Isolation and Characterization of *Streptococcus agalactiae* from Woman Patients with Vaginitis in Hilla Province

A Thesis

Submitted to the Department of Microbiology, College of Medicine, University of Babylon, in Partial fulfillments of the Requirements for the Master of Degree in Medical Microbiology

By

Lamees Abdul Razzak Abdul Lateef

٢٠٠٥ A.D ١٤٢٥ A.H

عزل وتشخيص بكتريا *Streptococcus agalactiae* من النساء المصابات بالتهاب المهبل في مدينة الحلة

أطروحة

مقدمة إلى فرع الأحياء المجهرية/كلية الطب/ جامعة بابل

كجزء من متطلبات

نيل شهادة الماجستير في الأحياء المجهرية الطبية

من قبل

لميس عبد الرزاق عبد اللطيف

١٤٢٥هـ ٢٠٠٥م

بِسْمِ اَللّٰه اَلرْحمَنْ اَلْرَ حْيم

وفَوْقَ كُلِ ذِيْ عِلْمٍ عَلِيْمٌ

صدق الله العظيم

سورة يوسف, من الآية ٧٦

Dedication

To My Family

Aknowledgements

I am deeply indebted to my supervisor, Assistant Prof. Dr. Mohammed Sabri Abdul-Razzak Head of Microbiology Department, College of Medicine, University of Babylon for his guidance patience and supervision. This thesis would not have been possible, without his sincere help and assistance.

I am also gratful to Dr. Suzan Taha , a gynecologist in Babylon Hospital of Maternal and Delivery for her invaluable advice and faithful help.

My thanks are also due to all members of the Department of Microbiology for their generous co-operation.

Lamees

CONTENTS

Chapter II: Materials and Methods

Chapter III: Results and Discussion

List of Tables

List of Figures

List of Abbreviations

****

GBS	Group B *Streptococcus*
STD	Sexual Transmitted Disease
CFA	Colonization Factor Antigen
DPPC	dipalmotyl phosphotidylcolin
GAS	Group A *Streptococcus*
BLIS	Bacteriocin like-inhibitory substance
PBPs	Penicillin-binding proteins
LGT	Lower genital tract
TSA	Trypicas soy agar
TCA	Trichloro acetic acid
CAMP	Christie, Atkins, and Munch-Peterson
CNS	Central nerve system
MIC	Minimal inhibition concentration

Abstract

In this study, ١٢٠ vaginal swabs obtained from women patients suffering from vaginitis, and admitted to Babylon Hospital of Delivery and Maternal in Hilla Province were included. It was found that only three isolates of *Streptococcus agalactiae* were identified. All isolates underwent culture and biochemical tests to confirm diagnosis, and it was reveled that the all isolates gave the same cultural and biochemical characters except in their ability to grow in ٦.٥٪ NaCl. However, other types of bacteria and yeasts were also isolated. *Streptococcus agalactiae* isolates were isolated mainly from non-pregnant women but there was no isolates obtained from pregnant women.

The effect of some antibiotics on *Streptococcus agalactiae* was investigated, and the results showed that all isolates were entirely resistant to Amoxillin (١٠٠٪), and lesser to Gentamycin, Tetracycline, and Erythromycin (٦٦.٦٪), and much lesser to Clindamycin (٣٣.٣٪). On the contrary, all Group B *Streptococcus* isolates were found to be susceptible to Lincomycin, Penicillin, and Chloramphenicol.

Some virulence factors of bacteria were also studied, and the results showed that all bacterial strains possessed capsules, which were regarded as the most virulent factor of the bacteria.

The results also showed that all strains did not have the first and second Colonization Factor Antigen (CFA/I & CFA/II) whereas all isolates contained (CFA/III).

In respect of the ability of the isolates to produce Haemolysin, Siderophore, and Extracellular proteases, the results of the work showed that all the isolates were able to produce bacterial haemolysin, but were not able to produce siderophore and extracellular proteases.

The ability of the bacterial isolates to produce bacteriocin was also investigated and it was found that only one isolate had the ability to produce bacteriocin that had effect on other *Streptococcus agalactiae* strains isolated in this study.

The effect of lactic acid on the bacterial growth was likewise studied. It was stated that lactic acid at high concentration >٢٠μg/ml could *cause inhibition to Streptococcus agalactiae* growth.

الخلاصة

تم في هذه الدراسة إخضاع ١٢٠ مسحة مهبلية للعزل والتشخيص, استحصلت من النساء اللواتي يعانين من التهاب المهبل والمراجعات إلى مستشفى الولادة والأطفال في الحلة. وقد تم عزل وتشخيص ثلاث عزلات من بكتيريا *Streptococcus agalactiae*، ولوحظ بان جميعها تمتلك نفس المواصفات الزرعية والبايوكيماوية باستثناء قابليتها على النمو بوجود كلوريد الصوديوم بنسبة ٦.٥%, ولوحظ أيضاً بان جميع العزلات قد تم عزلها من النساء المتزوجات غير الحوامل في حين لم تعزل أي عزلة من النساء الحوامل.

درس تأثير بعض المضادات الحياتية على عزلات بكتريا *Streptococcus agalactiae* وأظهرت النتائج بأن جميع العزلات ذات مقاومة كاملة للأموكسيلين, وبدرجة أقل لكل من الجنتاماسين والتتراسايكلين والأرثرومايسين (٦٦.٦%), اضافة الى الكلندامايسين (٣٣.٣%), في حين وجد أن جميع العزلات كانت على العكس, حساسة للبنسيلين والينكوماسين والكلورامفينيكول.

درست بعض عوامل الضراوة لهذه البكتريا وقد وجد أن جميع العزلات حاوية على المحفظة فضلا على عامل الأستيطان الثالث (CFA/III) وقدرتها على إنتاج الهيمولايسين البكتيري, في حين لم تظهر العزلات قابلية على إنتاج السايدروفورات وأنزيمات البروتيز الخارجية.

كما تم الكشف عن قابلية هذه البكتريا على إنتاج البكتريوسين, وقد أظهرت عزلة واحدة تتحسس لتأثره.

وأظهرت النتائج بأن حامض اللاكتيك عند التراكيز التي تفوق ٢٠ مايكروكرام/مل يمتلك تأثير تثبيطيا على نمو عزلات البكتريا.

Introduction and Literature Review

١.١ INTRODUCTION

Streptococcus agalactiae or Group B *Streptococcus* (GBS) is gram-positive coccus which appears in chain or pairs. It is usually beta-hemolytic and reliably identified by its production of Lancefield group B antigen (Ruoff, *et.al.*, ١٩٩٥). *Streptococcus agalactiae* has been classified serologically into ٩ serotypes (Ia, Ib, and II-VIII) according to difference in capsular polysaccharide (Perch, *et.al.*, ١٩٧٩). The gastrointestinal tract is the most likely human reservoir of *Streptococcus agalactiae*, whereas the genitourinary tract is the most common site of secondary spread. It is a member of the normal flora of the female genital tract, and in most studies from ١٠٪-٣٠٪ of pregnant women are colonized with *Streptococcus agalactiae* in the vaginal or rectal area (Regan, *et.al.*, ١٩٩١). However, this agent is frequently implicated as an important cause of a severe invasive disease primarily in newborns, pregnant women, and adults with underlying disease (Harrison, *et.al.*, ١٩٩٥).

In newborns, the most frequent presentation are bacteraemia, pneumonia, or meningitis (Baker, *et.al.*, ١٩٩٠). In pregnant women, *Streptococcus agalactiae* infection causes urinary tract infection, amnionitis, endometritis and wound infection postpartum (Anonymous, ١٩٩٦). In non-pregnant women, bacteraemia, genitourinary infection and pneumonia are the most frequent manifestations (Farley, *et.al.*, ١٩٩٣).

The rate of invasive *Streptococcus agalactiae* is twice as high in black adults as in whites. Black race has been identified as an important risk factor for both early-onset and late-onset Group B *Streptococcal* disease in neonates (Schuchat, *et.al.*, ١٩٩٠). However, these associations may be due to socioeconomic conditions.

Most of the infection with this bacteria can be prevented by using antibiotics such as penicillin or ampicillin (Betriu, *et.al.*, ١٩٩٤).

There is no independent study on this bacteria conducted in Iraq; this work therefore, aims to study the isolation and identification of *Streptococcus agalactiae* associated with vaginitis, to examine some virulence factor such as (Capsule, Hemolysin, Colonization Factor Antigens (CFA), Siderophores and Extracellular Protease), and to show the effect of some antibiotic on Group B *Streptococcus* isolates.

١.٢ Literature Review

١.٢.١ Group B *Streptococcus* (GBS)

Group B *Streptococcus*, taxonomically known as *Streptococcus agalactiae*, is a gram-positive cocci, non-motile, non-spore forming, and spherical or ovoid. Less than ٢ μm in diameter, catalase negative, oxidase negative, facultatively anaerobic, fermentative metabolism with lactic acid being primary product, it grows poorly on nutrient media, and prefer media enriched with blood or serum (Edward and Baker, ١٩٩٥).

On blood agar, hemolysis is usually of the β type, though the zones are usually narrow and smaller than those produced by group A, C, or G *Streptococci*. In some culture, hemolysis is of α type and in

some there is no hemolysis. Colonies are gray, mucoid and larger (about ٢ mm) than those of other *Streptococci*. They appear orange on some media, but pigment is most reliably formed on Islam's medium (Islam, ١٩٧٧).

Cell wall structure possesses a typically gram-positive cell wall consisting of mucopeptide (peptidoglycan) and teichoic acid. In immature cultures, a capsule may also be present .Group B *Streptococcus* has a layer of C carbohydrate which can be used to serologically classify an isolate (Colman, ١٩٨٨).

Resistance to bacitracin is a common feature of β-hemolytic strains of Group B *Streptococcus*. About ٨٠٪ of the strains develop a characteristic orange pigment on anaerobic incubation and this is seen best on Columbia agar plates. Biochemical tests are useful for differentiating it from other *Streptococci* are growth on ٤٠٪ bile agar, splitting of sodium hippurate and inability to hydrolysis easculin. Group B *Streptococcus* show a positive CAMP reaction, that is, it produce substance which acting with *Staphylococcal* β-hemolysin, completely lyses sheep red blood cell (MacFaddin, ٢٠٠٠).

Streptococcus agalactiae has a variety of potential virulence factors including a polysaccharide capsule occurring in ٩ distinct structural and antigenic types (Ia, Ib and II-VIII), secretion of enzyme hyaluronidase (formerly incorrectly identified as neuraminidase (Pritchard and Lin, ١٩٩٣)) and C٥a-peptidase, and the ability to bind human immunoglobulin A via the Fe protein to the surface-associated

beta-antigen protein. The serotype-specific capsular polysaccharides are essential for pathogenesis (Rubens, *et.al.*, ١٩٨٧).

Group B *Streptococci* may be found in the genitourinary tract and/or the gastrointestinal tract of humans. In adults, the gastrointestinal tract appears to be the major reservoir of Group B *Streptococcus* infection, with frequent spread to the genitourinary tract. Most people are unaware that they have the bacteria because these are usually have show no clinicaly symptoms (Edward and Baker, ٢٠٠٠).

Group B *Streptococcus* disease occurs when the bacteria gets past a person's immune system defenses. Health conditions that decrease the person's immunity like chronic or debilitating (i.e. diabetes, liver disease, and malignancy) infections also make invasive disease more likely (Christensen, *et.al.*, ١٩٨٢).

Streptococcus agalactiae causes invasive disease primarily in newborns and in women in the postpartum period (Anonymous, ١٩٩٦). Adults with severe infections unrelated to pregnancy are usually elderly and have underlying illness such as diabetes mellitus, liver failure, malignancy, acquired immunodeficiency syndrome, or renal failure (Jackson, ١٩٩٦). In non-pregnant adults, skin or soft-tissue infection, bacteraemia, genitourinary infection, and pneumonia are the most common manifestations of disease (Farley , *et.al.*, ١٩٩٣).

An infant may become infected by passing through the birth canal of a woman with symptomatic or asymptomatic GBS. Infant

may get severe form of Group B *Streptococcus* disease, including meningitis and bacteraemia, transmitted through the birthing process (Bakers and Edward, ١٩٩٠).

The relative risk of vaginal infection with *Streptococcus agalactiae* in patients with purulent vaginal discharge was greater than that of *Candida* sp. infection, and lower than that of *Trichomonas* sp. infection (Maniatis, et.al., ١٩٩٦). But Group B *Streptococcus* infection is not classified within sexually transmitted disease (STD) (Honig, *et.al.*, ١٩٩٩).

١.٢.٢ Physiopathology of *Streptococcus agalactiae*

Streptococcus agalactiae is an invasive encapsulated organism capable of producing severe disease in hosts that are immunocopromised. Virulence is related to the polysaccharide toxin produced by Group B *Streptococci.* Immunity is mediated by antibody to the capsular polysaccharide and is serotype-specific. Several serotypes are known: Ia, Ib, II, III, IV, V, VI, VII, and VIII (Perch, *et.al.*, ١٩٧٩).

Group B *Streptococci* colonize the vagina, gastrointestinal tract, and the upper respiratory tract of healthy humans. The portal of entry is not apparent, but possible areas include the skin, genital tract, urinary tract, and the upper respiratory tract.

The physiopathology of Group B *Streptococci* implies that this bacteria can

١- evade the host defense,

٢- adhere to and invade various types of epithelial cells, including those constituting the brain blood barrier, and

٣- rapidly adapt to various growth condition (pH, temperature variations and nutritional starvation).

The pathogenesis of Group B *Streptococci* infections is a multifactorial process that includes the ability to adhere, colonize, invade epithelial cell, and then replicate and evade host defenses (Nizet, *et.al.*, ٢٠٠٠).

Adherence is thought to be initiating entry into host cell, promoting invasion of deeper tissue and ultimate dissemination of the bacteria to the blood stream and multiple organ systems (Tamura and Rubens, ١٩٩٤). The process of colonization involves microbial and host receptor-lignad interaction.

Surface protein of pathogenic bacteria plays an important role during the infection process by mediating interactions between the pathogen and the host cell and/or evasion from the host defense. Surface proteins are predicated to be necessary for adherence because protease treatment of the bacteria reduces adherence and invasion (Bagg, *et.al.*, ١٩٨٢).

١.٢.٣ Incidence of *Streptococcus agalactiae*

Streptococcus agalactiae strains were once considered pathogens of domestic animals causing mastitis in cows.

Streptococcus agalactiae is now best known as a cause postpartum infection as well as the most common cause of neonatal sepsis. More recently, the role of this organism is found to be the cause of infection in non-pregnant women (Farley, ٢٠٠١).

Group B *Streptococci* colonize the vaginal and gastrointestinal tract in healthy women. Neonates acquire this organism as a result of vertical transmission from the maternal genital tract to the infant in utero or at delivery. Carriage rate in women may range from ٥٪-٤٠٪. Whereas acquisition of the organism by the neonate is efficient, the rate of subsequent clinical disease is quite low, ١٪-٢٪. Neonatal sepsis from Group B *Streptococci* is a rare event, but it is more common in the setting of prematurity and prolonged rupture of the membranes. The large number of women who carry this organism and the fact that neonatal sepsis is a rare event make the prevention approach to this problem difficult (Eickhoff, *et.al.*, ١٩٦٤).

Disease in the neonate is divided into early and late disease. Early neonatal sepsis with Group B *Streptococci* is often observed within ٢٤ hours of delivery, but it can become apparent as late as ٧ days after birth. Nothing specific regarding the clinical presentation in early disease differentiates Group B *Streptococci* as the etiology from other pathogens. Pneumonia with bacteraemia is common and meningitis less likely (Weisman, *et.al.*, ١٩٩٢). Late disease is defined as infection after one week and before three months after birth. Late disease is commonly serotype III, characterized by meningitis and bacteraemia (Stoll, *et.al.*, ١٩٩٦).

Group B *Streptococci* as a cause of infection in pregnant women can be manifested by chrioamnionitis, endometritis, or genitourinary infection with bacteraemia. Rarely can endocarditis or meningitis be observed (Berner, ٢٠٠٢).

Only in the last three decades has the role of Group B *Streptococci* as a serious pathogen in the non-pregnant adult been well defined. This organism is an extremely rare cause of infection in healthy individuals. It is almost always associated with underlying abnormalities. Diabetes is associated most commonly with Group B *Streptococci* in some series (Joshi, *et.al.*, ١٩٩٩). Malignancy is the most common association in a series from an institution with a large oncology population. Cardiovascular and genitourinary abnormalities have been observed as major factors for the acquisition of Group B *Streptococci.* Other conditions associated with Group B *Streptococci* in adults include neurologic deficits, cirrhosis, steroids, AIDS, renal dysfunction, and peripheral vascular disease (Waite, *et.al.*, ١٩٩٦).

Group B *Streptococci* in elderly people, aged ٧٠ years or more, is strongly linked to congestive heart failure and being bedridden. Urinary tract infection, pneumonia, and soft tissue infection are the most common illness in elderly people, possibly as a result of aspiration of Group B *Streptococcus* from the upper respiratory tract infection (Bayer, *et.al.*, ١٩٧٦).

Group B *Streptococci* colonize not only the female genital tract but are also found commonly in the gastrointestinal tract. They have been described as symptomatic colonizers of the urethra in both men

and women (Mathew, *et.al.*, ١٩٩٣). Group B *Streptococci* can colonize the upper respiratory tract. Group B *Streptococci* are very invasive but produce little inflammation at the entry site (Schuchat, ١٩٩٩).

