An-Najah National University

Faculty of Graduate Studies

PREVALENCE AND MOLECULAR CHARACTERIZATION OF GROUP B STREPTOCOCCUS (GBS) FROM NABLUS AREA

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Dedication

I GRATEFULLY DEDICATE THIS WORK TO:

The Soul of my grandmother and my aunt

My parents Mr. and Mrs. Awwad

My husband Mr. Qawareeq

My sisters and brothers

Thank you for bringing me up to be where I am today

Thank you for your support and ambition. I adore you

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I wish to extend my appreciation to the following:

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Declaration

I, Alaa Ahmad Awwad, declare that submitted the thesis entitled:

PREVALENCE AND MOLECULAR CHARACTERIZATION OF GROUP B STREPTOCOCCUS (GBS) FROM NABLUS AREA

Unless otherwise referenced, I declare that the work provided in this thesis is the researcher's work, and that hasn't been submitted elsewhere for any other degree or qualification.

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Date: **19/09/2023**

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Abstract

Background: Group B streptococci (GBS) are gram-positive bacteria, normally colonize human's genital and gastrointestinal tract asymptomatically, they colonize 18% of women worldwide, they could be transmitted to 50% of newborns from their colonized mothers causing invasive diseases.

Objectives: The objectives of this study are to find out GBS prevalence in Nablus, Palestine, the serotypes present, the antibiotics susceptibility and antibiotics resistance genes present on isolated GBS.

Methodology: It is a descriptive cross-sectional study, conducted from September 2022 to January 2023 at Rafidia Governmental Surgical Hospital and Al-Itihad Hospital, a convenient sampling technique was used to study 184 pregnant women with gestational age of 33 weeks and above. Descriptive data of participants was collected using questionnaires. A total of 184 vaginal swabs were taken from participants. Swabs were cultured on Uriselect 4 chrom media, bale blue colonies were confirmed by CAMP test and by PCR. Susceptibility pattern was performed. Antibiotic resistance genes and capsular polysaccharide antigens were also tested using PCR.

Results: GBS prevalence was 11.4%, a significant association had been found between GBS colonization and residency in city (P-value: 0.014). 100% of GBS isolates were sensitive to penicillin, ampicillin, amoxicillin, vancomycin, ceftriaxone, linezolid, cefepime, and ceftriaxone, 71.4% were resistant to tetracycline, 28.5% resistant to erythromycin, 23.8% resistance to clindamycin and 4.7% resistant to levofloxacin. ermB resistance gene presented in 19% of isolates, mefA presented in 4.7% of isolates and the majority 90% presented with the tetM gene. Serotype III accounted for 42.8% of isolates, serotype V 23.8%, 14.2% were serotype II, 9.5% were serotype Ib, 4.7% were serotype IV whereas 4.7% were non-typeable.

Conclusions: This study used combined methodology comparable to the international CLSI guidelines to confirm GBS isolates, serotypes, and the antibiotics profile. GBS isolates accounted for 11.4% of screened pregnant, GBS colonization was significantly associated with living in city (P-value 0.014), 100% of isolates were sensitive to penicillin, and 71.4% were resistant to tetracycline. Most isolates possessed the tetM resistance gene. Serotype III was the predominant (42.8%).

Keywords: GBS, Streptococcus agalactiae, serotypes, antibiotics resistance genes

Chapter One

Introduction and Theoretical Background

1.1 Overview

Streptococci, are the family of gram-positive bacteria recognized by the end of the 19th century, several diseases were recognized anciently due to *Streptococci* bacteria like; puerperal sepsis, scarlet fever, and erysipelas (1), by 1879, Pasteur reported streptococcus as a cause of puerperal sepsis (2), *Streptococci* started to be classified by 1903 according to their hemolysis pattern on blood agar, in 1919, Brown established a classification system according to the bacterial hemolysis pattern on into beta group (complete hemolysis), alpha group (partial hemolysis) and gamma (no-hemolysis), then by 1930s, lady Lancefield classified *streptococci* serologically according to the polysaccharides they possess on their cell surface (1). Which classified streptococci according to the results of the precipitin test which depends on the carbohydrate C substance commonly present in most hemolytic streptococci, and it serologically specifies and verifies streptococci into groups according to the chemical variation of the carbohydrate C among groups. The results of this test classified bacterial groups into 5 distinct groups, classification appears to possess a strong relationship between bacterial groups and their origin; most group A members were of human origin, group B where of animal origin mostly causing mastitis, C group was from lower animal sources, group D was from cheese products origin and group E were from certified milk origin (3).

Group B *streptococcus* (GBS) which is represented by the species *streptococcus agalactiae* encapsulated (4) facultative anaerobic, opportunistic gram-positive bacterium (5), catalase-negative, diplococci, forming small colorless colonies on blood agar (6) nearly 3-4 mm in diameter (7), CAMP test positive, bacitracin resistant, hydrolyzes sodium hippurate (8). By 1887, Nocard and Mollrereau identified *Streptococcus nocardi* renamed later to *Streptococcus agalactiae* as a cause of bovine mastitis (9). In 1937, GBS was isolated from women's vaginal samples and the first human post-partum fatal GBS infection cases were reported (10). By the 1960s, it started to be isolated and considered a maternal cause of infection (11), and by the 1970s, it started to be considered a major cause of neonatal meningitis (12). It normally colonizes human lower gastrointestinal tract and genital tract without causing harm

(13), 18% of women worldwide are suspected to be colonized with GBS asymptomatically however, it is considered the major risk factor for transmission of infection to their neonates and development of neonatal invasive disease (6).

1.2 GBS characteristics and its serotypes

GBS was first classified by Lancefield using two defined cell wall carbohydrate antigens: first, is the group B-specific carbohydrate antigen (GBC) which is commonly present in all strains of GBS and the second is the surface capsular polysaccharide which classifies GBS into sub-groups known as serotypes, currently 10 serotypes have been identified and classified according to their immunological reactivity into; Ia, Ib, II, III, IV, V, VI, VII, VIII and IX (6).

The complex GBC is structured of four oligosaccharides: galactose, rhamnose, glucitol, and N-acetyl glucosamine, and GBC has been found to be bound by phosphodiester bond to the cell peptidoglycan at the site of N-acetylmuramic acid (14). The capsular polysaccharide which divides GBS into serotypes is composed of three to four of only five sugars: galactose, N-acetyl neuraminic acid (sialic acid), glucose, N-acetyl glucosamine, and rhamnose (14). There are also the C proteins that aid in serotype identification, which are present in all Ib strains (14). Studying serotypes and their capsular components is very important since vaccine development depends on circulating serotypes (15).

1.3 Virulence factors of GBS

GBS possesses several virulence factors that aid in host cells evasion, colonization, adhesion to cells surface, and progression of infection into invasive disease, some of these mechanisms are listed below:

1. GBS capsular polysaccharide, the main virulence factor in GBS, is rich in sialic acid, which resembles that present in hosts' cells. The presence of sialic acid on the cell surface prevents activation of the alternative pathway of the complement system and so prevents phagocytation. From this point, it's clear how sialic acid acts as a virulence factor and explains the need for antibodies to mediate phagocyte-mediated uptake and killing by opsonization of GBS cells with antibodies (14).

2. Production of the enzyme C5a-ase by GBS, an enzyme belonging to class serine esterase, inactivates the complement C5a component which plays a role in neutrophils attraction and thus reduces neutrophils accumulation at the infection site mediating GBS virulence (6).

3. Presence of pili on the surface of the GBS capsule which facilitates the attachment of bacteria to host cells (6).

4. Production of beta-hemolysin toxin by GBS which results in the destruction and hemolysis of the host's RBCs (6).

5. The surface protein C facilitates GBS adhesion to women's vaginal cells (16).

1.4 Colonization and Epidemiology

GBS colonization refers to the isolation of GBS from a vaginal-, rectal, or peri-anal area of women by cultural method (15). Colonization in pregnant could be transient, persistent or it could be intermittent (17).

Usually, GBS colonizes the intestine, and the genital tract also occasionally the throat and urethra asymptomatically (18), however, it could progress to infection and invasive disease. GBS colonization rates vary among countries and within the same country and this could be attributed to several factors affecting the prevalence and GBS colonization results such as variation in sampling methodology; time of screening pregnant women whether at delivery or during pregnancy, sampling site if it was only vaginal or rectal and vaginal swabs, culture method (15); researchers found that the use of selective enrichment media for GBS gives better results (19).

In addition to the previous factors, some factors are considered risk factors for the higher rate of GBS colonization, including ethnicity (African Americans having higher risk), obesity, poor hygiene, and multiple sexual partners (6). GBS colonization worldwide among women is estimated to be 18% with a regional variation of 15-40% (20).

There are 10 GBS serotypes (Ia, Ib, II – IX) identified till now. In addition to variation in the GBS colonization rate among countries, there is also variation in serotypes distribution rate even though some regions show a similar prevalence rate of GBS

colonization, the serotypes present there may vary significantly (21), serotypes Ia, Ib, II, III, and V account for nearly 98% cases of colonized women worldwide (15) with regional variation, for example IV and V serotypes are the most prevalent in Egypt and UAE whereas serotypes VI and VIII were the predominant serotypes present in Japan (21).

1.5 Transmission and Pathophysiology of GBS in pregnant women

GBS could asymptomatically colonize pregnant women without causing harm, however, asymptomatic colonization could progress into infection because of the bacterial virulence properties and its ability to bind and adsorb to placental chorionic villi (22). Maternal colonization of GBS is considered a clinical source of concern because upstream ascending infection may occur, causing serious disease in pregnant or postpartum women like bacteriuria, cellulitis, amnionitis, fasciitis, wound infection, and endometritis in cesarean section cases. Also, it could progress into invasive diseases like osteomyelitis, endocarditis, sepsis, and meningitis (21) which in turn is associated significantly with preterm birth and miscarriages (22). However, GBS should be commensal and asymptomatic, if colonized it could switch to be pathogenic and cause disease as mentioned earlier, this could be attributed to several factors like the presence of virulent clones among some strains making them more virulent than other strains like serotype III which possess the phylogenetic lineage clonal complex 17 (CC17), a hyper-virulent clone of serotype III and this is why it seems that serotype III is linked to invasive cases more than other GBS serotypes (15), also the transition of GBS from the acidic medium in the vagina to the neutral blood medium has an effect in switching on the virulence genes and so promoting the progression of infections to be invasive, most virulence genes in GBS are regulated by the Cov-RS two-component regulatory system (TCS) which is pH dependent meaning, bacterial translocation to the neutral environment will switch on the virulence factors it regulates (21).

