلات (*Staphylococcus aureus*) تمتلك القابلية على إنتاج أنزيمـات البروتييـز الخارجية .

5. كذلك تم الكشف عن قابلية هذه العـزلات البكتيريـة علـى امـتلاك عوامـل الاسـتيطان (Adherence factors) ، ووجـد أن جميـع عـزلات (*Haemophilus influenzae*) و (*Streptococcus pneumoniae*) و (*Neisseria gonorrhoeae*) و (*Klebsiella pneumoniae*) تمتلـك عوامـل الاسـتيطان ، فـي حـين وجـد أن جميـع عـزلات (*Staphylococcus aureus*) لا تمتلك عوامل الاستيطان .

وتم دراسة تأثير بعض المضادات الحياتية على هذه العـزلات البكتيريـة,وقـد أشارت النتائج بأن سيبروفلوكساسين وكلورامفنيكول أكثر حـساسية مـن المـضادات الحياتيـة الأخـرى ، إذن إنهمـا أول مـا يجـب أن يـتم اختيارهمـا فـي عـلاج (Bacterial conjunctivitis) .

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