Primary bacteraemia without an obvious source with Group B *Streptococcal* infection is a common presentation in adults. Group B *Streptococci* can be a cause of acute destructive endocarditis, which may require emergency valve replacement (Duma, *et.al.*, ١٩٦٩). Other sources of bacteraemia include pneumonia in elderly people, genitourinary and soft tissue infection. Polymicrobial bacteraemia with Group B *Streptococci* is observed in disease related to infected lines and can also reflect a genitourinary source (Lerner, *et.al.*, ١٩٧٧).

١.٢.٤ Epidemiology of *Streptococcus agalactiae*

Transmission of Group B *Streptococci* from mother to child can occur during birth in the case of vaginal colonization, or before birth in the setting of maternal infection. Colonization of newborns may occur without infection; organisms can be isolated from neonatal skin and mucous membranes. Vertical transmission from mother to baby is well described in early onset neonatal disease whereas the horizontal transmission can also occur through sexual intercourse (Baker, *et.al.*, ١٩٧٣).

Nosocomial spread of *Streptococci* from baby to baby via health care workers has been demonstrated to occur in nurseries (Noya and Rench, ١٩٨٧).

Studies of carriage of Group B *Streptococci* suggest that the presence of these organisms in vagina may be intermittent, leading to the hypothesis that the normal habitat of Group B *Streptococci* is the intestine. Group B *Streptococci* can be isolated from faeces and the contents of the small intestine (Anthony, *et.al.*, ١٩٨٣), but the rectum and anorectal areas appear to be the major sites of the colonization (Islam and Thomas, ١٩٨٠; Easmon and Tanna, ١٩٨١). In pregnant women, anorectal specimens are more likely to be positive than those obtained from the urethra or vagina (Ross and Cumming, ١٩٨٢). Vaginal carriage rates have been found to be higher in the earlier part of the menstrual cycle, in teenagers compared to older women, in sexually active women and in women with a history of three or fewer pregnancies. Published carriage rate values have ranged from approximately ٥٪ to ٢٥٪ in surveys of pregnant women (Finch, *et.al.*, ١٩٧٦).

Asymptomatic bacterial colonization can be documented in the vagina, in the lower gastrointestinal tract and occasionally in the upper respiratory tract. It is estimated that up to ٢٠٪ of asymptomatic pregnant or non-pregnant women carry the organism (Oxtoby, *et.al.*, ١٩٨٦).

Vaginal carriage rates vary from ٥٪ to ٣٥٪, but are the same in pregnancy as in sexually active non-pregnant women. Carriage rates do not appear to be affected by age, race, and socioeconomic status. The majority of two thirds of pregnant women who carry Group B *Streptococci* do so intermittently or transiently, and only one third of

all pregnant Group B *Streptococci* carriers have the organism chronically (Regan. *et.al.*, ١٩٩١).

١.٢.٥ Vaginitis

Vaginitis is a name given to describe swelling, itching, burning or infection in the vagina that can be caused by several different germs. This is a common gynecological problem found in women of all ages, with most women having at least one form of vaginitis during their lives. Vaginal infections often occur when a women's natural resistance is lowered by anxiety, tension, lack of sleep, poor diet, and sexual activity with an infected partner. Vaginal infections are also caused by the proliferation of organisms such as *Candida albicans* as part normal commensal flora (Quan, ٢٠٠٠).

In addition, the vaginal environment is influenced by a number of different factors including a woman's health, her personal hygiene, medications, hormones (particularly estrogen), and the health of her sexual partner. A disturbance in any of these factors can trigger vaginitis. Vaginal infection can produce a variety of symptoms, such as abnormal or increased discharge, itching, fishy odor, irritation, and painful urination or vaginal bleeding (Spaker, ١٩٩١).

Vaginitis caused by *Streptococcus* presents a creamy, white discharge that is normally clear or white cloudy. There is usually no burning of the vulva as is the case with yeast infection, and the discharge is not very odorous like a bacterial vaginosis. There are several *Streptococcal* species that can be present in the vagina: groups

A, B and D. About ٧٠٪ of *Streptococcus* is due to group B and about ٣٠٪ is due to group D. Group A infection is rare. *Streptococcus* vaginitis can occur spontaneously but very often it is caused by the antibiotic treatment given for bacterial vaginosis (Frederick and Jelovesk, ٢٠٠١).

Beta hemolytic *Streptococci* cause purulent vaginal discharge (vaginitis), and the organism that cause purulent infection in the urethra may also infect the epithelial cell in the cervical opening (Baron, *et.al.*, ١٩٩٤).

١.٢.٦ Virulence Factors of *Streptococcus agalactiae*

The factors that determine the initiation, development, and outcome of an infection involve a series of complex and shifting interaction between the host and the parasite, which can vary with different infecting microorganisms (Brogden, *et.al.*, ٢٠٠٠). The microbial factors that contribute to the virulence of a microorganism can be divided into three major categories:-

١- Those that promote colonization of host surface.

٢- Those that evade the host's immune system and promote tissue invasion.

٣- Those that produce toxins that result in tissue damage in the human host.

Group B *Streptococcus* can produce many virulence factors associated with its pathogenicity. Some Group B *Streptococcus* virulence factors are secreted directly into the medium; others may be

associated with the bacterial surface but can be removed in an active form by proteolysis or binding to host cell component (Nizet and Rubens, ٢٠٠٠).

١.٢.٦.١ Capsule:

A capsule is a loose, relatively unstructured network of polymers that covers the surface of an organism. Capsule is a major virulence factors and the clinical isolates of *Streptococcus agalactiae* produce a polysaccharide capsule. A total ٩ different capsular serotypes (Ia, Ib, and II-VIII) have been demonstrated (Perch, *et.al.*, ١٩٧٩). They are all polysaccharides composed of galactose and glucose, combined with ٢-acetoamido-٢-deoxyglucose, rhamnose or N-acetylglucosamine and with terminally positioned sialic acid which gives them a net negative charge (Kogan and Uhrin ١٩٩٤).

The capsular polysaccharides are essential virulence factors (Rubens, *et.al.*, ١٩٨٧). They inhibit phagocytosis and complement activation in the absence of specific antibody. Capsular polysaccharides of Group B *Streptococcus* are not required for adherence to, or invasion of, epithelial cells and may, in fact, attenuate these cellular interactions to some degree (Tamura, *et.al.*, ١٩٩٤).

Of the nine serotypes, the type Ia, Ib, II, III, and V are responsible for the majority of neonatal human Group B *Streptococcus* disease. Serotype III is particularly important because it causes a significant percentage of early-onset disease (infection occurring in the first week of life) and the majority of late-onset disease (infection occurring after the first week of life) in human

neonates. It is also responsible for the majority (٨٠٪) of neonatal Group B *Streptococcus* meningitis cases (Harrison, *et.al.*, ١٩٩٨).

Type V is the most common capsular serotype associated with invasive infection in non-pregnant adults, and the emergence of type V strains over the past decade has been temporally linked to an increase in *Streptococcus agalactiae* disease in population (Tyrrell, *et.al.*, ٢٠٠٠).

١.٢.٦.٢ Hemolysin:

Streptococcal hemolysins can affect other kinds of cell and are better termed cytolysins. A hallmark phenotype of Group B *Streptococcus* in the clinical laboratory is the appearance of beta-hemolysis surrounding colonies growning on the surface blood agar plates. Colonies are gray, mucoid and larger (about ٢mm) than those of other *Streptococci.* They appear orange on some media, but pigment is reliably formed on Islam's medium (Islam, ١٩٧٧). The Group B *Streptococcus* beta-hemolysin has been particularly interesting to researchers for decade because of its ability to lyse not only red blood cells but a broad range of eukaryotic cell type (Nizet, *et.al.*, ١٩٩٦).

GBS hemolysin activity is normally associated with the bacterial cell surface (Platt, ١٩٩٥) and not present in abundance in the supernatant. However, Group B *Streptococcus* hemolysin activity can be extracted into the supernatant if a large carrier molecule such as

albumin, starch, or lipotechoic acid is present in the medium (Marchlewicz and Duncan, ١٩٨٠; Tsaihong and Wennerstrom, ١٩٨٣).

In addition to direct cytotoxity, the Group B *Streptococcus* hemolysin exhibit proinflammatory, proapoptotic, and proinvasive properties that could contribute to disease pathogenesis. The cytolytic properties of the Group B *Streptococcus* hemolysin are inhibited in a dose-dependent fashion by phospholipids such as dipalmotyl phosphatidylcholin (DPPC), the major component of pulmonary surfactant (Tapsall and Phillips, ١٩٩١; Nizet, *et.al.*, ١٩٩٦). DPPC can also inhibit Group B *Streptococcus* mediated cytokine activation (Doran, *et.al.*, ٢٠٠٢; Talati, *et.al.*, ٢٠٠١), macrophage apoptosis (Fettucciari, *et.al.*, ٢٠٠٠) and epithelial cell invasion (Doran, *et.al.*, ٢٠٠٢).

A weaker zone of β-hemolysis encircling colonies on selective culture of a rectovaginal swab obtained during pregnancy would probably indicate colonization with Group B *Streptococcus*, and guide intrapartum prophylactic antibiotic therapy to prevent neonatal infection (Keufhold and Ferrieri, ١٩٩٣; Garey, ٢٠٠١).

It appears that Group B *Streptococcus* hemolysin is a pluripotent virulence factor that contributes to disease pathogenesis by cytotoxity and inflammatory activation.

١.٢.٦.٣ **Adherence and Colonization Factor**:

The first host barrier for many invading pathogens is usually a mucosal surface, and since epithelial cell turnover is around ٤٨ hours in these environments, the bacterium must attach and replicate

sufficiently to avoid being swept away. Therefore, many have evolved motile or attachment elements like flagella and pilli to cross the barrier and invade (Sauer, *et.al.*, ٢٠٠٠). Simple attachment is mediated through a receptor on the host cell surface, and an adhesion on the bacterial one. Some may be species or even strain specific, while others exhibit tissue tropism i.e. *Streptococcus mutans* will colonize teeth, but not the tongue (Mouricout, ١٩٩٧).

The fimbriae mediated haemagglutination could be resistant or sensitive to blocking by D-mannose (Duguid, *et.al.*, ١٩٩٦). The mannose-specific fimbriae are named type I fimbriae and are widespread in most species of enterobacteriaceae.

The first colonization factor discovered is the colonization factor antigen (CFA/I) isolated from *E. coli.* This factor causes the agglutination of red blood cell for human group (A). Another colonization factor called (CFA/II) has also been discovered and found to cause the agglutination of chicken blood. The two factors are non-inhibited in the presence of mannose sugar and these two factors have specialized host (Al-zaag, ١٩٩٤).

The third colonization factor (CFA/III) causes the agglutination of red blood cell in the presence of Tannic acid. This factor underlyies control of two genetic chromosomes: one of them is responsible for piliation, and the other for adhesion (Hornick, *et.al.*, ١٩٩٥).

For *Streptococcus agalactiae,* no adhesion to epithelial vaginal cell is observed, but the mechanisms of adherence to vaginal cell are still unknown (Soledad, *et.al.*, ١٩٩٨).

١.٢.٦.٤ 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. High concentration of iron not only benefits invading cells, they may also mediate antimicrobial activities of defense cell (Weinberg, ١٩٩٨). In most environments, the level of soluble iron is too low for sufficient iron to be acquired by passive diffusion of ions into the cell. Bacteria have evolved a number of different strategies to combat this problem and one of the most of these is the secretion of iron-chelating compounds termed siderophores, which are small (MW ٥٠٠-١٠٠٠) lignand that are specific for ferric iron and thus they supply iron to the bacterial cell (Griffiths, *et.al.*, ١٩٨٧). This chelate ferric iron in the environment and ferrisiderophore complexes are taken up by the bacteria through specific cell-surface receptor proteins. The iron is then released from the ferrisiderophore complex for incorporation into cellular protein.

About ٥٠٠ siderophores have been identified, and most bacterial genera contain siderophore producers (Drechsel, *et.al.*, ١٩٩٧). The biological importance of siderophore has been demonstrated for a number of species; for example, siderophore-deficient mutants of pathogenic bacteria are invariably less virulent in disease models (Lamont, *et.al.*, ٢٠٠٢). Thus, siderophores are compounds secreted under low iron stress, that act as a specific ferric iron chelate agent and due to their potentialities in the biological control of phytopathogenic fungi and bacteria (Diaz de, *et.al.*, ٢٠٠٢).

E. coli has been reported to be able to synthesize at least five different siderophore- ferric iron transport systems (Touati and Jacques, ١٩٩٥).

Its known that the bacteria which able to produce hemolysin have no ability to produce siderophore and vice versa (Al-zaag, ١٩٩٤). So, *Streptococci* groups, including *Streptococcus agalactiae,* have the ability to produce hemolysin but are not able to produce siderophore (Eichenbaum, *et.al.*, ١٩٩٦).

١.٢.٦.٥ Extracellular Protease Production:

Proteases represent a class of enzymes that are involved in essential biological processes like blood clotting, controlled cell death, and tissue differentiation. They catalyse important protelytic steps in tumor invasion or in infection cycle of a number of pathogenic microorganisms and viruses. Proteases assist the hydrolysis of large polypeptides cell. The extracellular enzymes play a major role in nutrition due to their depolymerizing (Beynom and Bond, ١٩٨٩).

Microorganisms represent an excellent source of enzymes owing to their broad biochemical diversity and their susceptibility to genetic manipulation.

Numerous proteases are produced by microorganisms depending on the species of the producer or the strain even belonging to the same species. Several proteases are also produced by the same strain under various culture conditions (Rae, *et.al.*, ١٩٩٨).

Most bacteria proteases excreted into infected host exhibit a wide range of pathogenic potentials ranging from pain, edema or even stock to translocation of bacteria from the site of infection into systemic circulation, thus resulting in septicemia (Maeda and Yamamoto, ١٩٩٦). Some bacterial proteases are involved in activation of the other host protease zymogens such as plasminogen, procollagenase (Lähteenmakia, *et.al.*, ٢٠٠١).

Several species of beta-hemolytic *Streptococci* are known to produce a surface-bound protease that specifically inactivates the human phagocyte chemotaxin C٥a. All Group A *Streptococci* (GAS) serotype and Group B *Streptococci* (GBS) of human origin produce the C٥a peptidase (Hill, *et.al.*, ١٩٨٨).

The high degree of specificity for human C٥a and the exquisite sensitivity of this chemotaxin to the enzyme suggest that peptidase activity is important in human infection.

Several lines of evidence indicate that the protease is important in the pathogenesis of Group B *Streptococci* (Cheng, *et.al.*, ٢٠٠٢).

Other Virulence Factors of Group B *Streptococci:-*

١- **Hyaluronate lyase:-**

Hyaluronate lyase is part of a large family of enzymes called Hyaluronidases, found in many gram-positive pathogen. These enzymes are postulated to facilitate spread of bacteria by breaking down the hyaluronan polymers ubiquitously present in the extracellular matrices of the host.

Group B *Streptococci* produce hyaluronate lyase, the Group B *Streptococci* hyaluronate lyase is encoded by the gene *hylB* and is expressed as a ١١٠ Kd secreted protein (Lin, *et.al.*, ١٩٩٤; Gase, *et.al.*, ١٩٩٨). This enzyme was initially misidentified as a neuraminidase because it was routinely used with bovine mucin in an assay to measure sialic acid release.

The placenta and lung are especially rich in hyaluronan, and may represent preferential targets during the early stages in the pathogenesis of vertical transmission and early-onset infection (Miyake, *et.al.*, ١٩٩٠).

٢- CAMP Factors:

The CAMP reaction is eponymously named for its original descriptors: Christie, Atkins, and Munch-Peterson (Christie, *et.al.*, ١٩٤٤). This reaction refers to the synergistic hemolytic zones produced by colonies of Group B *Streptococci* streaked adjacent to colonies of *Staphylococcus aureus* on sheep blood agar plates.

Group B *Streptococci* CAMP has been shown to carry out two functions. In the CAMP reaction, the toxin damages sheep red cell presensitized to *Staphylococcus aureus* sphingomyelinase (Bernheimer, ١٩٧٩). In a two-step process, *Staphylococcus aureus* sphingomyelinase treatment appears to enhance binding of the CAMP protein to the sheep red cell membrane, which is followed by a high cooperative porin formation and subsequent cell lysis.

These Group B *Streptococci* factors contribute to virulence by direct cytolytic injury to host tissue, disruption of extracellular matrix components, promotion of cellular invasion, impairment of neutrophil recruitment or phagocytosis, or activation of host inflammatory factors in the sepsis cascade.

١.٢.٧ Bacteriocin production

Microorganisms naturally produce a range of protein component from simple polypeptides to very complex macromolecules such as toxins, pili, adhesions, siderophores, flagella, etc. Bacteriocins are grouped under the term toxins and provide a means of defense against other microorganism in the same environment (Leslie, *et.al.*, ١٩٩٨).

Tagg, *et.al.*, (١٩٧٦) have defined bacteriocin, a subgroup of bacterial toxins, as proteinaceous compound that kill closely related bacteria. Although this is true for most bacteriocins, it is evident that these molecules take many forms and may have bactericidal actions beyond closely related species.

Frequently bacterial species carry genes that encode both the production of one or more bacteriocin and immunity to them on chromosome or on plasmids (Frank, ١٩٩٤).

Bacteriocins have a spectrum of size; generally proteinaceous agents, they are sometimes complexed with lipids, carbohydrates or other distinctive proteins (Nissen-Meyer and Holo ١٩٩٢; Jimines-Diaz, *et.al.*, ١٩٩٣). There are three general classes:-

١- The microcins, which are small molecules produced in stationary phase by gram-negative bacteria.