Transmission of infection could be mediated by several routes: vertical transmission from mother to infant which is the primary route, fecal-oral route within the family members, sexual transmission, or nosocomial infection (23).

1.6 Pathogenesis of GBS in Newborn

As mentioned earlier in the study, by the 1970s, GBS started to be considered a major pathogen for neonatal meningitis (12), now GBS is considered the most common cause of neonatal sepsis and meningitis (24) and is known to cause two distinct syndromes and are classified according to newborn age into; Early-Onset Disease (EOD), a condition occurs within the first 7 days of life and Late-Onset Disease (LOD), a condition occurs between the 8^{th} day and 90^{th} day of life (23), in Palestine, according to an unpublished article *streptococcus* species accounts for 7.9% of newborn admitted to NICU due to sepsis according to positive blood culture result of newborn aged between 0-28 days of life. Infection could be transmitted vertically to newborns from their mothers during labor; if the infection spread systematically it could result in EOD, especially within the first 72hrs of birth or a few hours before delivery, if infant aspirates from the infected amniotic fluid of his mother, and this is possible as 5% of positive GBS women possessed GBS DNA in their placenta (25) or from mothers' vaginal secretions during delivery, if aspirated GBS reached pulmonary epithelial cells and vessels it could progress to pneumonia if the inflammation of lung tissue occurs and becomes systematically. It destroys the alveolar lining with its hemolysin toxin resulting in sepsis and meningitis (6), and this is affected also by the inoculum; (the number of organisms transmitted to infants). Infants of heavily colonized women are more susceptible to infection, moreover, infants who catch a larger number of organisms are more likely to develop invasive EOD disease (23). GBS infection is suspected to be transmitted to 50 % of infants of colonized mothers, however, only 1-2 % of infected infants are susceptible to developing invasive EOD (26), and the rest 98% remain healthy (27).

It is estimated that nearly 0.3-0.6 infants per 1000 are suspected to develop EOD due to GBS infection (28), most newborns with EOD present with respiratory distress syndrome and bacteremia, which are the most common features of EOD (6) with meningitis being less frequent, nearly 5% of neonates (17). Nearly 10% of infected neonates who develop EOD die due to infection and 20% survive with pregnancy disability (28).

Till now, the LOD mode of transmission is still not understood clearly, however, intestinal colonization is a risk factor for developing LOD as LOD involves the

transmission of bacteria from epithelial cells to the bloodstream progressing to systematic infection (23). Nosocomial infection and transmission via breastfeeding could be also routes of transmission for developing LOD (23). Bacteremia and meningitis are the most common features of LOD (6). Maternal GBS colonization is considered the main risk factor for developing LOD (29). Serotype III is responsible for 40-60% of EOD and 60-80% of LOD and this is why it seems to be associated with meningitis (28).

1.7 Diagnosis

The golden standard for evaluating and diagnosis of GBS infection is by isolation of GBS from body sites (6).

However, there is not an established method for screening maternal GBS colonization, the Centers for Disease Control and Prevention (CDC) had recommended a method for screening GBS colonization in pregnant women through culturing vaginal-rectal swabs from pregnant women at 35^{th}-37^{th} gestational weeks using selective enrichment media (30),however, this protocol is not effective for preterm labor, and according to Japan Society of Obstetrics and Gynecology (JSOG), screening could be conducted at 33-37 weeks of gestation (31), in France its recommended to perform the GBS screening test between 34-38 weeks of gestation (32).

In 2019, CDC transferred the management system of GBS lab testing to the American Society of Microbiology and the American College of Obstetricians and Gynecologists (ACOG) which then established and recommended gestational age for GBS detection from the week 36 0/7 to 37 6/7 of gestation, shifting the previous GBS detection age one week because 7% of USA pregnant deliver at the 41^{st} week and so the test still valid, the validity of GBS culture method is 5 weeks according to (ACOG); meaning that if the pregnant delivered more than 5 weeks of negative GBS screening test doesn't rule out a new infection (22, 33) negative GBS culture result may indicate negative results for 5 weeks with a predictive value of 95%-98%, if delivery happened 5 weeks later after screening the predictive value will decrease (33).

Vaginal swabs could be cultured on an enrichment selective media for GBS, here are two types of selective GBS media; the aerobic CHROM agar StrepB media which provides 87.7% detection sensitivity (34), and the anaerobic Granada media (26) also

has a comparable sensitivity 89.2% (35), these media decrease turnaround time in GBS detection because GBS could be directly identified due to high selectivity without need for confirmatory tests (34). Also, general media like blood agar could be used followed by confirmatory tests to confirm GBS (36).

Researchers found that using selective media has shown better results, however, false negative results are also possible due to the growth of the competitive *enterococcus faecalis* which is also present in the vagina and suppresses the growth of GBS (7). Selective broth media like Todd-Hewitt broth supplemented with gentamicin (8μg/ml) and nalidixic acid (15μg/ml) has a good impact on GBS growth and provide better results, then samples should be sub-cultured into 5% blood agar or chromogenic media and incubated for 24hrs to visualize B-hemolysis or colored colonies, suspected GBS colonies are further confirmed by biochemical tests available commercially like: latex agglutination tests, genetic detection of capsular polysaccharides, or through CAMP test for presumptive identification to avoid false positive results, then susceptibility test for antibiotics should be performed according to published CLSI reference method for confirmed positive GBS samples (33).

The golden method for screening GBS is by recto-vaginal swab culture followed by conformation methods such as biochemical profiling, latex agglutination, or direct antigen detection(17). Moreover, different techniques have been used to decrease turn-around time to enhance detection specificity and sensitivity such as matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF), in situ fluorescence hybridization, real-time PCR assay, rapid PCR methods are also being developed that doesn't depend on harvested colonies, saving time and providing reliable results however they are very expensive (7).

Nucleic acid-based methods like PCR for the detection of GBS is a very important tool not only in GBS identification but also in the detection of small quantities of bacteria due to high sensitivity and specificity, also in the detection of virulence genes, and antibiotics resistance genes (37).

1.8 Prevention of GBS infection

1. Screening pregnant women for GBS colonization (19).
2. Intrapartum Antibiotics Prophylaxis (IAP): In 1997, it was adopted as a preventive mechanism for invasive GBS in neonates for at-risk pregnant women of being colonizers of GBS or GBS-positive culture (12). Accordingly, antibiotics would be administered to pregnant women who were colonized with GBS at the period extended from 35-37 weeks of gestation. Intravenous penicillin G is the drug of choice used at least 4 hours before delivery (6), administration of IAP has decreased EOD infection incidence significantly by 50-80% (38). However, IAP has limitation because around 61% of infants who develops EOD, their mothers were negative for GBS colonization at the screening time and this suggests that infection could occur at the time interval between screening time and delivery, or due to false negative result for GBS at lab department (25). Therefore, IAP is becoming a source of concern due to the spread of antibiotic resistance GBS strains due to IAP (39).
3. Vaccination: Vaccines are being developed to protect from invasive GBS using the serotypes' capsular polysaccharides as vaccine antigenic epitopes, and it's thought to reduce invasive disease due to GBS by 30-54%, by reducing colonization in pregnant women and reducing neonatal infection, so the target is to immunize pregnant women. Nowadays, the developing vaccines target 6 serotypes out of 10 which are the most spread (18). Immunization also seems to have a positive effect in reducing resistance due to the widespread use of IAP in addition to reducing the need for GBS screening (25). The presence of IgG antibodies against capsular GBS antigens in mothers' blood would protect and reduce morbidity in their neonates as IgG antibodies would transfer to neonates through the placenta, therefore, enhancing neonates' immunity, and reducing morbidity (12). However, it is less effective in protecting preterm infants against invasive EOD due to the transmission of antibodies after week 34 of gestation (40).

However, these methods haven't decreased LOD incidence (25) or its clinical manifestations (32).

1.9 Treatment

1.9.1 Infection treatment

Generally, Penicillin G is the drug of choice for GBS infections. However, ampicillin is administered in combination with gentamycin in suspected sepsis due to GBS infections in neonates due to the better activity of both drugs together than using penicillin or ampicillin alone, if GBS infection is confirmed, penicillin alone is used for a minimum of 14 days (6). In general, GBS is susceptible to penicillin and ampicillin 100%, in addition to cephalosporin (first, second, and third generation) which could be used in treatment (41), clindamycin and erythromycin are used as a second choice of treatment for allergic patient to penicillin. Fluoroquinolone and vancomycin also have been used (42).

1.9.2 Preventive IAP dosage

According to the ACOG Committee Opinion, the protocol for IAP administration is; Penicillin G starting with 5 million units as a loading dose then 2.5-3million units every 4hrs, or ampicillin 2g IV as a loading dose followed by 1g every 4hrs until delivery (33).

For low-risk women with penicillin allergy, cefazolin 2g is given as a loading dose followed by 1g every 8 hours until delivery.

For high-risk women with penicillin allergy, women are given 900 mg IV clindamycin every 8hrs until delivery, and for women with resistance to clindamycin; vancomycin is given 20mg/Kg every 8hrs (17).

1.10 Risk factors for developing EOD

The primary risk factor for developing EOD is maternal colonization with GBS during delivery, there are also several factors considered as risk factors for developing EOD:

1. Prolonged rupture membrane during delivery of more than 12hrs.
2. Gestational age lower than 37 weeks.
3. Amniotic fluid infection.
4. Black race.
5. Maternal age less than 20 years of age.
6. Increased maternal temperature above 37.5^{o}C during delivery,

7. GBS bacteriuria which suggests heavy colonization.
8. Having a previous infant with EOD.

Women with these risk factors are at risk of birthing a child with EOD 6.5 times compared to those without risk factors (43).

1.11 Antibiotics resistance genes

In general GBS strains should be 100% susceptible to penicillin and ampicillin, however, resistance to both penicillin and ampicillin have been reported and erythromycin and clindamycin are used commonly as a second choice however, resistance has been also reported and mostly it's attributed to the presence of antimicrobial resistance genes (41).