٢- The lantibiotics, which are small molecules produced by gram-positive bacteria.

٣- Bacteriocins, which are a group encompassing medium to large phage-like structure.

These proteins share many characteristics. Many have a low molecular weight, are cationic, tend to aggregate and are benign to the producing organism.

The bacteriocin produced by lactic acid bacteria exhibit relatively broad antimicrobial spectrum and are active against several food spoilage and heath-threatening organism (Kim, ١٩٩٣).

E. coli can produce many types of bacteriocins such as colicins and microcins, which destroy the cell membranes (Lau, *et.al.*, ١٩٩٢).

Many strains of *Streptococci* produce bacteriocin-like inhibitory substance (BLIS) which inhibit growth of a wide range of bacteria (Tagg, ١٩٩٢). BLIS activity among *Streptococcus* species associated with human is believed to play a role in their ability to interfere with growth of other bacteria in the local environment along with other released inhibitory substance such as hydrogen peroxide and lactic acid (Jack, *et.al.*, ١٩٩٥).

The majority of BLIS characterized from *Streptococcus* are relatively small heat-stable proteins termed lantibiotics because they contain the unusual amino acid lanthionine and/or ٣-methyl lanthionine (Jung, ١٩٩١).

Streptococcus bovis, Streptococcus suis (Melacon and Grenier, ٢٠٠٣) and *Streptococcus agalactiae* (Mariela and Marcelo, ٢٠٠٢) have the ability to produce bacteriocin.

١.٢.٨ Bacterial Resistance to antibiotic

Streptococcus agalactiae is a well known cause of invasive infections in neonates and pregnant women. It has increasingly been recognized as a significant pathogen in non-pregnant adults especially among patients with underlying conditions (Tyrrell, *et.al.*, ٢٠٠٠). Penicillin and ampicillin are the drugs of choice for prevention or treatment of *Streptococcus agalactiae* infections, and clindamycin and erythromycin are the recommended alternatives for patients who are allergic to beta-lactam agents. In the majority of susceptibility studies (De Mouy, *et.al.*, ٢٠٠١) penicillin remains uniformly active against *Streptococcus agalactiae* although there are scattered reports of non susceptibility to penicillin or ampicillin (de Azavedo, *et.al.*, ٢٠٠١).

Beta-lactam antibiotics such as amoxicillin are mainly bactericidal like other penicillins. Amoxicillin inhibits the third and final stages of bacterial cell wall synthesis by preferentially binding to specific penicillin-binding proteins (PBPs) that are located inside the bacterial cell wall. Thus, the intrinsic activity of amoxicillin, as well as the other penicillins, against particular organism depends on their ability to gain access to and bind with the necessary PBPs. Amoxicillin's gram-positive spectrum is similar to the natural penicillins, though amoxicillin is slightly less active than penicillin G

against *S. pyogenes, S. pneumonia, S. agalactiae* and slightly more active against *enterococci*. The mechanism of resistance is mediated via the development of altered PBPs and penicillin-resistance strains will generally be resistant to amoxicillin (Morales, *et.al.*, ١٩٩٩).

Beta-lactam agents are the treatment of choice for these infections. But macrolide and related drugs provide useful alternative therapy for allergic patients. *Streptococcus agalactiae* is considered to be susceptible to beta-lactam antimicrobial agents, but the emergence of strains resistance to macrolides and tetracycline has been increasingly reported (Lin, *et.al.*, ٢٠٠٠).

Tetracycline resistance remains a clinically significant determinant to the utility of tetracycline, doxycycline, minocycline, and other commercially available tetracycline. There are two major mechanisms of tetracycline resistance: efflux and ribosome protection. Both mechanisms have been described in gram-positive and gram-negative bacteria either separately or together, with the ribosome protection generally more common in gram-positives and efflux in gram-negatives (Roberts, ١٩٩٦).

Resistance of *Streptococcus agalactiae* to erythromycin and clindamycin has increased during the last decade in several countries with some geographical variations. Hsuch, *et.al.*, (٢٠٠١) have reported an increase in erythromycin and clindamycin resistance from ١٨٪ and ١٩٪ in ١٩٩٤ to ٣٧٪ and ٤٦٪ in ١٩٩٧, respectively. On other hand, Morales, *et.al.*, (١٩٩٩) have found that the rate of resistance has risen from ١.٢٪ to ١٨٪ during ١٩٩٧ and ١٩٩٨; the increase has appeared to be related to an

increase in macrolide usage. Murdoch and Rellar (٢٠٠١) has reported rates of resistance to erythromycin higher than those for clindamycin. In contrast, Ko, *et.al.*, (٢٠٠١) has found resistance to clindamycin to be more common than resistance to erythromycin. The increasing tend in the rates of resistance to erythromycin and clindamycin among *Streptococcus agalactiae* isolated has raised concerns about the use of these antibiotics as alternative agents for the prophylaxis or treatment of *Streptococcus agalactiae* infection Uh, *et.al.*, (٢٠٠١).

Lincomycin has bacteriostatic mode of action against mainly gram positive bacteria like *Streptococcus agalactiae, Staphylococcus aureus, Streptococcus pyogenes and Diplococcus pneumoniae.* Enterococci and gram negative bacteria are hardly sensitive. Culebras, *et.al.*, (٢٠٠٢) have reported that *Streptococcus agalactiae* have had resistance to lincomycin.

In addition to that, Gentamicin is also used for the treatment of Group B *Streptococcus* infection. However, some strains of Group B *Streptococcus* have shown resistant to such antibiotics. It is observed that gentamicin show synergy when used with penicillin for Group B *Streptococcus*. In neonates, the ill patient with sepsis and in certain situations, such as endocarditis, adding an aminoglycoside as a second drug may be helpful. The possible benefit must be weighed against the toxicity of renal and eighth nerve dysfunction, particularly in elderly people (Aronson and Reyenold, ١٩٩٢).

Furthermore, the other antibiotics are used for treatment of Group B *Streptococcus* infection are as follows:-

١- Vancomycin:-

It is a potent antibiotic directed against gram-positive organisms. Useful in the treatment of septicemia and skin structure infections. It is indicated for patients who cannot receive or who have failed to respond to penicillins and cephalosporins or who have infections with resistance *Staphylococci.* Vancomycin is the initial treatment of choice for Group B *Streptococcal* infection in the penicillin-allergic individual (Rang, *et.al.*, ١٩٩٩).

٢- Trovafloxacin:-

Trovafloxacin is a broad-spectrum antibiotic used in gynecologic or pelvic infections, including endometritis, parametritis, septic abortion and postpartum infections caused by *Streptococcus agalactiae* (Provenzano, ١٩٩٩).

٣- Cefotaxime:-

It is a synthetic, broad-spectrum cephalosporin antibiotic for parenteral administration. The bactericidal activity of cefotaxime sodium results from inhibition of cell wall synthesis. Cefotaxime sodium has in vitro activity against a wide range of gram positive such as *Streptococcus agalactiae* and *Staphylococcus aureus* and gram negative such as *Nisseriae gonorrhoeae* and *Nisseriae meningitides.* This antibiotic is used to treat pelvic inflammatory disease, endometritis and pelvic cellulitis (Mary, *et.al.*, ٢٠٠٠).

The most active antibiotic in treating Group B *Streptococcus* are penicillin G, third generation cephalosporins, and meropenem (Fernandez, *et.al.*, ١٩٩٨). However; ceftriazone, cefamandole, cefotaxime, and meropenem are as effective as penicillin G and more effective than cephalothin, refampicin, and vancomycin. The use of second and third generation cephalosporins and meropenem is recommended in case of penicillin G allergy (Fernandez, *et.al.*, ١٩٩٨). Macrolides should be avoided in Group B *Streptococcus* because of their widespread use for gynecological infection (Pearlman, *et.al.*, ١٩٩٨).

Materials and Methods

٢.١ Materials

٢.١.١ Laboratory Instruments:-

Table (١) show Laboratory Instruments used:-

No.	Instruments	Company
١-	Sensitive electronic balance	A&D, Japan.
٢-	Autoclave	Stermite, Japan.
٣-	Incubator	Memmert, Germany.
٤-	Distillator	GFL- Germany.
٥-	Centrifuge	Hermle, Japan.
٦-	Oven	Memmert, Germany.
٧-	Refrigerator	Concord, Italy.
٨-	Millipore filter	Satorius membrane filter Gm bH, W. Germany.
٩-	Light microscope	Olympus, Japan.

١٠-	Micropipette	Oxford, USA.
١١-	pH meter	Hoeleze & Cheluis, KG, Germany.
١٢-	Spectrophotometer	Bausch & Lomb

٢.١.٢ Chemical and Biological material:-

Table (٢) show Chemical & Biological material used

Name of material	Company
A-Chemical material:- ١-Na_2HPO_4, KH_2PO_4, NaCl, $MgSO_4$, $CaCl_2$, KOH, K_2HPO_4.	Merk-Darmstadt.
٢-alpha-nephthol, esculin, Tannic acid, Trichloroacetic acid, Tetramethyl-P-paraphylene diamine dihydrochloride, Casein peptone, Meat peptone, Starch, Chloroform.	B.D.H
٣-H_2O_2, D-mannose, Glucose, dipyridyl, ٩٩٪alcohol.	Fluka chemika-Switzerland
B-Culture media:- ١- Blood agar base, Brain heart infusion agar, agar-agar, Muller-Hinton infusion agar, MacConky's agar media.	Mast.
٢-Nutrient agar media, Nutrient broth.	Oxiod
٣-Tryptic soy agar, MR-VP broth.	Diffco-Michigan.

٢.٢ Patients and Methods

٢.٢.١ Patients

One hundred and twenty samples are collected from woman patients suffering from severe to moderate vaginitis. The period is from October ٢٠٠٣ to March ٢٠٠٤.

٢.٢.٢ Collection of specimens

The specimens are generally collected from patients with vaginitis. The swabs are inserted into the upper part of the vagina and rotated there before withdrawing it, so that exudate is collected from the upper as well as the lower vaginal wall. An endocervical swab must be collected. A vaginal speculum must be used to provide a clear sight of the cervix and the swab is rubbed in and around the introitus of the cervix and withdrawn without contamination from the vaginal wall.

Swab for culture should be placed in tubes containing normal saline to maintain the swab moist until taken to laboratory. The swab has been inoculated on culture media and incubated aerobically for ٢٤h. at ٣٧°C.

٢.٢.٣ Reagents

١- Catalase reagent:-

It is prepared by adding ٣ gm of $H_٢O_٢$ to ١٠٠ ml distill water and stored in dark container (Baron, *et.al.*, ١٩٩٥).

٢- Oxidase reagent:-

This is soon prepared by dissolving ٠.١ gm of Tetra-P-paraphenylene diamine dihydrochloride in ١٠ ml of distill water and stored in dark container (Baron, *et.al.*, ١٩٩٥).

٣- Voges-Proskauer reagent:-

A- ٥ gm of alpha-nephthol is dissolved in ١٠٠ ml of ٩٩٪ alcohol, and stored in refrigerater in a brown glass bottle away from light.

B- ٤٠ gm of KOH is dissolved in ١٠٠ ml of distill water (Collee, *et.al.*,١٩٩٦).

٢.٢.٤ The preparation of media

١- M٩ media:-

٦ gm of Na_2HPO_4, ٣ gm of KH_2PO_4, ٠.٥ gm of NaCl, and ١ gm of NH_4Cl; are all dissolved in ٩٥٠ ml of D.W. with ٢٪ agar, and then sterilized into autoclave. After cooling the mixture to ٥٠ ^oC, ٢ml of ١ M of $MgSO_4$, ١٠ ml of ٢٠٪ glucose and ٠.١ ml of ١ M of $CaCl_2$ (sterilized them separately by filtration) are added, then the volume is completed to ١٠٠٠ ml (Miniatis, *et.al.*, ١٩٨٢).

٢- Easculin media:-

Nutrient agar is prepared; then ١.٤ gm ferric citrate and ٤ gm easculin are added. the volume is then completed to ١٠٠٠ ml. After that pouring the media into tubes and sterilized them into autoclave, then slant of media is formed (Baron, *et.al.*, ١٩٩٥).

٣- Columbia agar:-

The media consist of:-

Casein peptone ١٢.٠ gm,

Meat peptone ١١.٠ gm,

Starch ١.٥ gm,

Sodium chloride ٥.٠ gm, and

Agar ١٥.٠ gm

The ingredients are added to ٩٥٠ml of distilled water and after boiling, the pH is adjust to ٧.٣. The media is then sterilized at ١٢١°C for ١٥ minutes. To obtain Blood Agar, it is cooled to ٤٥-٥٠°C and ٥٪ defibrinated blood is added (Sancho, *et.al.*, ٢٠٠٠).

٢.٢.٥ The Preparation of Solution

*** Phosphate buffer solution:**

٨٠ gm of NaCl, ٠.٣٤ gm of $KH_٢ PO_٤$, and ١.١٢ gm of $K_٢ HPO_٤$ are all dissolved in ١٠٠٠ ml of D.W. The pH is ٧.٣, then the solution is sterilized in autoclave(Baron, *et.al.*, ١٩٩٥).

٢.٢.٦ Isolation and Identification of *Streptococcus agalactiae*

A colony that is gray has been selected with hemolysis blood agar(growing on blood agar) and showing beta hemolysis on blood agar. It has been identified depending on its morphology (shape, size, color) and then examined under microscope after staining it with gram stain(Its appears in pair or in chain and gram positive).

After staining the bacteria, its specific shape, color, aggregation and specific interacellular compound have observed. Biochemical tests have been done to reach final identification according to Bergy's Manual For Determinative Bacteriology(Holt, *et.al.*, ١٩٩٤).

٢.٢.٧ Biochemical Tests

١- Catalase Test:-

A small amount of bacterial growth which grow on medium in age ٢٤hr. is transferred by sterile wooden stick onto the surface of a clean, dry glass slide, and one drop of (٣٪H_2O_2) is added to it. The formation of gas bubbles indicates the positive results(Collee, *et.al.*, ١٩٩٦).

٢- Oxidase Test:-

A small portion of the colony to be tested is transferred by a sterile wooden stick to filter paper saturated with indicator prepared soon. If the color around the smear turns rose to purple, the oxidase test is positive(Collee, *et.al.*, ١٩٩٦).

٣- Voges-Proskaure Test:-

The test is performed by using MR-VP broth. The inoculated media is incubated for ٢٤hr. at ٣٧°C. Afterwards, ١٥ drops of ٥٪ alpha-nephthol are added and followed by ١٠ drops ٤٠٪ KOH. The mixture is shaken well and allowed to stand up to ١٥ minutes before calling a reaction negative. If positive, the culture turns red at the surface of the liquid, and the color spreads gradually throughout the

tube. The positive result indicates a partial analysis of glucose which produce(Acetyl-carbonyl) (Collee, *et.al.*, ١٩٩٦).

٤- Easculin test:-

The organism is grown in esculin slant for ٢٤hr. at ٣٧°C. The dark brown color indicates a positive result. The unchanging of the color is a negative result (Baron, *et.al.*, ١٩٩٥).

٥- Growth in ٦.٥٪ NaCl:-

Two or three colonies are inoculated into a tube of nutrient broth containing various concentration of NaCl and the tube is then incubated at ٣٥°C for ٢٤ hour. The growth is judged by the turbidity seen after dispersing any sediment indicated to positive growth, otherwise the growth is negative (Collee, *et.al.*, ١٩٩٦).

٦- CAMP Test:-

Inoculate a β-lysin producing of *Staphylococcus aureus* as a streak across a blood agar plat containing ٥٪ sheep blood. Then inoculate a single streak of the *Streptococcus* perpendicular to that of *Staphylococcus*, leave ١ cm of space between the two streaks, and then incubated plate at ٣٧°C for ٢٤h. The positive result is appear an arrowhead-shaped zone of enhanced hemolysis at the juncture between positive *Streptococcus* and the *Staphylococcus* (Collee, *et.al.*, ١٩٩٦).

٧- Coagulase Test:-

Several colonies of bacteria are transferred with a loop to a tube containing ٠.٥ ml of plasma. The tube is covered to prevent evaporation and incubated at ٣٧°C overnight. The test is read by

tilting the tube and observing for clot formation in the plasma. A negative test results in the plasma remaining free-flowing with no evidence of a clot (MacFaddin, ٢٠٠٠).

٢.٢.٨ Virulence Factors Test

٢.٢.٨.١ Capsule stain:-

a- A smear slide is prepared from bacterial suspension on glass slide without fixing and is left to dry.

b- Flood gently with ١٪ the crystal violet and leave for about ٤ minutes.

c- The smear is washed with ٢٠٪ copper sulfate, allowed to dry in air, and examined under the microscope.

The organism should be deep purple, and the capsule a faint blue against a light purple background (Cruickshank, *et.al.*, ١٩٧٥).

٢.٢.٨.٢ Haemolysin production:-

Haemolysis production is shown on blood agar media. The results are obtained after the incubation of the non-cultured plates for ٢٤hr. at ٣٧°C to exclude any contamination of blood, then the organism is inoculated at this blood agar plates and incubated again for ٢٤hr at. ٣٧°C. Any hemolysis presence showed be detected around the colonies (either α or β hemolysis) (De Boy *et.al.*, ١٩٨٠).

٢.٢.٨.٣ Siderophores production:-

M٩ media is prepared and then supplemented with ٢٪ agar. After sterilization in autoclave and cooling at ٥٠°C, ٠.٢٥ gm/L

glucose(sterilized by filtration) and ٢٠٠µm of dipyridyl are added to it. Then the organisms are inoculated into this media and incubated for ٢٤hr. at ٣٧°C. The results are seen if the growth of organism is present or not (Nassif, *et.al.*, ١٩٨٩).

٢.٢.٨.٤ Production of excetracellular protease:-

This method is carried out by using M٩ agar supplemented with ٢٪ agar. After sterilization in autoclave and cooling at ٥٠°C, ٠.٢٥ gm/L glucose(sterilized by filtration) is added, and then the media is supported by ١٪ Gelatin. After the inoculation of this media with bacterial strain and incubation for ٢٤-٤٨hr. at ٣٧°C, ٣ml of Trichloroacetic acid(٥٪) is added to precipitate the protein. The positive result is read by observing a transparent area around the colony (Piret, *et.al.*, ١٩٨٣).

٢.٢.٨.٥ Colonization Factor Antigen(CFA):-

A- Detection of (CFA/I):-

After culturing the organism on Tryptic soy agar and incubating it for ٢٤hr. at ٣٧°C, the agglutination of RBC with bacteria occurs in presence of D-mannose as follows:-

١-RBC suspension is prepared from the human blood(group A) and washed with phosphate buffer saline (repeated ٣ times). ٣٪ suspension from RBC(v/v) is then prepared.

٢- A bacterial suspension is prepared by taking half of the bacterial growth for each strain from TSA and mixing it with ١ml of ٠.١٥M

NaCl, to determine RBC agglutination test and vasticated colonization factor antigen type١.

٣- On a clean slide, one drop of bacterial suspension is mixed with one drop of ٠.١M D-mannose on one side, and with one drop of ٣٪ suspension on the other side (without D-mannose).

The agglutination of RBC with bacteria is detected after ١/٢-٢ min in room temperature(Smyth, ١٩٨٢).

B- Detection of (CFA/II):-

To determine second colonization factor antigen, the same step is followed with CFA/I by using chicken blood instead of human blood (group A). This factor causes agglutination of chicken blood (Al-zaag, ١٩٩٤).

C- Detection of (CFA/III):-

To determine third colonization factor antigen the same step CFA/I is followed except for the replacement of Tannic acid instead of D-mannose.

٢.٢.٩ Bacteriocin Production

The method of Abbot and Shannon(١٩٥٨), developed by Abbot and Graham(١٩٦١) have been used:-

١- A medial streak of the test strain by vertical line is done on TSA and then incubated at ٣٧°C for ٤٨hr. to allow bacteriocin to spread around the growth line.

٢- On the second day, sensitive or indicator strain is inoculated on nutrient agar and incubated at ٣٧°C to the next day.

٣- On the third day, the petri-plate cover of the streaked plate is covered by filter paper impregnated with chloroform in an upright position, then plate culture is inverted on its cover for ١/٢hr. The culture is scarped by sterilized glass slide into disinfecting vessel, and the plate culture is expose to chloroform vapors and then left the plate open for ١hr. to remove the chloroform.

٤- Inoculated sensitive or indicator strain (which has grown on nutrient agar) is streaked crossing the original scraped streak line on TSA plat culture and incubated for ٣٧°C overnight . The becteriocin production is scored as growth inhibition at the medial streak line.

٢.٢.١٠ Antibiotic Sensivity Test

Antibiotic Diffusion tests(The Kirby-Bauer susceptibility test).

١- It is performed by using a pure culture of previously identified bacterial organism. The inoculum to be used in this test is prepared by adding growth from ٥ isolated colonies grown on blood agar plate to ٥ml of broth. This culture is then incubated for ٢hr. to produce bacterial suspension of moderate turbidity. A sterile swab is used to obtain an inoculum from the standardized culture. This inoculum is then streaked on a Mueller-Hinton plate.

٢- The antibiotic discs are placed on the surface of the medium at evenly spaced intervals with flamed forceps or a disc applicator.

٣- Incubation is usually overnight with an optimal time being ١٤hr. at ٣٧°C. Antibiotic inhibition zones are measured using a caliper.

Zone size is compared to standard zones to determine the susceptibility or resistance of the organism to each antibiotic (MacFaddin, ٢٠٠٠).

Antibiotic disc supplied from Oxiod Company can be disc potency by µg/ml concentration as followed:-

Gen*	Lin*	Chl*	Tet*	Cln*	Ery*	Amx*	P*	Bac*
١٠	١٥	٣٠	٣٠	٥	١٥	١٠	١٠	١٠

*Gen: Gentamicin; *Lin: Lincomicin; *Chl: Chloramphenicol; *Tet: Tetracycline; *Cln: Clinadmicin; *Ery: Erythromycin; *Amx: Amoxicillin; *P: Penicillin; *Bac: Bacitracin.

٢.٢.١١ Effect of Lactic acid on Bacterial Growth

١- Nutrient broth is prepared and distributed in tubes and lactic acid is added to each tube at various volumes to gain the final concentrations (١٠, ٢٠,٤٠, ٦٠,٨٠,١٠٠µg/ml).

٢- Positive control is prepared by using nutrient broth free from lactic acid.

٣- The tubes in item ١ and ٢ are inoculated with ٠.٥ ml of bacterial suspension and then incubation ٢٤hr. at ٣٧°C.

٤- After incubation, the absorbance is read at wave length ٥٢٠nm by using spectrophotometer to show the effect of lactic acid on the growth of bacteria strain.

Results and Discussion

٣.١ Isolation and Characterization

In this study, ١٢٠ vaginal swabs obtained from women (pregnant and non pregnant) who suffering from vaginitis and admitted to Babylon Hospital for Maternal and Delivery for the period from October ٢٠٠٣ to March ٢٠٠٤ in Hilla Province were included.

All swabs were subjected for culturing on available media and it was found out of the a total of ١٢٠ samples, only ٩٦ samples showed positive cultures, ٦٦ bacterial isolates and ٣٠ yeast isolates. No growth was seen in the other samples (٢٤ samples) which could indicate the presence of microorganisms that might be cultured with diffeclty such as viruses, chlamydia, and other agents (Table ٣).

Table ٣:- Frequency of Positive Culture and Negative Culture

WOMEN	POSITIVE CULTURE		NEGATIVE CULTURE
	BACTERIAL	Yeasts	
Pregnant	١٦	٦	٤
Non-pregnant	٥٠	٢٤	٢٠
Total	٦٦	٣٠	٢٤

Yeasts live normally in the vagina in small numbers. A yeast infection occurs when there is an overabundance of yeast, often caused by a change in the pH balance of the vagina (Monif and Carson, ١٩٩٨). Since yeast is normally present and well-balanced in the vagina, infection occurs when something in women's system upsets this normal balance. For example, an antibiotic to treat another infection may upset this balance. In this case, the antibiotic kills the bacteria that normally maintain the balance of the yeast in the vagina. In turn, the yeast overgrows, causing an infection. Other factors that can cause imbalance to occur include pregnancy, which change hormone levels, and diabetes, which allows too much sugar in the urine and vagina (Sobel and Chaim, ١٩٩٦).

Local studies have confirmed the presence of yeast in cases of vagintis (Habbeb, ٢٠٠٣; Ghaly, ٢٠٠١).

Monif and Carson(١٩٩٨) have isolated candida from women in the cases of vaginitis at a rate ٢٠٪ and demonstrated that the yeast have the ability to inhibit growth of selected bacteria.

Also, Perera (١٩٩٤) and Di-Bartolomea, *et.al.*,(٢٠٠١) have isolated the yeast from women in the cases of vaginitis at a rate ٣٦٪ and ٣٤.٣٪ respectively.

In addition to the yeasts, bacterial isolates are found in unhealthy vagina and it reveals that these bacteria are more predominant than yeast, some of them are pathogenic and the others might be normally isolated from intact vagina. Furthermore, the prevalence of bacterial isolates are observed to be higher among non-

pregnant women than in pregnant women (٥٠ and ١٦ isolates respectively).

The results of bacterial isolation (Table ٤) show that only three isolates of *Streptococcus agalactiae* have been isolated from non pregnant women suffering from vaginitis.

Table ٤:- Isolation of Bacteria from Non-pregnant Women with Vaginitis

ISOLATES	NON-PREGNANT	%	CLINICAL SIGNS
Staphylococcus epidermidis	١٧	٣٤٪	Most of infected woman has vaginal discharge and itching
Pseudomonas aeruginosa	١٥	٣٠٪	
Lactobacillus	٦	١٢٪	
Klebsiella pnumoniae	٤	٨٪	
Streptococcus agalactiae	٣	٦٪	
Moraxella catarrahlias	٣	٦٪	
Acinetobacter	٢	٤٪	
Total	**٥٠**	**١٠٠٪**	

In the Table(٤), the most common types of bacterial isolates from non-pregnant women is *Staphylococcus epidermidis* (١٧) followed by *Pseudomonas aeruginosa*(١٥), *Lactobacillus*(٦), *Klebsiella pnumoniae*(٤), *Streptococus agalactiae*(٣), *Moraxella catarrahlis*(٣), and *Acinetobacter*(٢). Whereas in pregnant women the

most common types of the bacteria isolated in this study is shown in Table(٥).

Table ٥:- Isolation of Bacteria from Pregnant Women with Vaginitis

ISOLATES	PREGNANT %		CLINICAL SIGNS
Staphylococcus epidermidis	٧	٤٣.٧٥٪	Vaginal discharge, itching
Lactobacillus	٥	٣١.٢٥٪	
Klebsiella pnumoniae	٢	١٢.٥٪	
Pseudomonas aeruginosa	٢	١٢.٥٪	
Total	١٦	١٠٠٪	

In Table (٥) the most common types of bacterial isolates from pregnant women is *Staphylococcus epidermidis*(٧) followed by *Lactobacillus*(٥), *Klebsiella pnumoniae*(٢), and *Psedomonas aeurginosa*(٢).

This result is correlated with the results obtained by Perera, (١٩٩٤) , Provenzano, (١٩٩٩) and Donder, (٢٠٠٢), who have pointed that the bacterial isolated in non-pregnant women are the most common types in vagina. Whereas the results of bacteria isolates among pregnant women are similar to those obtained by Curzik, *et.al.*, (٢٠٠١) and Rodringuez, *et.al.*, (٢٠٠١).

Seventeen isolates of *Staphylococcus epidermidis* is isolated in this study in non-pregnant and (٧) in pregnant women. This bacteria is considered normal flora of vagina (Baron, *et.al.*, ١٩٩٤).

A previous study performed in Najaf (Ghaly, ٢٠٠١) has shown that this bacteria is isolated from woman patients with vaginal discharge. Metha (١٩٨٢) has also isolated this bacteria from vagina at a rate ٦٠٪.

On the other hand, (١٥) *Pseudomonas aeruginosa* have also been isolated in non-pregnant and (٢) in pregnant women. All these women are suffering from offensive odor and from vaginal discharge. Besides, the non-pregnant women have an intrauterine device, this bacteria spread among those women. It is known that *Pseudomonas aeruginosa* can cause severe disease particularly in urinary tract (Bonadio, *et.al.*, ٢٠٠١; Takeyama, *et.al.*, ٢٠٠٢), it potentially opportunistic microorganism within the vagina (von Wintzingerode, *et.al.*, ١٩٩٩). Such microorganisms may become increasingly prevalent upon minor alterations of the vaginal microenvironment. Metcalf, (٢٠٠١) has isolated this bacteria from cases of vaginitis and shown that this bacteria is prevalent among non-pregnant women using intrauterine device.

Six isolates of *Lactobacillus* have been isolated in this study from case of vaginitis in non-pregnant, and (٥) in pregnant women. This bacteria is the major member of the normal flora of the vagina. The lactic acid production of their metabolism helps maintain the low pH of the normal adult female genital tract. They rarely cause disease (Redondo-López, *et.al.*, ١٩٩٠).

In local studies carried out in Najaf and Baghdad, it has been found that this bacteria is really present in cases of vaginitis at rate ٧٥٪ and ٤٩٪ respectively (Ghaly, ٢٠٠١; Habbeb, ٢٠٠٣).

Sobel (١٩٩٦) has also pointed that the major of bacterial strains isolated from normal vagina belong to *Lactobacilli* spp. This bacteria is normally present in healthy vagina and their presence is very essential for maintaining vaginal acid pH through its production, and hence the growth of unwilling and pathogenic bacteria will be prevented.

Four isolates of *Klebsiella pneumonia* have also been isolated in this study in non-pregnant and (٢) in pregnant women. This bacteria is rarely present in healty vagina. However, our study has confirmed the presence of such bacteria in cases of vaginitis in the absence of *Lactobacillus*. Rodriguez, *et.al.*, (٢٠٠١) have isolated this bacteria from cases of vaginitis and they have pointed that this bacteria may be the causative agent of vaginal due to their ability to produce various types of virulence factors.

Three isolates of *Moraxella catarrahlis* have also been isolated in this study in non-pregnant women. This bacteria is considered normal flora genitourinary mucosal surface of humans (Schrechenberger and von Graevnitz, ١٩٩٩). *Moraxella* is like *Nisseriae* and can be present in the oral cavity and upper respiratory tract.

Also, two isolates of *Acinetobacter* are investigated in this study in non-pregnant women. *Acinetobacter* is diplococci, catalase positive

oxidas negative and it recoveres from the female genital tract (Geo, et.al., ٢٠٠١).

Al-Shukri (٢٠٠٣) has pointed that *Acinetobacter* can be isolated from unhealthy vagina at a low rate. This study has been carried out in Hilla province, too.

However, Johnson (١٩٩٩) has isolated this bacteria by a rate ٣.١٪ from ٦٤ cases of vaginitis.

Also, threes isolate of *Streptococcus agalactiae* have been isolated from cases of vaginitis.

This study is concerned with *Streptococcus agalactiae* because there is no sufficient studies carried out on this bacteria in Iraq, although it has been isolated in Najaf and Baghdad (Ghaly, ٢٠٠١; Habbeb, ٢٠٠٣).

This bacteria has been isolated in previous studies and most of these studies have stated that this bacteria is mostly prevalent among pregnant women and less frequently in non pregnant women (Baker, ١٩٩٧).

Three isolates *Streptococcus agalactiae* have been isolated in this study from non-pregnant women.

This result is identical with the results obtained by Farley, *et.al.*, (١٩٩٣) and Schwart, *et.al.*,(١٩٩١) who have indicated that the prevalence of *Streptococcus agalactiae* among non pregnant women is higher than in pregnant women.

Zhu, *et.al.*,(١٩٩٦) have reported the isolation of this bacteria from non-pregnant women by a rate ١٠.٨٦٪ from a total of ٢٦٧ cases.

Whereas other studies have pointed that the prevalence of this bacteria among pregnant women is higher than it is in non pregnant women.

In the United States of America, Zangwill, *et.al.*,(١٩٩٢) were isolated this bacteria from pregnant women by a rate ٦٧(١١٪) from ٦٦٦ cases. Besides, Dillon, *et.al.*,(١٩٨٢) have isolated it from pregnant women by a rate ١٣٪ from ٢٢٢ cases and Lehorani, *et.al.*,(١٩٩٨) have isolated this bacteria from pregnant women by a rate ٢.٨٪ from ٢٠٠ cases.

On contrast Maniatis, *et.al.*,(١٩٩٦) have also isolated *Streptococcus agalactiae* from ٢٢٦ cases of vaginitis at a rate of ١٠٪.

However, Edward and Baker,(٢٠٠٠) have proved that the rate of isolation of *Streptococcus agalactiae* from vaginal swabs ranges from ٥-٤٠٪ due to difference in the sample sites and culture method employed.

The presence of microorganism in the female lower genital tract (LGT) in the concentrations that modify the established normal equilibrium produces different symptoms that make necessary to consult the gynecologist. It is currently accepted that infections of the LGT are due to microorganisms that are normally integrating the internal flora, except for the erroneously called sexually transmitted disease that are caused by external microorganisms. This means that all those microorganisms usually present without causing any disease (Provenzano, ١٩٩٩).

٣.١.١ The Characteristics of *Streptococcus agalactiae*

The most characteristics of *Streptococcus agalactiae* is Gram-positive coccus which appears in chain or pair, catalase negative, facultivaly anaerobic bacteria, contain capsule and resistance to bacitracin.

Streptococcus agalactiae is non-motile they are unable to synthesize heme compounds. *Streptococcus agalactiae* nutritionally fastidious with variable with nutritional requirements, and growth on complex media is enhanced by the addition of blood or serum. Glucose and other carbohydrates are metabolized fermentatively with production of lactic acid as a major metabolic end product. Gas is not produced as a result of glucose metabolism(Hardie, ١٩٨٦).

On blood agar, GBS produce translucent to opaque, whitish gray, soft, smooth, small colonies that are moist. Most, but not all strains produce a relatively narrow zone of beta-hemolysis. The best medium for isolation and identification of *Streptococcus agalactiae* is Columbia agar. The orange colonies may be considered a primary diagnostic feature for the isolation of this bacteria (Mosabi, *et.al.*, ١٩٩٧) and by this medium, it can differentiate this bacteria from other *Streptococci* which cannot give the same color on such medium.

Furthermore, Bacitracin (١٠ unit) is also used to differentiate this bacteria from group A *Streptococci* in that the latter is highly sensitive to this agent but the former GBS is resistant (Collee, *et.al.*, ١٩٩٦).

Other features of bacteria are summarized in Table ٦:

Table ٦:- Diagnostic features of *Streptococcus agalactiae*

TESTS	RESULS
Hemolysin	Beta (narrow zone)
Bacitracin	Resistance
Growth on blood agar	Gray color with narrow zone of hemolysis
Growth on Columbia	Orange color
Gram stain	Positive
Shape of cell	Coccus(chain or pair)
Catalase	Negative
Oxidase	Negative
Capsule	Positive
CAMP	Positive
Easculin	Negative
VP	Negative

This table is performed according to MacFaddin, ٢٠٠٠.