Antimicrobial resistance is an important issue to be studied well from both microbiology and molecular points of view for optimal antibiotic administration, to monitor resistance spread, and to find out whether this resistance is due to resistance genes possessed by organisms (37).

Resistance to antibiotics can be mediated through several mechanisms: mutation in the antibiotic target on the microorganism and so reducing or preventing its' effect, newly evolved resistance mechanism that hasn't been recognized yet, in addition to the expression of resistance genes that are still not discovered (37).

1.11.1 Macrolides and Lincosamides

Macrolides (erythromycin), lincosamide (clindamycin), and streptogramin-B (MLSB) antibiotics are considered protein synthesis inhibitor agents in bacteria, resistance to MLS_B has been reported via several mechanisms the first one is by ribosomal methylation by adenine-N^6-methyltransferase enzyme which results in posttranscriptional modification at the site of 23S RNA altering the site where MLS_B antibiotics bind at the 50S large ribosomal subunit and results in broad-spectrum resistance to MLSB phenotypes and this is could be encoded by the *erm* (37) (erythromycin resistance methylase) genes: *erm*B and *erm*A (subclass of *erm*TR) (44), researchers showed that there is around 9.6%-15% of tested isolates have shown resistance to macrolides (45). This gene induces resistance to MLS_B antibiotics and classified into two types: the consecutive MLS_B ($cMLS_B$) phenotype meaning there is a

permanent resistance towards these antibiotics and the second is the inducible MLS_B ($iMLS_B$) phenotype shows resistance to erythromycin and sensitivity to clindamycin (46) and can be detected by the D-test only which is demonstrated by D shape formed around the clindamycin in proximal to erythromycin disk (decrease in inhibition area in proximal to erythromycin; blunting) (47).

The second way of antibiotic resistance to macrolides is by *mef* genes (macrolide efflux), *mef*A and *mef*E have been identified, *mef*A has been identified in GBS. GBS possessing the *mef*A gene display resistance toward macrolides only in a phenomenon called the M phenotype, this resistance is mediated through the efflux of antibiotics out of the cell and keeping antibiotics concentration low inside the cell and thus ribosomes still free of antibiotics binding (37). GBS with this phenotype exhibit resistance to erythromycin only with sensitivity to clindamycin.

Also, clindamycin resistance could be mediated through ribosomal translocation by neocletidyl-transpherase that is encoded by *lin*B genes in GBS bacteria resulting in L phenotype (48) (46) with sensitivity pattern to erythromycin.

Usually, the D-test is used to determine resistance phenotypes of MLS_B.

1.11.2 Tetracycline

Tetracycline antibiotic works by preventing bacterial protein synthesis.

Tetracycline resistance has been reported in several ways, one of them is by ribosomal protection by a protein that is encoded by the resistance genes, *tet*M and *tet*O which produce resistance proteins like *tet*M which binds to ribosome making it insensitive to tetracycline inhibition (49),(37).

1.12 Problem statement

In the city of Nablus, the prevalence of GBS was 12% (26) however; there was no information about the bacterial molecular characterization.

Here in our study, we studied the prevalence of GBS isolates and its serotypes present among the participating women as it is an important issue in the health of pregnant women and their neonates. The result of this study is important to shed light on the suitable techniques to deal with GBS infection in pregnant and to shed light on the importance of GBS screening in pregnant to prevent neonate infection. In addition, it searched the associated risk factors with GBS colonization, since there is not a screening program for GBS in Palestine, even more, there is a scarcity of information available about GBS and its serotypes present here.

Also, we tested antibiotic susceptibility to recommend the best one to be administered, and to avoid the administration of resistant antibiotics. And we focused on antibiotic-resistance genes that could mediate resistance in GBS strains.

1.13 Objectives and Aims

1.13.1 Main objective

The main objective of this study is to find out the group B streptococcus among pregnant women who arrived for delivery at Rafidia Governmental Surgical Hospital and AL-Itihad Hospital in the city of Nablus, Palestine in the period extended from September 2022 to January 2023, in addition to finding out the serotype distribution, antibiotic susceptibility pattern and antibiotic resistance genes present on the strains isolated. In addition, study the associated risk factors for GBS colonization.

1.13.2 Specific Objectives, to find out the

1. Prevalence of GBS among pregnant women at admission time to labor in Nablus city.
2. Associated risk factors for GBS colonization.
3. Serotype distribution among GBS colonized women.
4. Antibiotic resistance patterns and associated antibiotic resistance genes they possess on positive samples.

1.14 Significance of the study

Very few studies have been conducted in Palestine about group B *streptococcus* and its molecular characterization among pregnant women, however, none of them studied antibiotic resistance genes, so the importance of this study is to shed light on the importance of testing pregnant women for GBS colonization and though the importance of recommending screening pregnant women against GBS and its role in the prevention of newborn infection and subsequent invasive disease.

1.15 Literature Review

1.15.1 Prevalence of Group B streptococcus

19.7 million pregnant women globally are suspected to be colonized of GBS, by 2020 and the global deaths in children due to invasive GBS infection were 91900 cases (39), 10-25% of stillbirths are due to infections, and around 61% of GBS positive pregnant have either miscarriage or stillbirth this is why GBS is considered a life-threatening pathogen (50).

According to a worldwide systematic review in 2017, the worldwide prevalence was estimated to be 18% ranging from (13% and 11%) in Southern Asia and Eastern Asia respectively to 35%-40%% in The Caribbean, In China and Bangladesh prevalence was 11% (15). In the Middle East, GBS prevalence ranges from 3.3% in Iran -31.6% in Saudi Arabia (51), and according to the review of a cohort study in 2019, the prevalence of GBS colonization in the Middle East and North Africa region ranged from 1.6% in Israel - 32% in Turkey (29). A systematic review conducted in 2019, compared the prevalence of GBS between Islamic and non-Islamic countries, and it was 14% in Islamic countries whereas 16.3% in non-Islamic countries (52).

A Saudi cross-sectional study conducted in 2018 in Makkah found that GBS prevalence was 15% (53), and another study in 2013 found that GBS colonization was 13.4% (54). In 2022, a Yemini cross-sectional study revealed that 10.95% of pregnant at 35-39 weeks of gestational age were GBS colonized (55). A study in UAE included 563 women who found GBS prevalence was 10.1% in 2002 (56) and another study in 2021 in UAE included 2295 women who found the prevalence to be 6.9% (51). According to a systematic review in Iran, 13.65% of pregnant women are GBS colonized (57), and a cross-sectional study in Iran, in 2017, GBS prevalence was 11.8% (58) followed by another study in 2021, the prevalence of GBS was 11% (59). 18.4% of Lebanese pregnant were colonized with GBS according to a study conducted there (60). In Jordan, 2019, GBS prevalence was 19.5% (29). In Turkey in 2016, GBS prevalence was 9.8% (29). And in Kuwait, the prevalence of GBS colonization was 16.4% (61).

In Morocco in 2018, GBS prevalence was 24% (29). A cohort study in Egypt conducted in 2014 revealed a prevalence rate of GBS of 11.3% (27). In Ethiopia in 2015, GBS prevalence was 13.7% in a cross-sectional study conducted on 139 pregnant women

using vaginal swabs only (24) followed by another study on 413 participants, and revealed a prevalence of 30.6% in 2016 using recto-high and low vaginal swabs (48). Then in 2022, a systematic review in Ethiopia revealed a prevalence rate of 15% (20).

Prevalence of GBS-colonized pregnant women in Zimbabwe was 10.2% (41).

The prevalence of GBS colonization in Hong Kong was 10.4% (62). Whereas, in Brazil in 2020, was 17.2% (47). In Seri Lanka in 2023, the GBS colonization rate was 25.7% (63).

In a study conducted among Arab Israeli pregnant women in Nazareth in 2018, GBS prevalence was 31% (64). A study in South Israel, in 2003 revealed a GBS prevalence of 12.3% (65) and according to the national Israeli Ministry of Health, the overall prevalence of GBS colonization rate is 21.6% (66).

A pilot study conducted in Nablus, Palestine in 2017, revealed a GBS prevalence of 12% (26). Whereas, in the Gaza Strip, Palestine in 2017, it was 21% (67).

Also, another study conducted in Namibia revealed a GBS colonization rate of 5.7% (68).

1.15.2 Distribution of GBS serotypes in pregnant women

According to a worldwide systematic review in 2017 conducted 98% of global serotypes are Ia, Ib, II, III, and V (15).

In Jordan, serotype III was the most prevalent accounting for 48% of detected GBS strains, followed by serotype Ia 24% and II 20% (29).

In UAE, 26.3% of detected GBS strains belonged to the IV serotype, 21% Ia, 17% III, and 12.3% V whereas 15.8% were non-typeable (56). In Iran 2021, serotype Ib was predominant at 44.4%, serotype III was 40.7% and serotype II was at 11.1% (69). In a cross-sectional study in 2015 in Saudi Arabia, the predominant serotype was Ia accounting for 30% of isolates followed by III 25% and V also 25% (53).

Serotype III was predominant in China accounting for 54.9% followed by serotype Ib 17.3% and V 10.1% (70).

In Israel, a recent study in 2020 revealed that the predominant serotype in pregnant colonization was VI 40.8% followed by III 17.5%, V and IV 12.5%, and 11.7% (28).

In a study in Palestine conducted on isolated GBS strains from different sources not just pregnant women, serotype III was the predominant 35% followed by V serotype 19%, Ia 15%, and II 6% (71).

In Namibia, Ia was found in 50% of isolated GBS, Ib in 5.5% and the rest were non-typeable (68).

1.15.3 Antibiotics resistance genes

In a cross-sectional study in 2021 in Zimbabwe, 97.7% were resistant to tetracycline and this was attributed to the presence of *tet*M resistance gene in 97.7% of GBS strains and 2.4% had *tet*O gene, 30.2% were resistant to erythromycin and 34.5% have found to possess the *erm*B gene and 10.3% *erm*TR gene, and 3.4% *mef*A (41).

In an Ethiopian study, 55% of GBS isolates possessed the *erm*B gene, *mef*A also the *erm*TR gene were found in 3.4% of isolates, also 38% possessed both *erm*B and *lin*B genes (48).