٣.١.٢ Effect of NaCl on the growth of *Streptococcus agalactiae*

Various concentrations of NaCl are used to show the ability of *Streptococcus agalactiae* to grow under osmotic pressure. It has been found that all *Streptococcus agalactiae* isolates can grow until ٦.٥٪ except one isolate which has failed to grow in ٦٪ or above. Furthermore, all the isolates have failed to grow in ٧٪ of NaCl or above (Table ٧).

Table ٧:- Effect of Different Concentrations of NaCl on the Growth of *Streptococcus agalactiae*

Isolation	Concentration of NaCl					
	٤.٥٪	٥٪	٥.٥٪	٦٪	٦.٥٪	٧٪
١	+	+	+	+	+	-
٢	+	+	+	+	+	-
٣	+	+	+	-	-	-

(+) growth (-) no growth

Numerous reference manuals indicate that *Streptococcus agalactiae* is either unable to grow in the media containing ٦.٥٪ NaCl (Quinn, et.al., ١٩٩٤) or has a variable capacity to do that (Holt, et.al., ١٩٩٤). Salasia, *et.al.*,(١٩٩٤) have pointed that *Streptococcus agalactiae* isolates are able to grow in ٦.٥٪ NaCl. However, this test is not performed routinely by the laboratories in the standard procedure for the identification of beta hemolytic *streptococci.* Therefore, to establish the proportion of *Streptococcus agalactiae* isolates that were

able to grow in ٦.٥٪ NaCl, all isolates selected, regardless of the type of hemolysis produced were submitted to this study (MacFaddin, ١٩٨٥).

Our results suggest that a considerable proportion of *Streptococcus agalactiae* strains have the ability to grow in ٦.٥٪ NaCl. Further research should be conducted to determine if other *streptococci* isolated from vagina can grow at frequently in NaCl.

٣.٢ Investigation of Virulence Factor of GBS

Many GBS virulence factors represent integral components of the bacterial surface. The current review focuses on virulence factors of GBS; i.e. those factors that can exert a virulence function even when physically separated from the bacterial cell. Some GBS virulence factors are secreted directly into medium; others may be associated with the bacterial surface but can be removed in an active from by proteolysis or binding to host cell components (Nizet and Rubens, ٢٠٠٠).

٣.٢.١ Capsule:-

Capsule detection by using negative stain technique is carried out for GBS isolates and it is found that all GBS had a capsule surround the bacterial cell.

These results are identical with those obtained by Wessels *et.al.*, (١٩٨٩) who have stated that strains of *Streptococcus agalactiae* consist of capsule and had mucoid polysaccharide which could protect the bacteria from phagocytosis. The function of capsule contributes to

virulence by inhibiting activation of the alternative complement pathway (Edwards, *et.al.*, ١٩٨٢).

Streptococcus agalactiae is serotyped on the basis of the capsular polysaccharide, for which nine serotypes have been described so far (Kogan, *et.al.*, ١٩٩٦).

All GBS capsule types contain sialic acid displayed in a terminal linkage similar to the way sialic acid is displayed on the surface of human cells. The sialic acid inhibit complement deposition and activation, and therefore is antiphagocytic. It resembles to host tissues and also appears to diminish immune recognition, perhaps a form of molecular mimicry that enhances pathogenicity.

Most Group B *Streptococcus* that causes human infection in United State are encapsulated by one of five antigenically distinct polysaccharides (serotype Ia, Ib, II, III, or V) (Jennings, *et.al.*, ١٩٨٣; Jennings, *et.al.*, ١٩٨٣; Wessels, *et.al.*, ١٩٩١; Wessels, *et.al.*, ١٩٨٧). The capsular polysaccharide of Group B *Streptococcus* is an important virulence factor (Baker, *et.al.*, ١٩٩٠) and is the target of protective antibodies. The capsular polysaccharide has antiphagocytic properties (Edwards, *et.al.*, ١٩٨٢; Edwards, et.al., ١٩٨٠; Marques, *et.al.*, ١٩٩٢), and the degree of encapsulation correlates directly with the virulence of the organism (Rubens, *et.al.*, ١٩٨٧).

The role of capsules in microbial virulence is to protect the organism from complement activation and phagocyte-mediated destruction. Although the host will normally make antibodies directed against the bacterial capsule, some bacteria are able to subvert this

response by having capsules that resemble host polysaccharide (Brogden, *et.al.*, ٢٠٠٠).

٣.٢.٢ Detection of Hemolysin, Siderophores and Extracellular Protease Production:-

Microorganisms evolve a number of mechanisms for the acquisition of iron from their environments(Litwin and Calderwood, ١٩٩٣). One of them is the production of hemolysins, which act to release iron complexed to intracellular heme and hemoglobin. Another mechanism for iron acquisition is to produce siderophores which chelate iron with a very high affinity and which compete effectively with transferrin and lactoferrin to mobilize iron for microbial use (Neilands, ١٩٩٥).

Iron can increase disease risk by functioning as a readily available essential nutrient for invading microbial and neoplastic cell. To survive and replicate in hosts, microbial pathogens must acquire host iron. Highly virulent strains possess exceptionally powerful mechanisms for obtaining host iron from health hosts (Weinberg, ١٩٩٨).

To investegate the ability of *Str. agalactiae* to produce hemolysin on human blood agar. The results are show that all isolates are able to produce hemolysin extracellularly and the type of hemolysin is beta (Table ٨). Hemolysin has also produced on Columbia agar, and the latter is used for the identification of this bacteria from other species of *Streptococci*.

Table ٨: Detection of Production of Hemolysin, Siderophore and Extracellular Protease by *Str. agalactiae* Isolates

Virulence factor	Isolation no. ١	Isolation no. ٢	Isolation no. ٣
Hemolysin	+	+	+
Siderophore	-	-	-
Extracellular protease	-	-	-

These results are identical with those obtained by Marchlewicz and Duncan (١٩٨١).

The type of haemolysis in the culture media is beta-haemolysis which is clear (lysis of red blood cells) in the medium. The zone of hemolysis is usually narrow. The beta-hemolysin of *Str. agalactiae* is a broad-spectrum cytotoxin that has long been postulated to play a role in the tissue injury and systemic spread associated with severe human infection(Nizet, ٢٠٠٢).

The function of hemolysin is to provide the microorganism with iron and it will make the bacteria unable to reproduce any factor for obtaining the iron from environment (Valvano, *et.al.*, ١٩٨٦).

Haemolysis has not a direct role in vagina although there is indication about its role in lung and CNS.

The beta-hemolysin lyses a variety of human cells including lung epithelial cells, macrophages, and blood-brain endothelial cells. Therefore it can contribute to bacterial spread into blood stream and CNS. The beta-hemolysin also provokes inflammatory responses and

cytokine release that are present in GBS septicemia. phospholipid in surfactant blocks the GBS beta-hemolysin.

Production of beta-hemolysin by Group B *Streptococcus* has been correlated with lung epithelial cell injury in vitro, suggesting a possible pathogenic role of this enzyme in the invasive step of early-onset Group B *Streptococcus* disease (Nizet, *et.al.*, ١٩٩٦).

Streptococcus agalactiae is also tested for ability to siderophore synthesis grown on M٩ media containing dipyridyl. The results are recorded according to the ability of the isolates to grow or not.

The results show that all isolates of *Streptococcus agalactiae* cannot produce siderophore (Table ٨), because non of the isolates has the ability to grow on M٩ media which containe dipyridyl.

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.*, ١٩٩٦).

Furthermore, it is known that the bacteria which is able to produce haemolysin have no ability to produce siderophore, so, the bacteria need only one mechanism for obtaining iron (Al-Saeed, ١٩٩٧).

In the ability of *Str. agalactiae* to produce extracellular protease by using M٩ media (supported by ٠.٢٥gm/L glucose and ١٪ gelatin) was investigated and it was found that all isolates are not able to

produce extracellular protease after ٢٤h. of incubation and there is no transparent area around the colony after the addition of ٣ml (٥٪) of trichloro acetic acid (TCA) (Table ٨).

Str. agalactiae cannot produce extracellular protease. GBS is known to produce a surface-bound protease that specifically inactivates the human phagocyte chemotaxin C٥a (Cheng, *et.al.*, ٢٠٠٢).

Intracellular Protease is expressed at higher concentration in GBS than extracellular protease among the isolates obtained from neonates with bloodstream infection (Milligan , *et.al.*, ١٩٧٨).

Analyses of the GBS genome have revealed two additional loci that potentially encode cell wall-bound serine proteases with ٥٥٪ and٤٩٪ similarity to C٥a peptidase. Degrade C٥a peptidase is an important host chemokine and also acts as an adhesin.

GBS protease functions in the evasion of opsonophagocytosis (Harris, *et.al.*, ٢٠٠٣). Several lines of evidence indicate that the protease is important in the pathogenesis of GBS.

٣.٢.٣ Detection of Colonization Factor Antigen(CFA):-

The isolates are tested for their ability to produce colonization factor antigens type ١, ٢, or ٣. The results show that the strains have revealed the ability to produce CFA/٣ in the presence of tannic acid but not CFA/١, CFA/٢ in the presence of D-mannose (table ٩).

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

Table ٩:- The ability of Bacteria to Produce CFA

Virulence factor	Isolation no.١	Isolation no.٢	Isolation no.٣
CFA I	-	-	-
CFA II	-	-	-
CFA III	+	+	+

Bacteria adhere to tissue by having pili or adhesion. Pili or fimbriae are rod-shaped structures that consist primarily of an orderd array of a single protein subunit called pilin. An important function of pilus replacement, at least for some bacteria, is that it provides a way for the bacterium to evade the host's immune response(Salyers and Whitt, ١٩٩٤).

Soledad, *et.al.*, (١٩٩٨) show that *Streptococcus agalactiae* do not have the ability to adhere the vaginal epithelial cell. This bacteria are just opportunistic pathogens, it may be deduced that adherence is an important virulence factor.

However, the lack of *Streptococcus agalactiae* to produce CFA/I and CFA/II may indicate why this bacteria is difficult to be obtained from vagina and also sub MIC concentration of some antibiotic will assist in losing the bacteria their adhesive characteristics in the vagina.

٣.٣ Effect of Some antibiotic on GBS

Some antibiotics are used to show their effect on GBS isolates such as clindamycin, gentamycin, tetracycline, erythromycin, lincomycin, penicillin, amoxillin and chloramphenicol.

It has been found that GBS isolates ae sensitive (١٠٠٪) to lincomycin, chloramphenicol and penicillin and resistant (١٠٠٪) to amoxicillin whereas some isolates have shown resistant in a lesser degree to gentamycin, tetracycline and erythromycin (٦٦٪) and then to clindamycin (٣٣٪)(table ١٠).

Table ١٠:- Effect of antibiotic on the Growth of GBS

Antibiotic	Isolation no.١	Isolation no.٢	Isolation no.٣	Rate of resistance
Clindamycin	+	-	-	٣٣.٣٪
Gentamycin	+	-	+	٦٦.٦٪
Tetracycline	+	-	+	٦٦.٦٪
Erythromycin	+	-	+	٦٦.٦٪
Lincomycin	-	-	-	٠
Penicillin	-	-	-	٠
Amoxillin	+	+	+	١٠٠٪
Chloramphenicol	-	-	-	٠

(+) Resistance (-) Sensitive

All the isolates have shown sensitivity to penicillin and this antibiotic, as known, is the drug of choice for GBS treatment. Severin and Wiley (١٩٧٦) have observed the same results about the

susceptibility of this bacteria to penicillin. Penicillin is the agent of choice for GBS prophylaxis and therapy because its antimicrobial spectrum, narrower than that of ampicillin, would reduce the likelihood of resistance developing in other organism (Schuchat, ١٩٩٩).

GBS isolates are sensitive to chloramphenicol. This result is identical with those obtained by Silverman, *et.al.*,(٢٠٠٠) who have pointed that this drug is rarely used in women especially in pregnant women because of its toxicity and also its being the cause of anemia and gray baby.

Also, the isolates are sensitive to lincomycin. This antibiotic is not widely used in the treatment of vaginitis. Culebras, *et.al.*, (٢٠٠٢) have observed that the isolates of GBS are resistant to lincomycin but this result is not correlated with the results obtained by this study.

The results also show that only one isolate of GBS is sensitive to erythromycin. This result is similar to those obtained by de-Azaved, *et.al.*, (٢٠٠١). The mechanism of erythromycin resistance in GBS include modification of target site and active drug efflux. Target modification is conveyed by the action of family of methyltransferase enzymes encoded by the erm genes. The erm genes found in GBS are ^{erm}(B) and ^{erm}(A). Both genes may be inducibly or constitutively expressed (Kataja, *et.al.*, ١٩٩٨). Active drug efflux is mediated by the mef(A) gene and causes resistance to ١٤-and-١٥ membered macrolide compounds (Clancy, *et.al.*, ١٩٩٦).

On the other hand, some GBS isolates have shown resistance to tetracycline. This result is identical with those obtained by Turnidge *et.al.*,(٢٠٠٣) and Roberts, (١٩٩٦) who have observed that tetracycline resistant genes are often found on the same mobile unit as erythromycin resistance genes. The most widely distributed tetracycline resistant determined in GBS is tet(M). The ^{tet}(O), ^{tet}(K), ^{tet}(L) genes also appear but are much lesser common. Seventeen different tetracycline resistant determinants have been characterized to date. Most of these determinants code either for protein which pumps tetracycline out of the cell or for the ribosomal protection protein which protects the ribosomes from the action of tetracycline (Chopra, *et.al.*, ١٩٩٢).

Furthermore, all isolates of GBS are resistant to amoxillin. This result is identical with those obtained by Morales, *et.al.*, (١٩٩٩). The mechanism of resistance is to decrease penetration of the antibiotic through the outer cell membrane and prevent the drug from reaching the target penicillin- binding proteins(PBPs) (Mary, *et.al.*, ٢٠٠٠).

Two isolates of GBS are sensitive to clindamycin and one isolate is resistant. This result is identical with those obtained by Ko, *et.al.*, (٢٠٠١). Resistant mechanism to clindamycin is the same as that of erythromycin (Betriu, *et.al.*, ٢٠٠٣).

They also study the effect of gentamycin on GBS isolates and observe that two isolates are resistant and one isolate is sensitive. The recurrent usage of gentamicin in treatment of vaginitis may be the

most important reason for revealing such resistance of GBS to gentamicin (Sharat, ٢٠٠٤).

٣.٤ The Effect of Lactic acid on the Growth of *Streptococcus agalactiae*

The effect of lactic acid at different concentrations (١٠-١٠٠µg/ml) on *Str. agalactiae* growth has been investigated (Figure١). Colorimetric method has been used for this purpose (Al-Shukri ,٢٠٠٣).

It has been observed that the growth absorbance of the bacteria without addition of lactic acid is ٠.٦٠٩; the growth rate was decreases when lactic acid is added at a concentration ١٠µg where the absorbance is ٠.٤٨٢. When the lactic acid concentration increases until ١٠٠µg/ml, the absorbance decreases to ٠.٠٧٥ (Table ١١).

Table ١١:- Effect of Lactic acid on the Growth of GBS

Concentration of lactic acid	*O.D at ٥٢٠nm*
Without lactic acid	٠.٦٠٩
١٠µg/ml	٠.٤٨٢
٢٠µg/ml	٠.٤٤٤
٤٠µg/ml	٠.٣٧٢
٦٠µg/ml	٠.٣١٩
٨٠µg/ml	٠.٢١٢
١٠٠µg/ml	٠.٠٧٥

The results above may interpret why the isolation of this bacteria is rare in vagina swabs.

This may be attributed to the presence of lactic acid bacteria and other lactic acid producing microorganism such as *Streptococcus* spp. and yeast.

Bruce and Reid (١٩٨٨) have pointed that lactic acid bacteria can prevent the growth of *Str. agalactiae*.

The same results are shown by Redondo-López, *et.al.*, (١٩٩٠), who have pointed that lactobacilli can control vaginal bacterial microflora through the production of the lactic acid. However, in this study, lactobacilli has been isolated without any other bacteria, where it prevents the growth of other bacteria and protects the vagina from invasive microorganism.

Lactic acid bacteria is considered a probiotics organism. Probiotics are defined as living organisms beneficial to health when ingested. Different species of microorganisms such as lactic acid bacteria or yeast have been proposed for human use. These microorganisms differ from each other and it is, therefore, unlikely that they act in the same way. Probiotics could be used for several conditions such as diarrhoea, candidal vaginitis, urinary tract infection, immune disorders, lactose intolerance, hypercholsterol--aemia and food allergy (Mobelli and Gismondo, ٢٠٠٠).

Instillation of probiotic lactobacilli has the potential to make a significant impact on the health of women, and therefore, it is important to understand how the vaginal microbe changes and adapts to the presence of these strain (Burton, *et.al.*, ٢٠٠٣).

The potential importance of lactobacilli is to protect the vagina from disease therefore, it has been used nowaday as probiotic to be determined (Reid, *et.al.*, ٢٠٠٠).

In brief, *Streptococcus agalactiae* colonization to the vagina may depend on several factors. The most important is the presence and absence of lactic acid, and the possession of the isolates to produce one or more than one colonization factors antigens. According to the results obtained in this study, the prevalence of *Streptococcus agalactiae* is very low if it is compared to other microorganisms. This may be attributed to inability to resistant the action of lactic acid and also its ability to produce sufficient types of colonization factors antigens.