In a cross-sectional study in Iran in 2021, 12 isolates were resistant to erythromycin however the gene *erm*B wasn't detected; 10 of them had the *erm*TR gene and 3 the *mef*A gene (69). A survey in Iran in 2016, conducted a resistance rate of 35% to erythromycin in which the *erm*B, *mef*A, and *erm*TR genes were detected in all resistance strains (46).

In Brazil in 2020, the *erm*B gene was detected in 44.4% of erythromycin and clindamycin resistant isolates, *mef*A also was found in 44.4% of resistance isolates to erythromycin and *erm*TR was found in 11.1% of resistance isolates whereas *lin*B was not detected (47).

In Seri Lanka 2023, the *erm*B gene was found in 15.5% of GBS isolates, *erm*TR in 35.6% of GBS isolates, *mef*A was found in 4.4% of GBS isolates, and *lin*B was not detected (63).In a study conducted in South Africa, 3.4% of isolates had the *mef*A gene and the *erm*B was found in 55% of isolates (48).

In a study in Namibia, the resistance gene, *tet*M was present in 88.9% of isolated strains (68).

In Palestine, there were not any previous studies that mentioned the antibiotic-resistance genes in GBS.

1.15.4 Antibiotic sensitivity

According to a meta-analysis review in 2021, 98.9% of GBS isolates were sensitive to penicillin, 98.2% sensitive to ampicillin, and 99.7% to vancomycin (52), In the systematic review compared between Islamic and non-Islamic countries about GBS prevalence, the resistance rate to tetracycline was 82.92% whereas 98.2%, 98.9% were sensitive to ampicillin and penicillin respectively (52).

In the Saudi cross-sectional study in 2015, GBS isolates were 100% sensitive to vancomycin, penicillin, and ampicillin whereas 15.7% and 5.1% were resistant to erythromycin and clindamycin respectively (54). In Yemini's study, 100% were sensitive to penicillin, ampicillin, vancomycin, levofloxacin, and cefotaxime however, 8.6% were resistant to clindamycin and 47.8% resistant to tetracycline (55). In an Iranian study in 2021, 44.4% and 29.6% of isolated GBS were resistant to erythromycin and clindamycin respectively (69).

In Zimbabwe in 2021 a study revealed 69.8% of isolates were resistant to penicillin, 55.8% were resistant to clindamycin and 58.1% were resistant to ampicillin (41). In Brazil in 2020, 100% of isolates were sensitive to penicillin, cefotaxime, vancomycin, and ampicillin with 18.8% resistance to clindamycin and 25% resistance to erythromycin and the D test was 100% negative (47). In Seri Lanka 2023, all detected GBS isolates were sensitive to penicillin, 24.4% were resistant to erythromycin, 13.3% exhibited intermediate sensitivity to erythromycin, also 22.2% of isolates were resistant to clindamycin, and 11.1% were intermediate in sensitivity (63).

In Namibia, a study revealed a 100% sensitivity pattern against penicillin, ampicillin, erythromycin, linezolid, clindamycin, vancomycin, chloramphenicol, and ceftriaxone whereas all isolates 100% were resistant to tetracycline (68).

In an Israeli study, 20.7% were resistant to erythromycin whereas 19.3% were resistant to clindamycin (28). In Ethiopia in 2015, 100% of GBS isolates were sensitive to

vancomycin, penicillin, ampicillin, erythromycin, and gentamycin (24), also followed by another study conducted 100% sensitivity to ampicillin, vancomycin, and penicillin however 17.2% and 21.1% were resistance to clindamycin and erythromycin respectively and 94.5% resistance to tetracycline (48).

In the Gaza Strip, only 57% were sensitive to penicillin, and 69% were resistant to tetracycline (67). In a Palestinian study, 91.7% of GBS isolates were resistant to ampicillin, 25% and 29.2% were resistant to clindamycin and erythromycin respectively and 8.3% were resistant to levofloxacin (26). In another Palestinian study, samples were of different sources not only pregnant, 26% of GBS isolates were resistant to clindamycin 16% were resistant to ampicillin, 19% resistant to ceftriaxone, 17% were resistant to cefotaxime and 11% were resistant to levofloxacin (71).

1.15.5 Risk factors for GBS infection

In the previous study conducted in Palestine, in 2020, preterm birth was significantly associated with GBS colonization, however, there was no association between GBS colonization and parity, UTI, vaginitis, smoking, age, or sociodemographic characteristics (26). A similar study conducted in Namibia found that there was no significant association between GBS colonization with obstetric findings or sociodemographic variables (68).

A study in Saudi Arabia revealed a significant relationship between parity and GBS colonization (54). Another study conducted in Australia showed a significant relationship between GBS colonization and spontaneous abortion (72).

A study held in Congo revealed a significant association between HIV-positive serum tests, abortion, premature childbirth, UTI, and low level of education with GBS infection (73). And there was an association between GBS infection and vaginitis (74). Also, a study in Iran revealed a significant relationship between GBS colonization with smoking (58).

Chapter Two

Methods

2.1 Study design

In this study, we performed a descriptive cross-sectional study in which participants were conveniently recruited to participate according to inclusion and exclusion criteria to measure the prevalence of GBS colonization among the selected group (75), in which descriptive data were gathered from participants using structured questionnaires, in which the questionnaire was structured into sections which asked about, socio-demographic characteristics, general health, infection-related variables, and obstetric related variables of participants. This study was based on a quantitative method as it involves the calculation of the prevalence rate of GBS.

This study was carried out at Rafedia Governmental Surgical Hospital (a governmental obstetric hospital in the city of Nablus, Palestine) and AL-Itihad Hospital (a private hospital in Nablus, Palestine) over five months extended from September 2022 to January 2023.

2.2 Consent forms and questionnaire

Approval from the institutional review board (IRB) (appendix E) has been obtained from the An-Najah National University committee, in addition to approval consent from the General Directorate of Health Education and Scientific Research in the Ministry of Health (appendix D) to be approved for collecting samples from hospitalized patients. Also, approval from Al-Itihad Hospital (appendix D) was obtained before collecting samples.

A written consent form (appendix A) has been obtained from each participant who has agreed to participate and a written questionnaire (appendix B) already prepared in Arabic language was filled by face-to-face interview. The questionnaire questions were divided into two parts, the first asked about demographic variables like education level, employment, living area, family income, and smoking, and the second asked about gestational variables related to general health and obstetric variables like covid-19 infection, and vaccination, maternal age, gestational age, antibiotic dedication within the last 2 weeks, presence of diabetes or chronic disease, body mass index, number of

labor, amniotic fluid status, vaginal infections, discharges, burning, number of times of urinate, number of miscarriages, stillbirth, children with birth defects and allergy to penicillin.

2.3 Study sample

Pregnant women in the late third trimester of their gestation were the target of our study because infection during early pregnancy is not a sign of developing EOD in their children.

2.4 Sample size

The sample size was calculated based on the prevalence of GBS colonization found in a study conducted by Qadi in which the prevalence was 12% (26).

The sample size was calculated using the following formula (76):

$$n = \left(\frac{(Z)^2 * P(1-P)}{(d)^2}\right) \qquad n = \left(\frac{1.96^2 * 0.12\ (1-0.12)}{(0.05)^2}\right) \qquad n = \frac{3.8416 * 0.12(0.88)}{0.0025} \tag{2.1}$$

$$n = 162 \text{ participants}$$

Where:

n = sample size

Z = value of 95% confidence interval (1.96)

P = previous prevalence of GBS colonization = 0.12

d = margin of error (5%) = 0.05

A 10% had been added as a non-response rate to the sample size (i.e. $\frac{10}{100} * 162 = 16.2$) a total of 178 participants (162+ 16.2) were recruited.

2.5 Inclusion criteria

Pregnant women with a gestational age of 33 weeks or more were asked to participate in the study. In our country there is no established protocol for screening pregnant women for GBS, however, we accepted participants with a gestational age of 33 weeks and above due to an increased rejection rate among pregnant to participate in the study, this may be because it seems like a sensitive issue in our community. However, it is accepted according to the guidelines of JSOG to screen pregnant women for GBS colonization by the beginning of week 33 of gestation (31).

Also according to the JSOG, screening could be conducted between 33-37 weeks (31) so our inclusion criteria are within the accepted range globally.

2.6 Exclusion criteria

Pregnant women who took antibiotic treatment through the previous two weeks of admission were excluded. Also, pregnant women with gestational age lower than 33 weeks were excluded from the study.

35 women were excluded due to antibiotic treatment and 26 women refused to participate in the study. Also, 19 women of gestational age lower than 33 weeks were excluded. The overall number of pregnant women who were included in the study was 184.

2.7 Collection of samples

A vaginal swab was obtained by a doctor from each participant without a speculum, in the lithotomy position (26), a cotton swab was inserted 2cm (33) into the lower vagina and rotating it around the vaginal wall then immediately placed in the Aimes transport gel media and identified with a code number identical to the code of the questionnaire, samples were stored at $4^{o}C$ till transport to the microbiology laboratory and cultured within 24hrs of collection (33), sampling technique was set as suitable to the recommendation of CDC organization.

In this study, a total of 184 pregnant were recruited with a gestational age range from 33 to 41 weeks.

2.8 Microbiology

Vaginal swabs were inoculated within 24hrs of collection on UriSelect 4 chromogenic, differential media and incubated 18-24hrs at 37^{o}C in ambient air, bale blue colonies were suggested for further confirmation using CAMP test and PCR assay method as these colonies were suspected to be GBS (77).

UriSelect 4 agar media is a recently developed deferential, non-selective, chromogenic medium based on the detection of the two products B-glucosidase and B-galactosidase, it's used usually for the detection of urinary tract infection pathogens the sensitivity of this media in detection microorganisms is 98.3% (77),(78).

The cervical and speculum were not used for culture collection (33).

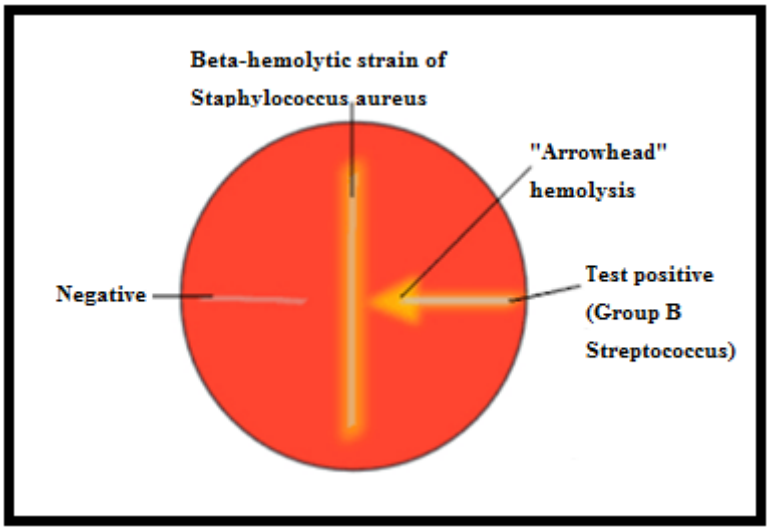
2.9 CAMP test

CAMP test: is a test used for presumptive confirmation of GBS, nearly all GBS strains produce the cytolytic toxin, *cfb* gene encodes the CAMP factor (Christie, Atkins, Munch-Petersen) (79), this toxin is unable to lys sheep RBCs by itself but it could lys RBCs in the presence of sphingomyelinase (a staphylococcal B-lysin toxin) enzyme produced by *staphylococcus aureus* in a synergistic interaction form. This test is performed through streaking *staphylococcus aureus* perpendicular to the suspected GBS on a 5% sheep blood agar plate, then incubated at 37^{o}C for 24 hours. Positive results appear as a clear zone of hemolysis resembling an arrowhead shape adjacent to the proximity zone of the two bacterial lines (7).

In this study, the CAMP test has been used to confirm suspected GBS isolates, by streaking a line of suspected bacteria perpendicularly to a line of *staphylococcus aureus* on blood agar and stored overnight at 37^{o}C at ambient air.

Figure 1

Illustration figure of CAMP test



Note. Hanson A. CAMP Test Protocols. American Society for Microbiology. Monday, 09 October 2006.

2.10 Preservation of GBS confirmed by CAMP test

Samples confirmed as positive GBS after conformation by bale blue colored colonies and positive CAMP test were sub-cultured on blood agar media to obtain pure colonies and then were preserved in LB (Luria-Bertani) broth with 30% glycerol and stored in 2 ml cryogenic vials at −80°C for further molecular testing according to manufacturer instructions (80),(81).

2.11 Antibiotic susceptibility test

Confirmed positive GBS samples by CAMP test were tested for their susceptibility pattern against the following antibiotics: ampicillin 6 (10µg), penicillin 10 units, amoxicillin /clavulanic acid, clindamycin (2µg), erythromycin (15µg), tetracycline (30µg), cefepime (30µg), cefotaxime (30µg), ceftriaxone, vancomycin (30µg), levofloxacin 5ug and linezolid, using disk diffusion method on Mueller Hinton agar according to the Clinical and Laboratory Standards Institute (CLSI) M100, Kirby Bauer method, 3-4 colonies of confirmed positive GBS were suspended in 3-5 ml of sterile normal saline and adjusted to prepare 0.5 McFarland's standard then antibiotic disks were added and incubated at 35^{o}C-37^{o}C for 18-24hrs and read for susceptibility (82).

Results were classified according to the size of the clear zone formed around each of the antibiotic disks into sensitive (S), intermediate (I), and resistant (R).

2.12 D- Test

The D-test in this study was performed to identify erythromycin and clindamycin resistance phenotype, it was performed for all positive GBS isolates according to (CLSI) M100, Kirby Bauer method, erythromycin (15µg) and clindamycin (2µg) were placed apart 12 mm on Mueller Hinton agar enriched with 3% blood, incubated for 24hrs at 37^{o}C with 5% CO_2 (47, 48).

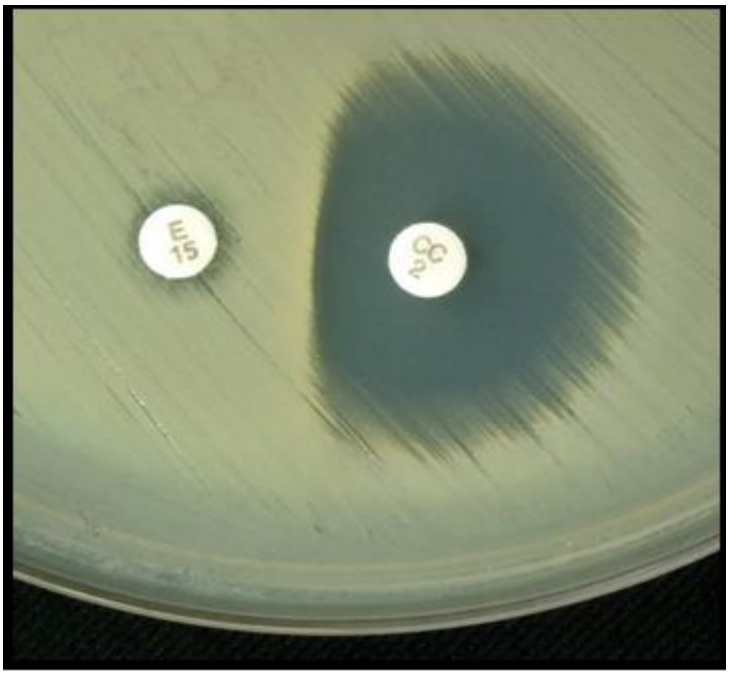
Resistance to both clindamycin and erythromycin was an indicator of cMLSB phenotype.

Whereas the growth of bacteria within the inhibition area of clindamycin proximal to the side of erythromycin (blunting) indicates iMLSB phenotype (48), indicating a positive D-test.

Resistance to erythromycin only with sensitivity to clindamycin indicates M phenotype, resistance to clindamycin only with sensitivity to erythromycin indicates L phenotype (83), also sensitivity pattern to both clindamycin and erythromycin, indicates negative D-test.

Figure 2

Illustration figure of D-test



Note. Tankeshwar A. Inducible Clindamycin Resistance (D Test). Microbe online. 2022;Bacteriology.

2.13 DNA extraction

Confirmed positive GBS isolates were cultured on blood agar overnight and harvested for DNA extraction for further presumptive identification of GBS using molecular PCR assay method also for serotype categorizing and detection of antibiotics resistance genes they possess.

Bacterial DNA was extracted using the boiling method; a few colonies cultured on 5% human blood agar were emulsified in 1000 µL of 0.9 normal saline using sterile plastic loops in an Eppendorf tube. Each tube was labeled according to its isolate sample label, and then these tubes were centrifuged for 5 min at 13000 rpm at 18^{o}C. The supernatant was discarded and the sediment was kept (84), 200 µL of distilled water was added and boiled for 10-15 min in a water bath (85), then centrifuged for 10min at 10000 rpm at 18.8^{o}C (68). The supernatant here contained the bacterial DNA, and then we separated it into 2 aliquots (41) and labeled them according to sample codes.

2.14 Conformation of positive CAMP-GBS isolates by PCR

In this study, the PCR assay method was used for presumptive confirmation of positive CAMP GBS isolates using the *atr* gene which is a housekeeping gene, expressed in all GBS isolates, it is GBS specific and encodes a gs0538 transporter amino-acid protein: 'CAACGATTCTCTCAGCTTTGTTAA' as the forward primer (F) and 'TAA GAA ATC TCT TGT GCG GAT TTC' as the reverse primer (R) (85).

The PCR reaction mixture was set of a total volume of 20 µL; 2 µL of sample DNA, 0.5 µL of forward primer, 0.5 µL of reverse primer, 10 µL of HY Taq Ready mix (hylabs), and 7 µL of sterile nuclease-free water. This solution mixture was prepared in a total volume of 378 µL, the solution was mixed gently and dispended into 21 PCR tubes in which 18 µL aliquots of the solution were transferred into each of the 21 tubes. Then 2µL of DNA sample was added to each PCR tube and labeled as suitable. Then the tubes were loaded in the thermal cycler (Prime).

The PCR amplification thermal conditions were set as follows: initial denaturation at 94°C for 3 min followed by 35 cycles at 94 °C for 30sec for denaturation, annealing temperature 50^{o}C for 3 seconds, extension at 72°C for 1min, and the final elongation at 72°C for 10 min (86).

1.5% agarose gel was prepared by adding 1.5gm of agarose into 100ml of 1X TAE (Tris-Acetate EDTA) buffer, the mixture was heated by microwave until dissolved completely, then the gel was set to cool down and 50 µL of ethidium bromide dye was added and mixed gently. Then the gel was poured into a cast tray and set until solidified.

The amplicons then were separated by electrophoresis techniques by adding 7.5 µL samples of PCR reaction mixture to the wells of 1.5% agarose gel. 6 µL of a 100-base pair DNA ladder was included to compare the DNA bands size of samples in proximity to the similar size of the ladder bands which works as a reference for DNA samples (87).

The gel was run for 1hr at 90 volts, then the gel was visualized under ultraviolet light and the amplicon size was determined using standard DNA molecular weight. The conformation for GBS by PCR targeting 780bp amplicon size corresponds to the *atr* gene (88).

2.15 The PCR amplification thermal conditions for serotypes identification

Confirmed positive GBS isolates by PCR performed in the previous step were further tested for identification of their serotype according to the capsular polysaccharide they possess on their cell wall.

Capsular polysaccharide genotyping was performed using pairs of primers listed in the table below using multiplex PCR assay for detection of GBS serotypes: Ia, Ib, II-IIIV. We tested GBS isolates against 9 serotypes out of ten.