٣.٥ Bacteriocin Production

Bacteriocin is antimicrobial protein produced by bacteria that kill or inhibit the growth of other bacteria related to the same group or species (Cleveland, *et.al.*, ٢٠٠١).

Bacteriocin production is investigated by using GBS isolates and it is found that only one isolate (no.١) is able to produce bacteriocin by using cross streaking technique, that only one isolate (no.٢) is sensitive to it (Figure ٢).

This result is identical with the result obtained by Mariela and Marcelo (٢٠٠٢) who have pointed that GBS is able to produce bacteriocin and it is considered a virulence factor for GBS.

The use of cross streaking technique for the production of the bacteriocin has many benefits. The most important one is the period of incubating the bacteria for ٤٨hr. to give enough time to secrete high quantity of bacteriocin and then, to spread easily through the media.

Bacteriocin plays a role in spreading the bacteria inside the host body. This bacteriocin is also produced and secreted without using inducible agents such as mitomycin C widely used in bacteriocin induction. Bacteriocin produced by indigenous bacteria may be critical for the maintenance of normal microflora and host health by preventing invasion by exogenous pathogens (Brook, ١٩٩٩).

Bacteriocins are of interest in medicine because they are made by non-pathogenic bacteria that normally colonize the human body. Loss of these harmless bacteria following antibiotic use may allow pathogenic bacteria to invade human body.

This agent is considered as bacteriocin like agent because further studies should be needed to prove that this agent is bacteriocin. However, some studies have pointed that these substances produced by *Streptococcus* spp. which can effect on wide range of bacteria are considered bacteriocin like inhibitor substance (BLIS) (Ragland and Tagg, ١٩٩٠). This, the BLIS, has been demonstrated in occasional strains of *Streptococcus agalactiae*, *Streptococcus dysagalactiae* and *Streptococcus equi* (Schofield and Tagg, ١٩٨٣).

The majority of BLIS characterized from *Streptococcus* are relatively small (<٥ KDa) heat-stable proteins termed lantibiotics because they contain the unusual amino acids lanthionine and/or ٣-methyllanthionine (Jung, ١٩٩١).

Bacteriocin produced by *Streptococcus agalactiae* is also considered a spreading factor, which facilitates the spreading and competing with other bacteria present in the vagina.

There are no previous studies about bacteriocin production by this bacteria and this study confirms the ability of this bacteria to produce this agent. This study is considered as the first study that detects of the presence of this agent.

Conclusions and Recommendations

٤.١ Conclusions

The results of this study can yield the following conclusion:

١- The optimal method for Group B *Streptococcus* screening is collection of a single standard culture swab or two separate swabs of the distal vagina.

٢- The incidence of *Streptococcus agalactiae* infection in ١٢٠ woman patients (pregnant and non-pregnant) suffering from vaginitis is three isolates obtained from non-pregnant only.

٣- The isolates have shown multi-resistant to antibiotics.

٤- *Streptococcus agalactiae* is revealed to possess more than one virulence factor such as capsule, CFA/III, haemolysin production, etc.

٥- The ability of *Streptococcus agalactiae* to produce bacteriocin like inhibitor substance without induction was detected.

٦- Lactic acid have potent effect on the growth of *Streptococcus agalactiae*, therefore, this bacteria cannot be isolated in the presence of lactic acid bacteria.

٤.٢ Recommendations

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

١- Study of Group B *Streptococcus* prevalence in disease other than vaginitis.

٢- Study of molecular basis of Group B *Streptococcus* resistance to antibiotics.

٣- Evade of using antibiotics which can effect on vaginal normal flora and then facilitate the colonization of unwilling bacteria.

٤- Current studies must be performed to describe the bacteriocin produced by such bacteria.

References

Abbot, J. and R. Shannon. ١٩٥٨. A method for typing *Shigella sonnei*, Using Colicin Production as a Marker. *J. Clin. Path.* ١١:٧١-٧٧.

Abbot, J. and J. Graham. ١٩٦١. Colicins typing of *Shigella sonnei. Mon. Minist. Hilth. Lab Servo.* ٢٠:٥١-٥٨.

Al-Saeed, M.S. ١٩٩٧. Upper respiratory tract infection in Babylon province. PhD. Thesis. Baghdad University.

Al-Shukri, M. ٢٠٠٣. A Molecular and Clinical Study of Some Strains of *Acinetobacter* Isolated from Patients in Hilla Province. MSC. Thesis. Collage of Science. Babylon University.

Al-Zaag, A. ١٩٩٤. Molecular biology of bacterial virulence. ١st ed. Baghdad University.

Anonymous. ١٩٩٦. Prevention of prenatal group B *Streptococcal* disease: a public health perspective. Center for Disease Control and Prevention Guidelines. *MMWR-Morbidity and Mortality Weekly Report.* ٤٥(RR-٧):١-٢٤.

Anthony, B.F. and J.A. Carter et.al. ١٩٨٣. Isolation of group B *Streptococcus* from the proximal small intestine of adults. *J InfectDis.*١٤٧:٧٧٦-٧٧٩.

Aronson, J.K. and DJM. Reynolds. ١٩٩٢. ABC of monitoring drug therapy. Aminoglycoside antibiotics. *British Medical Journal.* ٣٠٥:١٤٢١.

Bagg, J., I.R. Poxton, D.M. Weir and P.W. Ross. ١٩٨٢. Binding of type-III group B *Streptococci* to buccal epithelial cells. *J. Med. Microbiol.* **١٥:** ٣٦-٣٧٢.

Baker, C.J. and F.F. Barrett. ١٩٧٣. Transmission of group B *Streptococci* among parturient women and their neonates. *J Pediater.* **٨٣**:٩١٩-٢٥.

Baker, C.J., M.A. Rench and D.L. Kasper. ١٩٩٠. Response to type III polysaccharide in women whose infants have had invasive group B *Streptococcal* infection. *N Engl J Med.* ٣٢٢:١٨٥٧-١٨٦٠. Baker, C.J. and M.S Edwards. ١٩٩٠. Group B *Streptococcal* infection. In: J.L. Remington and J.O. Klein, ed. Infectious disease of the fetus and newborn infant. ٣rded. Philadelphia: W.B. Saunders. ٧٤٢-٨١١.

Baker, C.J. ١٩٩٧. Group B *Streptococcal* infection. *Clin. Perinatol.* **٢٤**:٥٩-٧٠.

Baron, E.J., L.R. Peterson and S.M. Finegold. ١٩٩٤. Bailey and Scott's Diagnostic Microbilogy. ٩thed. The C.V. Mosby company, U.S.A.

Baron, E.J., L.R. Peterson and S.M. Finegold. ١٩٩٥. Bailey and Scott's Diagnostic Microbilogy. ١٥thed. The C.V. Mosby company, Toronto. Canada.

Bayer, A.S., A.W. Chow, B.F. Anthony and L.B. Guze. ١٩٧٦. Serious infections in adult due to group B *Streptococci.* Clinical and serotypic characterization. *Am J Med.* **٦١**:٤٩٨-٥٠٣.

Berner, R. ٢٠٠٢. Group B *Streptococci* during pregnancy and infancy. *CurrOpinInfectDis.* **١٥**:٣٠٧-١٣.

Bernheimer, A.W., R. Linder and L.S. Avigad. ١٩٧٩. Nature and mechanism of action of the CAMP protein of group B *Streptococci*.*InfectImmun.* **٢٣**:٨٣٨-٨٤٤.

Betriu, C., M. Gómez, A. Sánchez, A. Cruceyra, J. Romero and Picazo.١٩٩٤. Antibiotic resistance and penicillin tolerance in clinical isolates of group B *Streptococci*. *Antimicrob. Agents Chemother.* **٣٨**:٢١٨٣-٢١٨٦.

Betriu, C., E. Cluerbras, M. Gomez, I. Rodriguez-Avial, B.A. Sanchez, M.C. Agreda and J.J. Picazo. ٢٠٠٣. Erythromycin and Clindamycin Resistance and Telithromycin susceptibility in *Streptococcus agalactiae*. *Antimicrob. Agents Chemother.* **٤٧**:١١١٢-١١٤.

Beynom, R.H. and J.S. Bond. ١٩٨٩. Proteolytic enzymes: A pratical aproach. Academic press. Oxford.

Bonadio, M., M. Meini, P. Spitaleri and C. Gigli. ٢٠٠١. Current microbiological and clinical aspects of urinary tract infections. *Eur. Urol.* **٤٠**:٤٣٩-٤٤٤.

Brogden, K.A. J.A. Roth, T.B. Stanton, C.A. Bolin, C.F. Minion and M.J. Wannemuehler (ed). ٢٠٠٠. Virulence mechanism of Bacterial Pathogens. ASM Press, Washingston, D.C.

Brook, I. ١٩٩٩. Bacterial interference. *Crit. Rev. Microbiol.* **٢٥**:١٥٥-١٧٢.

Bruce, A.W. and G. Reid. ١٩٨٨. Intravaginal instillation of *Lactobacilli* for prevention of recurrent urinary tract infections. Can. J. Microbiol. **٣٤**:٣٣٩-٣٤٣.

Burton, J., P. Cadieux and G. Reid. ٢٠٠٣. Improved Understanding of the Bacterial Vaginal Microbiota of Women before and after Probiotic Instillation. *Appl Environ Microbiol.* **٦٩(١)**:٩٧-١٠١.

Cheng, Q., D. Stafslien, S.S. Purushoth and P. Cleary. ٢٠٠٢. The Group B *Streptococcal* C٥a peptidase IS Both a specific protease and an Invasive, Infection and Immunity. **٧٠(٥)**:٢٤٠٨-٢٤١٣.

Christie, R., N. Atkins and E. Munch-Peterson. ١٩٤٤. A note on a lytic Phenomenon shown by group B *Streptococci*. *Aust J Exp Biol Med. Sci.* **٢٢**:١٩٧-٢٠٠.

Christensen, K.K., P. Christensen and I. Hagerstrand ١٩٨٢. The clinical signicance of group B *Streptococci*. *J Perinat Med.* **١٠**:١٣٣-١٤٦.

Chopra, I., P.M. Hawkey and M. Hinton. ١٩٩٢. Tetracyclines, Molecular and Clinical aspect. *J. Antimicrob Chemother.* **٢٩**:٢٤٥-٢٧٧.

Clancy, J., J. Petitpas, F. Dib-Haij, W. Yuan, M. Cronan, A.V. Kamath, J. Bergeron and J.A. Retsema. ١٩٩٦. Molecular cloning and Functional analysis of a novel macrolide resistance determinant, *mefA*, from *Streptococcus pyogens.* Mol. Microbiol. **٢٢**:٨٦٧-٨٧٩.

Cleveland, J., T.J. Montville, I.F. Nes and M.L. Chikindas. ٢٠٠١. Bacteriocins: Safe, natural antimicorbe for food preservation. *Inf. J. Food. Microbiol.* **٧١(١)**:١-٢٠.

Collee, J.G., A.G. Fraser, B.P. Marmian and S.A. Simmon. ١٩٩٦. Mackin and McCartney. Practical Medical Microbiology. ١٤th ed. The Churchill Livingston. Inc. U.S.A.

Colman, G. ١٩٨٨. Typing of *Streptococcus agalactiae*(Lancefield group B). European Journal of Clinical Microbiology and Infectious Disease. **٧**:٢٢٦-٢٣١.

Cruickshank, R., J. Duguid, B.Marmion and R. Swain. ١٩٧٥. Medical Microbiology. A book of the practice of medical microbiology. ٥th ed. Logram Grouplimitted Britain.

Culebras, E., I. Rodriguez-Avial, M. Redondo and J.J. Picazo. ٢٠٠٢. Macrolide and Tetracycline Resistance and Molecular Relation Ships of Clinical Strains of *Streptococcus agalactiae*. *Antimicrob. Agents Chemother*. **٤٦(٥)**:١٥٧٤-١٥٧٦.

Curzik, D., A. Drazancic and Z. Hrgovic. ٢٠٠١. Nonspecific aerobic vaginitis and pregnancy. Fetal. Diagn. Ther. **١٦(٣)**:١٨٧-٩٢.

de Azavedo, J.C., M. McGavin, C. Duncan, D.E. Low and A. McGeer. ٢٠٠١. Prevalence and mechanisms of macrolide resistance in invasive and non invasive group B *Streptococcus* isolates from Ontario, Canada. *Antimicrob. Agents Chemother.* **٤٥**:٣٥٠٤-٣٥٠٨.

De Boy, J., K. Wachsumth and B. Davis. ١٩٨٠. Hemolysin activity in enterotoxigenic and non- enterotoxigenic strains of *E. coli. J. Clin. Microbiol.* **١٢**:١٩٣-١٩٨.

De Mouy, D., J.D. Cavallo, R. Leclercq, R. Fabre ٢٠٠١. Antibiotic susceptibility and mechanisms of erythromycin resistance in clinical isolates of *Streptococcus agalactiae*. French multicenter study. Antimicrob. Agents Chemother. **٤٥**:٢٤٠٠-٢٤٠٢.

Diaz de, VME., P. Villa and A. Frias. ٢٠٠٢. Evaluation of the siderophores production by *Psudomonas aeruuginosa* sp. *Rev Latinoam Microbiol.* **٤٤(٣-٤)**:١١٢-١١٧.

Di-Bartolomeo, S., M. Rodriguez, D. Sauka and R. Alberto-De-Torres. ٢٠٠١. Microbiologic profile in symptomatic pregnant women's genital secretions in Gran Buenos Aires Argentia. *Enferm. Enfect Microbiol. Clin.* **١٩(٣)**:٩٩-١٠٢.

Dillon, H.C., E. Gray, M.A. Pass and B.M. Gray. ١٩٨٢. Vaginal carriage of group B *Streptococcus* during pregnancy. *J. Infect. Dis.* **١٤٥**:٧٩٤-٩.

Donder, G.G., V. Annei, B. Eugene, B. Alfons, D. Geert and S. Bernard. ٢٠٠٢. Definition of a type of abnormal vaginal flora is distinct from bacterial vaginosis: aerobic vaginitis. BJOG. **١٠٩(١):**٣٤-٤٣.

Doran, K.S., J.C. Chang, V.M. Benoit, L. Eckmann and V.Nizet. ٢٠٠٢. Group B *Streptococcal* beta-hemolysin/cytolysin promotes invasion of human lung epithelial cells and the release of interleuken-٨. *J. Infect. Dis.* **١٨٥**:١٩٦-٢٠٣.

Drechsel, H., G. Winkelmann and C.J. Carrano. ١٩٩٧. In Transition Metalsin Microbial Metabolism(Harwood Academic).

Duguid, J.P., E.S. Anderson and I. Campbell. ١٩٩٦. Fimbriae and adhesive properties in *Salmonella*. *J Pathol Bacteriol.* **٩٢**:١٠٧-٣٨.

Duma, R.J., A.N. Weinberg, T.F. Medrek and L.J. Kunz. ١٩٦٩. *Streptococcal* infections: a bacteriologic and clinical study of *Streptococcal* bacteraemia. Medicine. **٤٨**:٨٧-١٢٧.

Easmon, CSF. and A. Tanna. ١٩٨١. Group B *Streptococci* gastrointestinal organisma. *J. Clin. Pathol.* **٣٤**:٩٢١-٣.

Edwards, M.S., W.A. Nicholson, C.J Baker and D.L. Kasper. ١٩٨٠. The role of specific antibody in alternative complement pathway by type III, group B *Streptococci*. *J. Immunol.* **١٢٨**:١٢٧٨-١٢٨٣.

Edwards, M.S., D.L. Kasper, H.J. Jennings, C.J. Baker and A. Nicholson- Weller. ١٩٨٢. J. Immunol. ١٢٨:١٢٧٨-١٢٨٣.

Edwards, M.S. and C.J. Baker. ١٩٩٥. *Streptococcus agalactiae*. In: Mendell, G.L., J.E. Bennett, R. Dolan. Eds. Mandell, Douglas and Bennett. Principles and Practice of Infectious Disease.٤th ed. Churchill Livingstone Inc. New York. ١٨٣٥-٤٥.

Edwards, M.S. and C.J. Baker. ٢٠٠٠. *Streptococcus agalactiae*. P.٢١٥٦- ٢١٦٥. In Mandell, Douglas and Bennett(eds). Principles and Practice of Infectious Disease. ٥th ed. Churchill Livingstone of perinatal group B *Streptococcal* disease: a public health prespective. MMWR. **٤٥(RR-٧)**:١-١٤.

Eichenbaum, Z., E. Mull, S.A. Morse and J.R. ١٩٩٦. Acquisition of iron from host proteins by *Streptococcus*. Infect Immun. ٦٤:٥٤٢٨-٩.

Eickhoff, T.C., J.O. Klein, A.L. Daly et.al. ١٩٦٤. Neonatal sepsis and other infections due to group B beta-hemolytic *Streptococci*. *N. Eng. J. Med.* ٢٧١:١٢٢١-١٢٢٥.

Farley, M.M., C. Harvey and T. Stull. ١٩٩٣. A population based assessment of invasive disease due to group B *Streptococcus* in non-pregnant adults. *N Engl J Med.* ٣٢٨:١٨٠٧-١٨١١.

Farley, M.M. ٢٠٠١. Group B *Streptococcal* disease in non pregnant adults. *Clin Infect Dis.* ٣٣:٥٥٦.

Fernandez, M., M.E. Hickman and C.J. Baker. ١٩٩٨. Antimicrobial susceptibilities of Group B *Streptococci* isolated between ١٩٩٢ and ١٩٩٦ from patients with bacteremia or meningitis. *Antimicrob. Agents Chemother.* ٤٢:١٥١٧-١٥١٩.

Fettucciari, K., E. Rosati, L. Scaringi, P. Cornacchione, G. Migliorati, R. Sabatini, I. Fetriconi, R. Rossi and P. Marconi. ٢٠٠٠. Group B *Streptococcus* induces apoptosis in macrophages. *J. Immunol.* ١٦٥:٣٩٢٣-٣٩٣٣.

Finch, F.G., G.L. French and S.I. Phillips. ١٩٧٦. Group B *Streptococci* in the female genital tract. *Br Med J.* ١:١٢٤٥-٧.

Frank, S.A. ١٩٩٤. Spatial polymorphism of bacteriocins and other allelopathictraits, *Evol Ecol.* ٨:٣٦٩-٨٦.

Frederick, R. and M.D. Jelovesk. ٢٠٠١. Bacterial vaginal Infections in Pregnancy. Woman's Diagnostic Cyber. BMJ. ١٨:٢٥-٢٨.

Garey, J.C. ٢٠٠١. Screening and mangement protcol for group B *Streptococcus* in pregnancy. *Curr. Womens Health Rep.* ٢:٢٣٨-٢٤٤.

Gase, K., J. Ozegowski and H. Malke. ١٩٩٨. The *Streptococcus agalactiae* hyl B gene encoding hyaluronate lyase: Completion of the sequence and expression analysis. *Biochim Biophys Acta.* ١٣٩٨:٨٦-٩٨.

Geo, F.B., S.B. Janet and A.M. Stephen. ٢٠٠١. Medical Microbiology. *Psudemonads, Acinetobacters* and uncommon Gram-negative Bacteria ٢٢ed. Acid-Free paper. P:٢٣٢.

Ghaly, K. ٢٠٠١. Epidemiological study of STD among women with abnormal vaginal discharge in Najaf. MSC. Thesis. College of Medicin. University of Kufa.

Griffiths, E., J.J. Bullen and E. Griffiths.١٩٨٧. ed. In Iron and Infection (Wiley. Chichester. U.K.).

Habbeb, F. ٢٠٠٣. Isolation and identification of most common pathogen in vagina of non-pregnant and pregnant women in

Baghdad. MSC. Thesis. Collage of medical and health technology.

Hardie, J.M.١٩٨٦. Genus *Streptococcus* Rosenbach ١٨٨٤, ٢٢AL. In Sneath P.H.A, N.S. Mair, M.E. Sharpe and J.C. Holt (ed.). Bergey's Manual of Systematic Bacteriology. The William and Wilkins Co., Baltimore, Md. ٢:١٠٤٣-١٠٧١.

Harris, O., D.W. Shelver, J.F. Bohnsack and C.E. Rubens. ٢٠٠٣. A novel *Streptococcal* protease promotes virulence, resistance to opsonophagocytosis, and cleavage of human fibrinogen. J. Clin. Invest. ١١١(١):٦١-١٠٧١.

Harrison, L.H., A. Ali and D.M. Dwyer. ١٩٩٥. Relapsing invasive group B *Streptococcal* infection in adults. *Ann Int Med.* ١٢٣:٤٢١- ٤٢٧.

Harrison, L.H., J.A. Elliott, D.M. Dwyer, J.P. Libonati, P. Ferrieri, L. Billmann and A. Schuchat. ١٩٩٨. *Streptococcus agalactiae* infection. *J. Infect. Dis.* ١٧٧:٩٩٨-١٠٠٢.

Hill, H.R., J.F. Bohnsack, E.Z. Morris, N.H. Augustine, C.J. Parker, P. Cleary and J.T. Wu. ١٩٨٨. Group B *Streptococci* inhibit the chemotactic activity of the fifth component of complement. *J. Immunol.* ١٤١:٣٥٥١-٣٥٥٦.

Holt, J.C., N.R. Krieg, A. Sneath, J.T. Stachley and S.T. William. ١٩٩٤. Bergy's manual of determinative bacteriology. ٩th ed. USA.P:٥٥٢.

Honig, E., J.W. Mouton and W.I. van-der-Meijden. ١٩٩٩. Can group B *Streptococci* cause asymptomatic vaginitis. *Infect. Dis. Obstet. Gynecol.* **٧(٤)**:٢٠٦-٩.

Hornick, B.D., J. Thommandru, W. Smith and S. Clegg. ١٩٩٥ Adherence properties of an mrkD-negative mutant of *Klebsiella Pnumoniae. Infect. Immun.* **٦٣(٥)**:٢٠٢٦:٣٢-٣٩.

Hsuch, P.R., L.J. Teng, L.N. Lee, S.W. Ho, P.C. Yang and K.T. Luh. ٢٠٠١. High incidence of erythromycin resistance among clinical isolates of *Streptococcus agalactiae* in Taiwan. *Antimicrob. Agents.Chemotherapy*.**٤٢**:٣٢٠٥-٣٢٠٨.

Islam, AKMS. ١٩٧٧. Rapid recognition of Group B *Streptococci*. Lancet i :٢٥٦-٢٥٧.

Islam, AKMS and E. Thomas. ١٩٨٠. Fecal carriage of Group B *Streptococci. J Clin Pathol.* **٣٣**:١٠٠٦-٨.

Jack, R.W., J.R. Tagg and R. Bibek. ١٩٩٥. Bacteriocin of Gram-positive bacteria. Microbiol. Rev. **٥٩**:١٧١-٢٠٠.

Jackson, R. J., M.L. Dae and D.V. Lim. ١٩٩٤. Cell-associated collagenolytic activity by group B *Streptococci*. *Infect Immun.* **٦٢**: ٥٦٤٧-٥٦٥١.

Jackson, L.A., M.M. Farley and A. Schuchat. ١٩٩٦. Adult Group B *Streprococcal* Disease. *Ann Intern Med.* **١٢٥**:١٥٢-١٥٣.

Jennings, H.J., E. Katzenllenbogen, C. Lugoweski and D.L. Kasper. ١٩٨٣. Structure of the native polysaccharide antigens of type Ia and type Ib group B *Streptococci*. *Biochemistry.* **٢٢**:١٢٥٨-١٢٦٣.

Jennings, H.J., K.G. Rosell, E. Katzenllenbogen and D.L. Kasper. ١٩٨٣. Structural determination of the capsular polysaccharide antigen of type II group B *Streptococci*. *J. Biol. Chem.* **٢٥٨**:١٧٩٣-١٧٩٨.

Jiminez-Diaz, R., R.M. Rios-Sanches et.al., ١٩٩٣. Plantaricins S and T, two new bacteriocins produced by *Lactobacillus pluntarum* LPC٠١٠ isolated from green olive fermentation. *APP Environ Microbiol.* **٥٩**:١٤١٦-٢٤.

Johnson, K. ١٩٩٩. Normal Vaginal Flora May Hamper IVF Success. *Lancet.* **٣٥٤(٩١٧٩)**:٦٥١-٥٢.

Joshi, N., G. Caputo, M.R. Weitekamp and A.W. Karchmer. ١٩٩٩. Infection in patients with diabetes mellitus. *N. Engl. J. Med.* **٣٤١**:١٩٠٦-١٩١٢.

Jung, G. ١٩٩١. Lantibiotics ribosomally synthesized biologically active polypeptides containing sulfide bridges and α, β-didehydroamino acid. Angew. Int. Ed. Engl. **٣٠**:١٠٥١-٦٨.

Kataja, J., H. Seppälä, M. Skurnik, H. Sarkkinen and P. Huovinen. ١٩٩٨ Different erythromycin resistance mechanisms in *Streptococcus*. *Antimicrob. Agent Chemother.* **٤٢**:١٤٩٣-١٤٩٤.

Keufhold, A. and P. Ferrieri. ١٩٩٣. The microbiologic aspects, including diagnosis of beta-hemolytic streptococcal and enterococcal infections. *Infect. Dis. Clin.* North Am. **٧**:٢٣٥-٢٥٦.

Kim, W.J. ١٩٩٣. Bacteriocins of lactic acid bacteria-their potentials as food. *Food Rev Int.* **٩**:٢٩٩-٣١٣.

Ko, W.C., H.C. Lee, L.R. Wang, C.T. Lee, A.J. Liu and J.J. Wu. ٢٠٠١. Serotyping and antimicrobial susceptibility of group B *Streptococcus* over an eight-year period in Southern Taiwan. *Eur. J. Clin. Microbiol. Infect. Dis.* **٢٠**:٥٢-٥٤.

Kogan, G. and D. Uhrin. ١٩٩٤. Structure of the type VI of GBS capsular polysaccharide determian by high resolution NMR spectroscopy. *J Carbohydr Chem.* **١٣**:١٠٧١-٨.

Kogan, G., D. Uhrin, J.R. Brisson, L.G. Paoletti, A.E. Blodgett, D.L. Kasper and H.J. Jennings. ١٩٩٦. Structural and immunological characterization of the type VIII group B *Streptococcus* capsular polysaccharide. *J. Biol. Chem.* **٢٧١**:٨٧٨٦-٨٧٩٠.

Lähteenmakia, K., K. Pentti and T.K. Korhonen. ٢٠٠١. Bacterial plasminogen activators and receptors. *FEMS Microbiol. Rev.* **٢٥**: ٥٣١-٥٥٥.

Lamont, I.L., P.A. Beare, A.I. Vasil and M.L. Vasil. ٢٠٠٢. Siderophore-mediated signaling regulates virulence factor

production in *Pseudomonas aeruginosa. Proc. Natl. Acad. Sci.* **٩٩(١٠)**:٧٠٧٢-٧٠٧٧.

Lau, PCK., M. Parsons and T. Uchimura. ١٩٩٢. Molecular evolution of colicin plasmids with emphasis on the endonuclease types, *Bacteriocin and Lanbiotics*, ed. James R, NATO Series, Springer-Verlag, Berlin-Heidelberg-NewYourk.

Lehorani, S., R. Kamungo, A. Omachigui and S. Badrinath. ١٩٩٨. Vaginal carrier state of *Streptococcus agalactiae* in healthy pregnant women and its effect on the neonates. Biomedicine. **١٨(٣)**:١٣٣-٦.

Lerner, P.I., K.V. Gopalakrishna and E. Wolinsky. ١٩٧٧. Group B *Streptococcus* bacteremia in adults: analysis of ٣٢ cases and review of the literature. Medicin (Baltimore).**٥٦**:٤٥٧-٧٣.

Leslie, C., B. Albert and S. Max. ١٩٩٨. Microbiology And Microbial Infection. ٢. Great Britain at The Bath Press, Avon p:١٨٥.

Lin, B., S.K. Hollingshed, J.E. Coligan, M.L. Egan, J.R. Baker and D.C. Pritchard. ١٩٩٤. Cloning and expression of the gene for group B *Streptococcal* hyaluronate lyse. *J Biol Chem*. **٢٦٩**:٣٠١١٣-٣٠١١٦.

Lin, F.Y., P.H. Azimi, L.E. Weisman, J.B. Philips, J. Regan, P. Clark, G.G. Rhoade, J. Clemens, J. Troendle, E. Pratt, R.A. Brenner and V. Gill. ٢٠٠٠. Antibiotic susceptibility prolifes for group B *Streptococci* isolated from neonates. *Clin. Infect. Dis.* **٣١**:٧٦-٧٩.

Litwin, C.M. and S.B. Calderwood. ١٩٩٣. Role of iron in regulation of virulence genes. Clin. Microbiol. Rev. **٦**:١٣٧-١٤٩.

MacFaddin, JF. ١٩٨٥. Media for Cultivation-Identification-Maintenance of Medical Bacteria. Vol ١. Baltimore: Williams and Wilkins.٣٦٥-٣٦٧.

MacFaddin, J.F.٢٠٠٠. Biochemical tests for the identification of medical bacteria. ٣rded. The Williams and Wilkins-Baltimor. USA.

Maeda, H. and T. Yamamoto. ١٩٩٦. Pathogenic mechanisms induced by microbial proteases in microbial infection. *Biol. Chem. Hoppe Seyler.* **٣٧٧(٤)**: ٢١٦-٢٦.

Maniatis, A.N., J. Palermos, M. Kantzanous, N.A. Maniantis, C. Christodoulou and N.J. Legakis. ١٩٩٦. *Streptococcus agalactiae*: a vaginal pathogen. *J. Med. Microbiol.* **٤٤(٣)**:١٩٩-٢٠٢.

Marchlewicz, B.A. and J.L. Duncan. ١٩٨٠. Properties of a hemolysin produced by group B *Streptococci. Infect. Immun.* **٣٠**:٨٠٥-٨١٣.

Marchlewicz, B.A. and J.L. Duncan. ١٩٨١. Lysis of erythrocytes by a haemollysin produced by a group B *Streptococcus* sp. *Infect. Immun.* **٣٤**:٧٨٧-٧٩٤.

Mariela, S. and G. Marcelo. ٢٠٠٢. Extracellular virulence factors of *Streptococci* Associated with Animal Diseases. *Frontiers in Bioscience.***٧**:٧٥٢-٧٦٤.

Marques, M.B., D.L. Kasper, M.K. Pangburn and M.R. Wessels. ١٩٩٢. Prevention of C٣ deposition is a virulance mechanism of type III group B *Streptococcus* capsular polysaccharide

Infect.Immun. ٦٠:٣٩٨٦-٣٩٩٣.

Mary, J., A. Richard and C. Pamela. ٢٠٠٠. Lippincott's IIIustrated Reviews: Pharmacology. ٢nded. Lippincott William and Wilkins London, New York.

Mathew, P., A.C. Justica, M.M. Farley, D.S. Stephens, J.D. Wenger, M.R., Wessels and D.L Kasper. ١٩٩٣. Group B *Streptococcal* Disease in Adult. *N Engl J Med.* **٣٢٩**:١٦٥٨-١٦٥٩.

Melancon, D. and D. Grenier. ٢٠٠٣. Production and properties of bacteriocin-like Inhibitory substances from the swine pathogen *Streptococcus suis* serotype ٢. *Appl Enviro Microbiol.* **٦٩(٨)**:٤٤٨٢-٤٤٨٨.

Metcalf, E.S. ٢٠٠١. The role of international transport of equine semen on disease transmission. *Anim. Reprod. Sci.* **٦٨(٣-٤)**:٢٢٩-٣٧.

Metha, P. ١٩٨٢. Vaginal flora: A dynamic ecosystem. *J. Reprod Med.* ٢٧:٤٤٥.

Milligan, T.W., C.J. Baker, D.C. Straus and S.J Mattingly. ١٩٧٨. Association of elevated levels of extracellular neuraminidase with clinical isolates of type III group B *Streptococci. Infect. Immun.* **٢١**:٧٣٨-٧٤٦.

Miniatis, T., E. Frtsch and J. Sambrook. ١٩٨٢. Molecular cloning; a laboratory manual. Cold Spring Harbour laboratory. New York.

Miyake, K., C.B. Underhill, J. Lesley and P.W. Kincade. ١٩٩٠. Hyaluronate can function as a cell adhesion molecule and CD٤٤ participate in hyalronate recognition. *J Exp Med.* ١٧٢:٦٩-٧٥.

Mombelli, B. and M.R. Gismondo. ٢٠٠٠. The use of probiotics in medical practice. *Int. J. Antimicrob. Agernts.* **١٦(٤)**:٥٣١-٦.

Monif, G.R. and H.J. Carson. ١٩٩٨. Female genital tract bacterial coisolates with *candida albicans* in patients without clinical vaginitis. *Infect. Dis. Obstet. Gynecol.* **٦(٢)**:٥٢-٦.

Morales, W.J., S.S Dickey, P. Bornick and D.V. Lim. ١٩٩٩. Change in antibiotic resistance of group B *Streptococcus*: impact on intrapartum mangement. *Am. J. Obstet. Gynecol.* ١٨١:٣١٠-٣١٤.

Mosabi, J.M., S.M. Arimi and E.K. Kang'ethe. ١٩٩٧. Isolation and characterization of group B *Streptococci* from human and bovine source within and around Nairobi. *Epidemiol Infect.* ١١٨:٢١٥-٢٢٠.

Mouricout, M. ١٩٩٧. Interaction between the enteric pathogen and the host. An assortment of bacterial lectins and a set of glyconjugate receptors. *Adv. Exp. Med. Biol.* ٤١٢:١٠٩-١٢٣.

Murdoch, D.R. and L.B. Reller. ٢٠٠١. Antimicrobial susceptibilities of group B *Streptococci* isolated from patients with invasive

disease: ١٠-year perspective. *Antimicrob. Agents Chemother.* **٥**:٣٦٢٣-٣٦٢٤.

Nassif, X., J. Fournier, J. Arnodel and P. Sansonetti. ١٩٨٩. Mucoid phenotype of *Klebsiella pnumoniae* is a plasmid encoded virulence factor. *Infect. Immun.* **٥٧(٢)**:٥٤٦-٥٥٢.

Neilands, J.B. ١٩٩٥. Siderophores: Structure and function of microbial Iron transport compounds. J. Biological Chemistry. **٢٧٠(٤٥)**:٢٦٧٢٣-٢٦٧٢٦.

Nissen-Meyer, J. and H. Holo. ١٩٩٢. A novel Lactococcal bacteriocin whose activity depends on the complimentary action of two peptides. *J. Bacteriol.* **١٧٤**:٥٦٨٦-٩٢.

Nizet, V., R.L. Gibson, E.Y.Chi, P.E. Framson, M. Hulse and C.E.Rubens. ١٩٩٦. Group B *Streptococcal* beta-hemolysin expression is associated with injury of lung epithelial cells. *Infect Immun.* **٦٤**:٣٨١٨-٣٨٢٦.