Table 1

PCR primers used for the detection of GBS serotypes

Primer name	Primer sequence (5′-3′)	Amplicon size
Ia-F	5′-GGTCAGACTGGATTAATGGTATGC – 3′	1826 &521
Ia-R(89)	5′- GTAGAAATAGCCTATATACGTTGAATGC-3′	
Ib-F	5′-TAAACGAGAATGGAATATCACAAACC-3′	770
Ib-R(89)	5′-GAATTAACTTCAATCCCTAAACAATATCG-3′	
II-F	5′-GCTTCAGTAAGTATTGTAAGACGATAG-3′	397
II-R (89)	5′-TTCTCTAGGAAATCAAATAATTCTATAGGG-3′	
III-F	5'-CGTTATTATGTTACACGCTC-3'	281
III-R (90)	5'-CAAGTATGCGATTATCTTCC-3'	
IV-F	5′ – GGTGGTAATCCTAAGAGTGAACTGT – 3′	579
IV-R (89)	5′ – CCTCCCCAATTTCGTCCATAATGGT– 3′	
V-F	5′ – GAGGCCAATCAGTTGCACGTAA – 3′	701
V-R (89)	5′ – AACCTTCTCCTTCACACTAATCCT – 3′	
VI-F	5′ – GGACTTGAGATGGCAGAAGGTGAA – 3′	487
VI-R (89)	5′ – CTGTCGGACTATCCTGATGAATCTC3′	
VII-F	5′ – CCTGGAGAGAACAATGTCCAGAT3′	371
VII-R (89)	5′ – GCTGGTCGTGATTTCTACACA – 3′	
VIII-F	5′ – AGGTCAACCACTATATAGCGA – 3′	282
VIII-R (89)	5′–TCTTCAAATTCCGCTGACTT – 3′	

Note. F: forward primer, R*: reverse primer.

The PCR reaction mixture was set at a total volume of 20 µL; 0.25 µL of each forward primer, 0.25 µL of each reverse primer, 10 µL of HY Taq Ready mix (hylabs), 2 µL of sample DNA and sterile nuclease-free water until preparation of 20 µL as a total volume. This solution mixture was prepared in a total volume of 378 µL, the solution was mixed gently and dispended into 21 PCR tubes in which 18 µL aliquots of the

solution were transferred into each of the 21 tubes. Then 2µL of DNA sample was added to each PCR tube and labeled as suitable. Then the tubes were loaded in the thermal cycler (Prime).

The PCR amplification thermal conditions were set as follows: initial denaturation at 95°C for 5 min followed by 35 cycles at 94 °C for 60sec for denaturation, annealing temperature 58^{o}C for 60 seconds for serotypes I_a, I_b, II, 59^{o}C for IV and V and 56^{o}C for VI, VII, VIII, extension at 72°C for 60 sec and the final elongation at 72°C for 10 min (91).

The PCR conditions for serotype III were set as follows: initial denaturation at 95°C for 2 min followed by 40 cycles at 95 °C for 10sec for denaturation, annealing temperature 50^{o}C for 30 seconds for serotypes, extension at 72°C for 20 sec and the final elongation at 72°C for 7 min (90).

The amplicons were then separated by electrophoresis techniques by adding 7.5 µL samples of PCR reaction mixture to the wells of 1.5% agarose gel. 6 µL of a 100-base pair DNA ladder was included to compare the DNA bands' size of samples in proximity to the similar size of the ladder bands which works as a reference for DNA samples (87).

The gel was run for 1hr at 90 volts, then the gel was visualized under ultraviolet light and the amplicon size was determined using standard DNA molecular weight. The conformation for GBS serotypes by PCR was established according to each serotype band size (88).

2.16 The PCR amplification thermal conditions for antibiotics resistance genes

GBS-positive isolates were tested for the presence of antibiotic-resistance genes.

The selected antibiotics resistance genes in this study were: *erm*B and TR, *mef*A, *lin*B, and *tet*M and O.

Table 2

PCR primers used for the detection of antibiotic resistance genes in GBS

Primer name	Primer sequence (5′-3′)	Amplicon
ErmB-F ErmB-R (48)	5′_GAAAAGGTACTCAACCAAATA-3′ 5′_AGTAACGGTACTTAAATTGTTAC-3′	640
ErmTR-F ErmTR-R (48)	5′_GAA GTT TAG CTT TCC TAA-3′ 5′_GCTTCAGCACCTGTCTTAATTGAT-3′	400
MefA-F MefA-R (48)	5′-AGTATCATTAATCACTAGTGC-3′ 5′-TTCTTCTGGTACTAAAAG TGG-3′	348
TetM-F TetM-R (68)	5′-GTCTTGCATATATACGCCTTTATAGTGGAGTACTACATTTACGAG-3′ 5′-CCACGTAATATCGTAGAAGCGGATCACTATCTGAG-3′	374
TetO-F TetO-R (68)	5′-CGTATATATAGCGGAACATTGCATTTGAGGG-3′ 5′-CGGCTCTATGGACAACCCGACAGAAG-3′	548
LinB-F LinB-R (48)	5′-CCT ACC TAT TGT TTG TGG AA-3′ 5′_-ATA ACG TTA CTC TCC TAT TC-3′	944

The PCR reaction mixture was set of a total volume of 20 µL; 0.5 µL of forward primer, 0.5 µL of reverse primer, 10 µL of HY Taq Ready mix (hylabs), 2 µL of sample DNA, and 7 µL of sterile nuclease-free water. This solution mixture was prepared in a total volume of 378 µL, the solution was mixed gently and dispended into 21 PCR tubes in which 18 µL aliquots of the solution were transferred into each of the 21 tubes. Then 2µL of DNA sample was added to each PCR tube and labeled as suitable. Then the tubes were loaded in the thermal cycler (Prime).

The PCR amplification thermal conditions for *mef*A, *erm*B, *erm*TR and *lin*B genes were set as follows: initial denaturation at 95°C for 3 min followed by 35 cycles at 95 °C for 60sec for denaturation, annealing temperature 57ºC for 60 seconds and extension at 72°C for 60 sec and the final elongation at 72°C for 5 min (48) whereas for *tet*O, *tet*M genes, the PCR amplification thermal conditions were set as follows: initial denaturation at 94°C for 4 min followed by 35 cycles at 93 °C for 60sec for denaturation, annealing temperature 57.6ºC for 60 seconds and extension at 72°C for 60 sec and the final elongation at 72°C for 7 min (68).

The amplicons then were separated via electrophoresis techniques by adding 7.5 µL samples of PCR reaction mixture to the wells of 1.5% agarose gel. 6 µL of a 100-base pair DNA ladder was included to compare the DNA band size of samples in proximity to the similar size of the ladder bands which works as a reference for DNA samples (87).

The gel was run for 1hr at 90 volts, then the gel was visualized under ultraviolet light and the amplicon size was determined using standard DNA molecular weight. The conformation for GBS antibiotic resistance genes by PCR targeting amplicon size corresponding to each antibiotic resistance gene (88).

2.17 Data analysis

Data were analyzed using the IBM Statistical Package for Social Sciences program (IBM SPSS) version 21. We used Pearson's Chi-square and Fisher's exact test to describe the relationship between GBS colonization and independent variables. The P value of less than 0.05 was used to describe a significant relationship between variables**.**

For continuous variables, we used median ± standard deviation to express data, and for categorical variables, we used frequencies and percentages.

Chapter Three

Results

3.1 Prevalence of GBS

A total of 184 pregnant women were screened in this study, 21 (11.4%) were colonized by GBS.

The overall number of pregnant women who were included in this study was 184, after excluding 26 women due to antibiotic treatment within the last 2 weeks of admission, and 35 women refused to participate in the study. Also 19, women of gestational age lower than 33 weeks were excluded.

120 (65.2%) participants were from Rafedia Governmental Surgical Hospital and 64 (34.8%) were from AL-Itihad Hospital.

42.8% of positive GBS samples were isolated from Al-Itihad Hospital and 57.2% of positive samples were isolated from Rafidia Governmental Surgical Hospital.

After calculation of the prevalence among each of the two hospital populations, it was 11.1% GBS colonization among the Rafidia Hospital population. And 16.4% of GBS colonization was among the Al-Itihad Hospital population. Since there was a difference GBS colonization rate between the two hospitals we calculated the P value for these hospitals to find out if there is a relationship between the colonization rate and the hospital. However, no significant relationship has been figured, P-value: 0.409.

3.2 Participants' variables obtained by questionnaire

In this study, participants were asked about their socio-demographic characteristics, general clinical status, clinical symptoms related to infection symptoms, and specific factors related to current pregnancy.

3.2.1 Socio-demographic status of participants

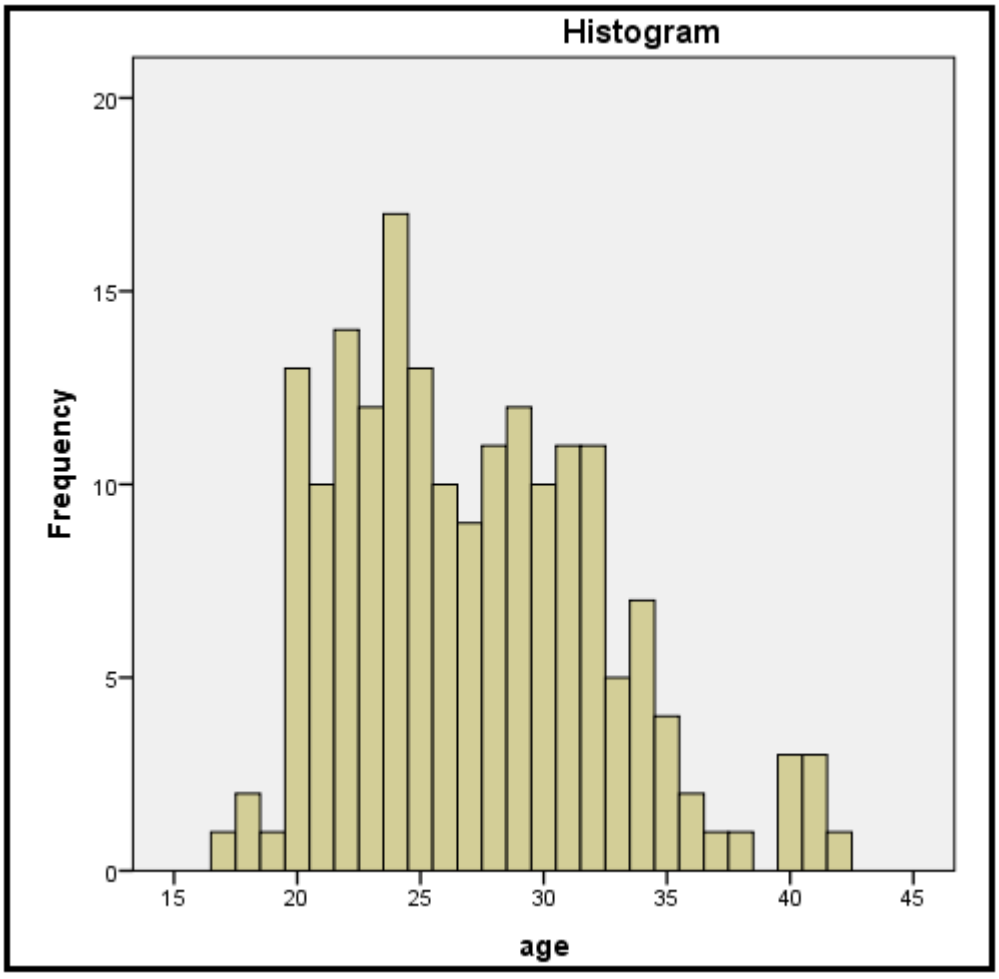
3.2.1.1 Maternal age

The age of pregnant women ranged between 17 and 42, the median age was 26 ± 5.3 years. The age was then categorized into 3 categories to find out if there is a statistically significant relationship between the age category and GBS colonization.