Nizet, V., P. Ferrieri and C.E. Rubens. ٢٠٠٠. Molecular pathogenesis of group B *Streptococcal* disease in newborns. In Stevens, D.L. and E.L. Kaplan (ed.). *Streptococcal* infections, clinical aspects, microbiology and molecular pathogenesis. Oxford University Press, New York, N.Y. P:١٨٠-٢٢١.

Nizet, V. and C.E. Rubens. ٢٠٠٠. Pathogenic mechanisms and virulence factors of Group B *Streptococci*. In: The Gram-positive pathogens. Eds: Fischetti JJ, Portnoy DA, Rood JI. ASM Press, Washington, D.C. ١٢٥-١٣٦.

Nizet, V. ٢٠٠٢. *Streptococcal* beta-hemolysin genetics and role in disease pathogenesis. TRENDS in Microbiology. **١٠(١٢)**:٥٧٨-٥٨٠.

Noya, FJD. and M.A. Rench. ١٩٨٧. Unusual occurrence of an epidemic of type Ib/c Group B *Streptococcal* sepsis in a neonatal intensive care unit. *J Infect Dis.* **١٥٥**:١١٣٥-٤٤.

Oxtoby, M.J., S.L. Cochi and A.W. Hightower et.al. ١٩٨٦. A population- based study of group B *Streptococcal* infections. In Program and Abstracts of the ٢٦th Interscience Conference on Antimicrobial Agents and Chemotherapy. *American Society for Microbiology*, Abstracts. ١٢٢١.

Pearlman, M.D., C.L. Pierson and R.G. Faix. ١٩٩٨. Frequent resistance of clinical group B *Streptococci* isolates to clindamycin and erythromycin. *Obstet Gynecol.* **٩٢(٢)**:٢٥٨-٦١.

Perch, B., E. Kjems and J. Henrichsen. ١٩٧٩. New serotypes of Group B *Streptococci* isolated from human sources. *J Clin Microbiol.* **١٠**:١٠٩-١٠.

Perera, J. ١٩٩٤. Microbiological patterns in vaginitis. *Ceylon. Med. J.* **٣٩(٢)**:٩١-٤.

Piret, J., J. Millet and A. Demain. ١٩٨٣. production of intracellular protease during sporulation of *Bacillus brevis. Eur. J. Appl. Microbiol. Biotecnol.* **١٧**:٢٢٧-٢٣٠.

Platt, M.W. ١٩٩٥. In vivo hemolytic activity of Group B *Streptococcus* is dependent on erythrocyte-bacteria contact and independent of a carrier molecule. *Curr Microbiol.* **٣١**:٥-٩.

Pritchard, D.G. and B. Lin. ١٩٩٣. Group B *Streptococcal* neuraminidase is actually hyaluronidase. *Infect. Immun.* **٦١**:٣٢٣٤-٣٢٣٩.

Projan, S.J. and R.P. Novick. ١٩٩٧. The molecular basis of pathogenicity. Churchill Livingstone. New York, USA. P:٥٥-٨١.

Provenzano, S.L. ١٩٩٩. Trovafloxcin in gynecology. *Medicin. B. Aires.* **٥٩(١)**:٥٥-٦١.

Quan, M. ٢٠٠٠. Vaginitis. *Clin. Cornestone.* **٣(١)**:٣٦-٤٧.

Quinn, P.J., M.E. Carter, B. Markey and G.R. Cater. ١٩٩٤. Clinical Veterinary Microbiology, London. Mosoby. P:١٣٤.

Rae, M.B., A.P. Tanksale, M.S. Ghathge and V.V. Deshpande. ١٩٩٨. Molecular and bioteclogical aspects of microbial proteases. *Microbiology and Molecular biology Reviews*. **٦٢(٣)**:٥٩٦-٦٣٥.

Ragland, N. and J. Tagg. ١٩٩٠. Application of Bacteriocin like inhibitory substance typing in a longitudinal study of the oral carriage of β-haemolytic *Streptococci* by a group of Dunedins Schoolchildren. ZbI Bakteriol. **٢٧٤**: ١٠٠-٨.

Rang, H.P., M.M. Dale and J.M. Ritter. ١٩٩٠. Pharmacology. ٤th ed. Churchill Livingston. London, New York.

Redondo-López, V., R.L. Cook, R.L. and J.D. Sobel. ١٩٩٠. Emerging role of *Lactobacilli* in the control and maintenance of the vaginal bacterial microflora. *Rev. Infect. Dis.* **١٢(٥)**:٨٥٦-٨٧٢.

Regan, J.A., M.A. Klebanoff and R.P. Nugent. ١٩٩١. The epidemology of Group B *Streptococcal* colonization in pregnancy. *Obstet. Gynecol.* ٧٧:٦٠٤-١٠.

Reid, G., D. Beuerman, C. Heinemann and A.W. Bruce. ٢٠٠٠. Probiotic *Lactobacillus* dose required to restore and maintain a normal vaginal flora. *FEMS Immunol. Med. Microbiol.* ١٣٥١:١-٥

Roberts, M.C. ١٩٩٦. Tetracycline resistance determinants: mechanisms of actions, regulation of expression, genetic mobility and distribution. *FEMS Microbiol. Rev.* ١٩:١-٢٤.

Rodriguez, R., R. Hernandez, F. Fuster, A. Torres, P. Prieto and J. Alberto. ٢٠٠١. Infection genital tract. Enferm-Infect. Microbiol. Clin. **١٩(٦)**:٢٦١-٦.

Ross, P.W. and C.G. Cumming. ١٩٨٢. Group B *Streptococci* in women attending a sexually transmitted disease. *J Infect Clinic.* ٤:١٦١-٦.

Rubens, C.E., M.R. Wessels et.al., ١٩٨٧. Transposon mutagenesis of type III GBS, correlation of capsule expression with virulence. Proc Natl Acad Sci USA. ٨٤:٧٢٠٨-١٢.

Ruoff, K.L. *Streptococcus.* In: P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover and R.H. Yolken(eds). ١٩٩٥. Manual of Clinical Microbiology. ٦ ed. American Society for Microbiology, Washington.٢٩٩-٣٠٧.

Salasia, S.I., I.W. Wibawan, C. Lämmar and M. Sellin. ١٩٩٤. Phase variation in *Streptococci* of serological group B characteristic

properties of isolates from human and bovine infection. *Acta Pathol Microbiol Immunol Scand*. ١٠٢:٩٢٥-٩٣٠.

Salyer, A.A. and D.D. Whitt. ١٩٩٤. Virulence factors that promote colonization. In Salyer, A.A. and D.D. Whitt (ed.). Bacterial Pathogenesis: A Molecular Approach. ASM Press, Washingston, D.C. P:٣٠-٤٦.

Sancho, J., R. Baldris and M. Sanchez. ٢٠٠٠. Handbook of Microbiological culture media. In Ellner, Stoessel, Darakeford and Vosi. ١٩٦٦. Blood agar base, Columbia. Scharlau Chemie. S.A. ٤٥:٥٠٢.

Sauer, F.G., M.A. Mulvey, J.D. Schilling, J.J. Martinez and S.J. Hultgren. ٢٠٠٠. Bacterial pili: molecular mechanisms of pathogenesis. *Curr Opin. Microbiol.* ٣:٦٥-٧٢.

Schofield, C.R. and J.R. Tagg. ١٩٨٣. Bacteriocin like activity of group B and group C *Streptococci* of human and animal origin. JHyg Camb. ٩٠:٧-١٨.

Schreckenberger, P.C. and A. von Graevenitz. ١٩٩٩. *Acinetobacter, Alcaligenes, Moraxella, Methylobacterium,* and other fermentative gram-negative rods. In: Manual of Clinical Microbiology. ٧th ed. Murray PR et.al.(editors). American Society for Microbiology.

Schuchat, A. M. Oxtoby and S. Cochi et.al. ١٩٩٠. Population-based risk factors for neonatal group B *Streptococcal* disease: results

of a cohort study in metropolitan Atlanta. *J Infect Dis.* **١٦٢**:٦٧٢-٢٧٧.

Schuchat, A. ١٩٩٩. Group B *Streptococcus*. Lancet ٢. **٣٥٣(٩١٤٥)**:٥١-٦.

Schwart, B., A. Schucht, M.J. Oxtoby, S.L. Cochi, A. Hightower and C.V. Broom. ١٩٩١. Invasive group B *Streptococcal* disease in adults: a Population based study in metropolitan Atlanta. JAMJ. **٢٦٦**:١١١٢-١١١٤.

Severin, M.J. and J.L. Wiley. ١٩٧٦. Change in susceptibility of Group B *Streptococci* to penicillin from ١٩٦٨ through ١٩٧٥. *Antimicob. Agents Chemother.* **١٠**:٣٨٠-٣٨١.

Sharat, K. ٢٠٠٤. Group B *Streptococcus* infection. Medicine. **١٦(٣)**:١٤٢٥.

Silverman, N.S., M. Morgan and W.S. Nichols. ٢٠٠٠. Antibiotic resistance patterns of Group B *Streptococcus* in antenatal genital cultures. *J. Reprod. Med.* **٤٥(١٢)**:٩٧٩-٨٢.

Smyth, C.J. ١٩٨٢. Two mannose resistant hemagglutinins on enterotoxigenic *Escherichia coli* of serotype o ٦:kis:Hi ٦ or H- isolated from traveler's and infantile diarrhaea. *J. Gene. Microbiol.* **١٢٨**: ٢٠٨١-٢٠٩٦.

Sobel, J.D. ١٩٩٦. Vaginitis and vaginal flora: controversies abound. *Curr. Opin. Infect. Dis.* **٩**: ٤٢-٤٧.

Sobel, J.D. and W. Chaim. ١٩٩٦. Vaginal microbiology of women with acute recurrent vulvovaginal candidasis. *J. Clin. Microbol.* **٣٤**: ٢٤٩٧-٢٤٩٩.

Soledad, B., E. Juan, V. Fernando and B. Covadogan. ١٩٩٨. Adherence of Human vaginal *Lactobacilli* to Vaginal Epithelial cells and interaction with Uropathogens. *Infect Immun.* **٦٦(٥)**:١٩٨٥-١٩٨٩.

Sparks, J.M. ١٩٩١. Vaginitis. *J. Reprod Med.* **٣٦**:٧٤٥-٧٥٢.

Stoll, B.J., T. Gordon, S.B. Korones, S. Shankaran, J.E. Tyson and C.R. Bauer. ١٩٩٦. Late-onset sepsis in very low birth weight neonates : a report from National Institute of Child Health and Human Development Neonatal Research Network. *J. Pediatr.* **١٢٩**:٦٣-٧١.

Tagg, J.R. ١٩٩٢. BLIS production in the genus *Streptococcus*, Bacteriocin, Microcins and Antibiotics, eds RJC. Lazdunski, F. Pattus. ٤١٧-٢٠.

Tagg, J.R., A.S. Dajani and L.W. Wannamaker. ١٩٧٦. Bacteriocins of Gram-positive bacteria, *Bacteriol. Rev.* **٤٠**:٧٢٢-٥٦.

Takeyama, K., Y. Kunishima, M. Matsukawa, S. Takahashi, T. Hirose, N. Kobayashi, I. Kobayashi and T. Tsukamoto. ٢٠٠٢. Multidrug-resistant *Pseudomonas aeruginosa* isolated from urine of patients with urinary tract infection. *J. Infect. Chemother.* **٨**:٥٩-٦٣.

Talati, A.J., D.T. Crouse, B.K. English, C. Newman, L. Harrison and E. Meals. ٢٠٠١. Immunomodulation by exogenous surfactant : effect on TNF-alpha secretion and luminol-enhanced chemiluminescence activity by murin macrophage stimulated with group B *Streptococci*. *Microbes Infect.* ٣:٢٦٧-٢٧٣.

Tamura, G.S. and C.E. Rubens. ١٩٩٤. Pathogenesis of group B *Streptococcal* infections. *Curr. Opin Infect Dis.* ٧:٣١٧-٣٢٢.

Tamura, G.S., J.M. Kuypers, S. Smith, H. Raff and C.E. Rubens. ١٩٩٤. Adherence of Group B *Streptococci* to cultured epithelial cells: roles of environmental factors and bacterial surface components. *Infect. Immun.* ٦٢:٢٤٥٠-٢٤٥٨.

Tapsall, J.W. and E.A. Phillips. ١٩٩١. The hemolytic and cytolytic activity of Group B *Streptococcal* hemolysin and its possibal role in early onset Group B *Streptococcal* disease. *Pathology* ٢٣:١٣٩-١٤٤.

Touati, D. and M. Jacques. ١٩٩٥. Lethal oxidative damage and mutagensis are generated by iron in Dfur mutants of *Escherichia coli;* protective role of superoxide dismutase. *J Bacteriol.* ١٧٧:١٣٠٥-١٤.

Tsaihong, J. and D. Wennerstrom. ١٩٨٣. Effect of carrier molecules on production and properties of extracellular hemolysin produced by *Streptococcus agalactiae. Curr. Microbiol.* ٩:٣٣-٣٨.

Turnidge, J.D., J.M. Bell and the SENTRY Asia-Pacific Group. ٢٠٠٣. High Rates and Macrolide Resistance and Emergining

Quinolone Resistance in *Streptococcus agalactiae* in the Asia-Pacific Region. Women's and Children's Hospital.٦١-٨.

Tyrrell, G.J., L.D. Senzilet, J.S. Spika, D.A. Kertesz, M. Alagaratnam, M. Lovgren and J. A. Talbot. ٢٠٠٠. Invasive disease due to group B *Streptococcal* infection in adults: results from Canadian, population based, active laboratory surveillance study. *J. Infect. Dis.* **١٨٢**:١٦٨-١٧٣.

Uh, Y., I.H. Jang, G.Y. Hwang, K.J. Yoon and W. Song. ٢٠٠١. Emerging erythromycin resistance among group B *Streptococci* in Korea. *Eur. J. Clin. Microbiol. Infect. Dis.* ٢٠:٥٢-٥٤.

Valvano, M.A., R.P. Silver and J.H. Crose. ١٩٨٦. Occurrence of chromosome or plasmid mediated aerobacter in iron transport invasive strain of *Escherichia coli* KI. *Infect. Immuno.* **٥٣(١)**:١٩٢-١٩٩.

von Wintzingerode, F., U.B. Gobel and E. Stackebrandt. ١٩٩٩. Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. *FEMS Microbiol. Rev.* **٢١**:٢١٣-٢٢٩.

Waite, D.C., E.J. Alper and B.J. Mady. ١٩٩٦. Adult Group B *Streptococcal* Disease. *Ann Intern Med.* **١٢٥**:١٥٢.

Weinberg, E.D. ١٩٩٨. Patho-ecological implication of microbial acquisition of host iron. *Review in Medical Microbiology*. **٩**:١٧١-٨.

Weisman, L.E., B.J. Stoll, D.F. Cruess, R.T. Hall, G.B. Merenstein, V.G. Hemming and G.W. Fischer. ١٩٩٢. Early-onset group B *Streptococcal* sepsis: acurrent assessment. *J. Pediater.* ١٢١:٤٢٨-٤٣٣.

Wessels, M.R., V. Pozsgay, D.L. Kasper and H.J. Jenning. ١٩٨٧. Structure and immunochemistry of an oligosaccharide repeating unite of the capsular polysaccharide of type III group B *Streptococci.* Arevised structure for the type III group B *Streptococcal* polysaccharide antigen. *J. Biol. Chem.* ٢٦٢:٨٢٦٢-٨٢٦٧.

Wessels, M.R., C.E. Rubens, D.L. Kasper et.al. ١٩٨٩. Definition of bacterial virulence factor: Sialylation of GBS capsule, Proc Nalt Acad Sei USA. ٨٦: ٨٩٨٣-٧.

Wessels, M.R., J.L. Difabio, V.J. Benedi, D.L. Kasper. F. Michon, J.R. Brisson, J. Jelinkova and H.J. Jennings. ١٩٩١. Structural determination and immunochemical characterization of the type V group B *Streptococci* capsular polysaccharide. *J. Biol. Chem.* ٢٦٦:٦٧١٤-٦٧١٩.

Zangwill, K.M., A. Schuchat and J.D. Wenger. ١٩٩٢. Group B *Streptococcal* disease in United States, ١٩٩٠: report from a multistate active surveillance system. CDC Surveillance Summaries. *Morbial. Mortal. Weekly Rep.* ٤١:٢٥-٣٢.

Zhu, Y.Z., Y.H. Yang and X.L. Zhang. ١٩٩٦. Vaginal colonization of group B *Streptococcus.* A study in ٢٦٧ cases of factory women.

Chung. Hua. Liu. Hsing. Ping. Hsuen. Tsa. Chinh. **١٧(١)**:١٧-٩.

We, the examining committee, certify that we have read this thesis and have examined the student in its content and that in our opinion; it is adequate as a thesis for the Degree of Master in Medical Microbiology

Signature:
Name: Dr. Mohammed Shamkhi Jaber
Professor
Chairman

Signature:
Name: Dr. Emad Mohammed Rashed
Assistant Professor
Member

Signature:
Name: Dr. Layla Abdul Amer
Assistant Professor
Member

Approved for the college Committee of graduate studies

Signature:
Name: *Dr. Hussein S. AL-Janabi*
Dean
Babylon University
College of Medicine

We certify that this thesis was prepared under my supervision at the Collage of Medicine, University of Babylon, as a partial requirement for the Degree of Master in Medical Microbiology.

Signature.....

Name: Mohammed Sabri

Assistant Professor of Microbiology

Supervisor

In view of the available recommendation. I put forward this thesis for debate by the examining committee.

Signature.....

Name: Mohammed Sabri

Assistant Professor of Microbiology

Head of Department of Microbiology