Most GBS colonized women (71.4%) were less than 26 years old.

Figure 3

Histogram of maternal age of participants



3.2.1.2 Socioeconomic status

The socioeconomic status of the participants in this study was analyzed according to family monthly income; the majority (63.5%) of participants had an income range between 1850 and 3500 NIS.

3.2.1.3 Living area

Pregnant women arrived at Rafidia Governmental Surgical Hospital and Al-Itihad Hospital located in Nablus City so the majority of pregnant women were from Nablus City with a few participants from adjacent cities, however, women living places were categorized into city, village, and camp. The majority were village citizens 66.3%.

3.2.1.4 Educational status of participants

Participants were categorized according to their educational level into; the school stage, diploma stage, and bachelor stage or post-graduate stage. Most participants had academic education higher than a school; 75 (40.8%) had school education, 106 (57.6%) had education to diploma and BA and 3 (1.6%) only had post-graduate education.

3.2.1.5 Employment status

The employment status of women was also analyzed and we categorized them into housewives or career women who did work outdoors; 174 (94.6%) were housewives and 10 (5.4%) were career women.

Table 3

Socio-demographic characteristics of 184 pregnant women classified according to the status of GBS colonization

Variable	Women with positive GBS result: 21 (11.4%)	Women with negative GBS result: 163 (88.6%)	P- value
Maternal age			0.102
< 26 years	15 (71.4%)	69 (42.3%)	
26-36 years	5 (23.8%)	84 (51.5%)	
>36 years	1 (4.8%)	10 (6.1%)	
Living area			0.014
City	9 (42.9%)	35 (21.6%)	
Village	8 (38%)	114 (69.9%)	
Refugees Camp	4 (19.1%)	14 (8.5%)	
Socioeconomic status			0.810
Less than 1850	5 (23.8%)	26 (16%)	
1850-3500	12 (57.1%)	105 (64.4%)	
3500-7000	4 (19%)	31 (19%)	
More than 7000	0 (0%)	1 (0.6%)	
Employment status			1.000
Housewife	20 (95.2%)	154 (94.5%)	
Work doors outdoors	1 (4.8%)	9 (5.5%)	
Education			0.317
School	5 (23.8%)	70 (42.9%)	
Diploma and BA	16 (76.2%)	90 (55.2%)	
Post-graduate	0 (0%)	3 (1.8%)	

3.2.1.6 Analysis of relationships between socio-demographic characteristics and GBS colonization

There was no significant correlation between GBS colonization with participants' socio-demographic characteristics variables, occupation (P-value: 1.000), maternal age (P-value: 0.102), educational level (P-value: 0.317), and family income (P-value: 0.810).

However, there was a significant relationship between place of residency with GBS colonization, (P-value: 0.014), 42.9% of colonized women were city citizens.

3.2.2 Factors related to the general clinical status of participants

3.2.2.1 Penicillin allergy

In this study, only one participant was allergic to penicillin 0.5% according to questionnaire answers.

3.2.2.2 Weight of participants

In this study, we asked participants about their weight in a plan to measure their body mass index (BMI) but many of them didn’t know their height and it was hard to measure it in the department of delivery because most participants were in the bed of labor suffering pain. Most participants were between 48Kg and 111 Kg the median was 73 ±11.5 Kg, and 85 (46.2%) of participants were above the median.

3.2.2.3 Covid-19 infection and vaccination status

In this study, 36 (19.6%) of participants had been infected with the virus, and 61 (33.2%) had been vaccinated.

3.2.2.4 Presence of chronic diseases

The majority of pregnant women were free of chronic diseases, and only 11 participants were suffering from chronic diseases.

3.2.2.5 Smoking status

A few proportion of participants were smokers of either cigarette or bubbly or non-smokers; 12 (6.5%) were smokers whereas the majority were non-smokers.

Table 4

General clinical health characteristics of 184 pregnant women classified according to the status of GBS colonization

Variable	Women with positive GBS result: 21 (11.4%)	Women with negative GBS result: 163 (88.6%)	P value
Covid-19 infection			1.000
Infected	4 (19.1%)	32 (19.6%)	
Hadn't been infected	17 (80.9%)	131 (80.4%)	
Smoking			0.366
Yes	0 (0%)	12 (7.4%)	
No	21 (100%)	151 (92.6%)	
Covid-19 vaccine			0.985
Yes	7 (33.3%)	54 (33.1%)	
No	14 (66.7%)	109 (66.9%)	
Chronic disease			0.618
Yes	0 (0%)	11 (6.7%)	
No	21 (100%)	152 (93.3%)	

3.2.2.6 Analysis of relationships between participants' general clinical status and GBS colonization

There was no significant correlation between GBS-colonized women and non-colonized women with their general clinical status variables, smoking (P-value: 0.366), chronic disease (P-value: 0.618), and covid-19 infection and vaccination (P-value: 1.000 and 0.985 respectively).

3.2.3 Factors related to symptoms of UTI or vaginal infection they suffer during the current pregnancy

3.2.3.1 Presence of UTI

Nearly one-quarter of participants had UTI to the best of their knowledge or were experiencing any UTI symptoms like dysuria, burning sensation, urgency, or frequency. Answers were categorized into Yes if they had UTI or any of UTI symptoms and No if they have none, 40 (21.7%) answered yes.

3.2.3.2 Presence of vaginal infection symptoms

Around one-third of participants were suffering vaginal infection during the screening test due to the presence of vaginitis symptoms like vaginal irritation, intercourse pain, or vaginal secretions, 62 (33.7%) answered yes at least to one symptom of the mentioned.

Table 5

Infection-related variables of 184 pregnant women classified according to the status of GBS colonization

Variable	Women with positive GBS result: 21 (11.4%)	Women with negative GBS result: 163 (88.6%)	P-value
UTI			1.000
Yes	4 (19%)	36 (22.1%)	
NO	17 (81%)	127 (77.9%)	
Vaginitis			0.598
Present	6 (28.6%)	56 (34.4%)	
Absent	15 (71.4%)	107 (65.6%)	

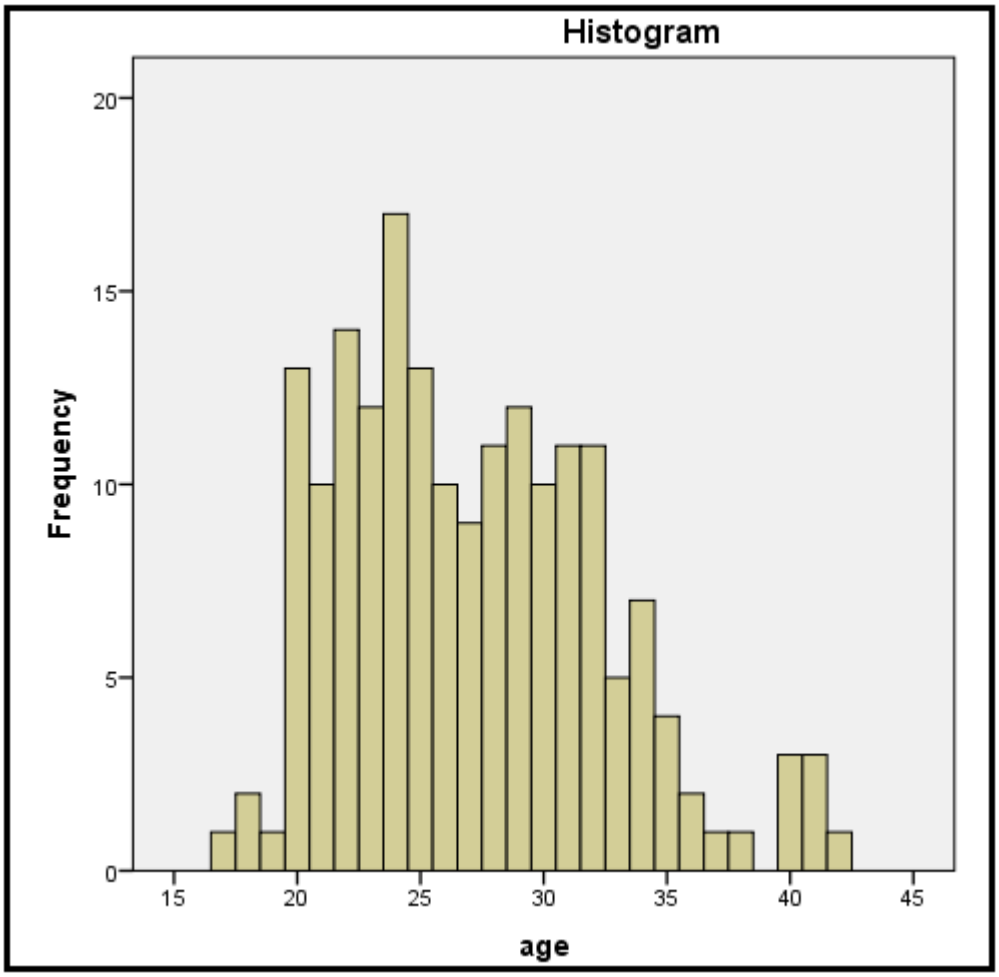
3.2.4 Factors related to the obstetric status of participants

3.2.4.1 Gestational age

Pregnant women with a gestational age of 33 weeks and above were screened for GBS infection. The gestational age of women ranged from 33 weeks to 42 weeks and the median gestational age was: 39 ±2.1 weeks.

Figure 4

Histogram of participants' gestational age



3.2.4.2 Parity status

Around 66 (35.9%) of participants were nulliparous and 118 (64.1%) of participants pregnant were multiparous.

3.2.4.3 Gravidity

The majority of participants had experienced pregnancy before. However, 53 (28.8%) of participants, this was their first pregnancy.

3.2.4.4 Amniotic Fluid Statue's

The majority 161 (87.5%) of participants had a normal amniotic fluid status, only 10 (5.4%) had abnormal amniotic fluid and 13 (7.1%) didn't know about its status according to their answers in the questionnaire.

3.2.4.5 Having a child with congenital defects

3 (1.6%) participants had a child with a congenital defect.

3.2.4.6 Number of miscarriages or pre-term delivery and delivery of dead baby

Also, in this study, we asked participants if they ever had previous miscarriages, stillbirths, or pre-term births to find out whether are these risk factors or not. 53 (28.8%) participants had a previous miscarriage; 36 (19.5%) had one miscarriage, 13 (7%) had 2 miscarriages 3 (1.6%) had 3 miscarriages and one (0.5%) had 4 miscarriages. And 16 (8.6%) participants had a pre-term delivery 3 (1.6%) of them had a preterm birth twice. And 6 (3.2%) had delivered a dead baby, and one of them had delivered 2 dead babies.

Table 6

Obstetric characteristics of 184 pregnant women classified according to the status of GBS colonization

Variable	Women with positive GBS result: 21 (11.4%)	Women with negative GBS result: 163 (88.6%)	P- value
Gestational age			0.604
<35 weeks	1 (4.8%)	12 (7.4%)	
35-37 weeks	5 (23.8%)	41 (25.2%)	
>37 weeks	15 (71.4%)	110 (67.4%)	
Gravidity			0.980
1	6 (28.6%)	47 (28.8%)	
>1	15 (71.6%)	116 (71.2%)	
Parity:			0.478
nulliparous	7 (33.3%)	56 (34.4%)	
Multiparous	14 (66.7%)	107 (65.6%)	
Amniotic fluid status			0.551
Normal	20 (95.2%)	141 (86.5%)	
Abnormal	1 (4.8%)	9 (5.5%)	
I Don't Know	0 (0%)	13 (8%)	
Pre-term birth	0 (0%)	13 (7.9%)	0.223
Miscarriages	5 (23.8%)	55 (33.7%)	0.520
Stillbirth	1 (4.8%)	5 (3.1%)	0.522
Birth with a congenital defect	0 (0%)	3 (1.8%)	1.000

3.2.4.7 Analysis of relationships between participants' clinical status related to pregnancy and infections and GBS colonization

There was no significant correlation between GBS-colonized women and non-colonized women with their clinical status and infection variables related to pregnancy, UTI (P-value: 1.000), presence of vaginal infection symptoms (P-value: 0.598), gestational age (P-value: 0.604), gravidity (P-value: 0.980), parity (P-value: 0.478), pre-term birth (P-value: 0.0.223), miscarriages (P-value: 0.520), stillbirth (P-value: 0.522), children with congenital defects (P-value:1.000) and amniotic fluid status (P-value: 0.551).

3.2.4.8 Associated risk factors with GBS colonization in pregnant women

As the above statistical analysis results of the different variables against GBS colonization, most of them had no statistically significant relationship between colonized women and non-colonized women, only the place of residency was statistically related to GBS colonization, it was higher in pregnant women from city origin. So the place of residency is the only variable that could be a risk factor for GBS colonization according to this study.

Despite there being no statistical relationship between GBS colonization and gestational age the highest proportion of colonization was among pregnant with gestational age above 37 weeks of gestation.

3.3 Confirmatory tests for GBS samples

3.3.1 CAMP test

The CAMP test was performed for all bale blue colonies noticed on UriSelect 4 chromogenic media to confirm positive GBS results, a total of 21 isolates were positive for CAMP test and identified as GBS.

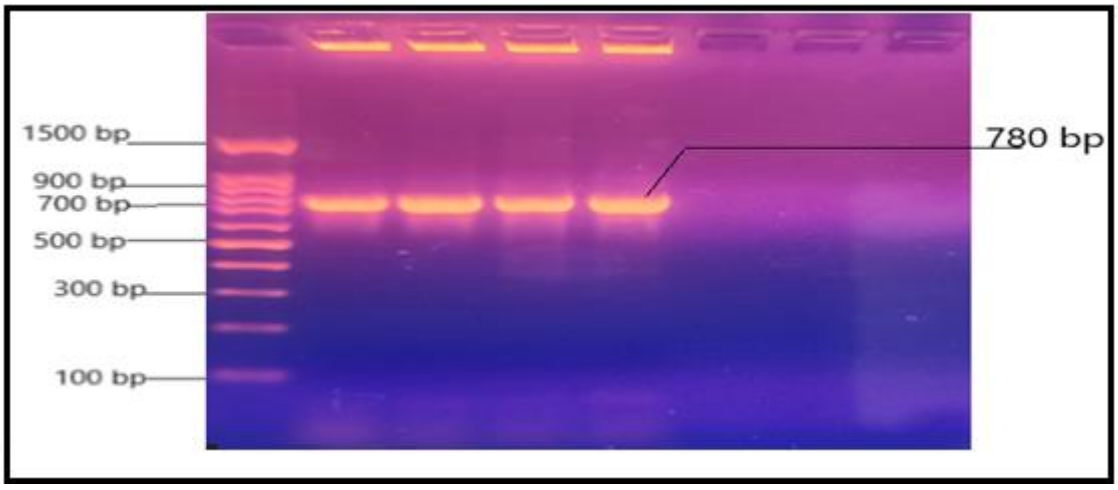
3.3.2 PCR amplification technique

Positive GBS results by chromogenic culture method and positive CAMP test samples were also confirmed by a molecular technique using the PCR amplification method.

The 21 isolates with positive CAMP test were positive by PCR assay method.

Figure 5

Illustration figure of positive GBS isolates using PCR



Note. Positive GBS isolates perform a band of DNA illustrating to 780 base pair DNA band size.

3.4 Antibiotic susceptibility test for isolated GBS

Confirmed positive samples were tested for their susceptibility pattern against the selected antibiotics by disk diffusion method, Kirby Bauer method according to CLSI M100.

All of the 21 (100%) samples were sensitive to ampicillin 6 (10µg), penicillin 10 units, amoxicillin /clavulanic acid, cefepime (30µg), cefotaxime (30µg), ceftriaxone, vancomycin (30µg) and linezolid.

6 (28.5%) samples were resistant to erythromycin (15µg), 5 (23.8%) of these were also resistant to clindamycin (2µg), 1 (4.7%) sample was resistant to levofloxacin 5ug, the majority of samples 15 (71.4%) were resistance to tetracycline (30µg).

Results were classified according to the size of the clear zone formed around each of the antibiotic disks into sensitive, intermediate, and resistant.

3.5 D-test results

D-test was performed to identify MLSB phenotype for resistance isolates to erythromycin and or clindamycin:

15 (71.4%) isolates were sensitive to both erythromycin and clindamycin and one isolate was resistant to erythromycin only, though their D test was negative. 5 (23.8) samples were resistant to both erythromycin and clindamycin in which 4 (19%) of them

had the cMLSB phenotype corresponding to negative D-test, 1 (4.7%) isolate had the M phenotype, resistance to erythromycin only and none had L phenotype. Thus, negative D-test.

Whereas 1 (4.7%) isolate had an iMLSB phenotype, corresponding to a positive D-test.

3.6 Presence of Antibiotics resistance genes

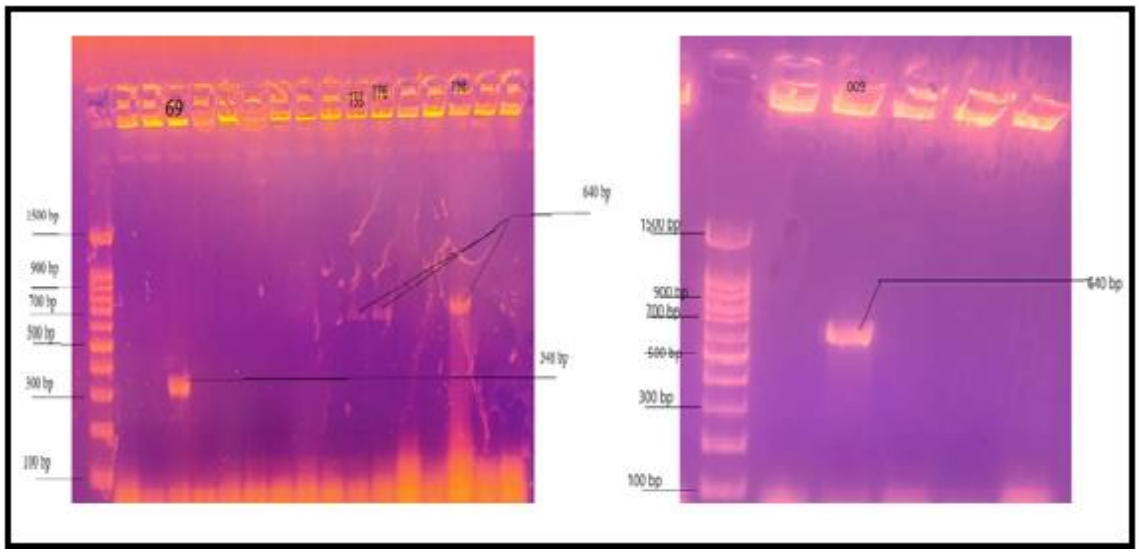
In this study, 4 antibiotic resistance genes were investigated; *erm* genes *erm*B and *erm*TR, *mef*A gene, *lin*B gene, *tet*M, and *tet*O.

4 (19%) isolates appeared to possess the *erm*B gene, 1 (4.7%) isolates had the *mef*A gene, and 19 (90%) isolates possessed the *tet*M gene.

None of the isolates exhibited any of the *erm*TR, *lin*B, or *tet*O resistance genes.

Figure 6

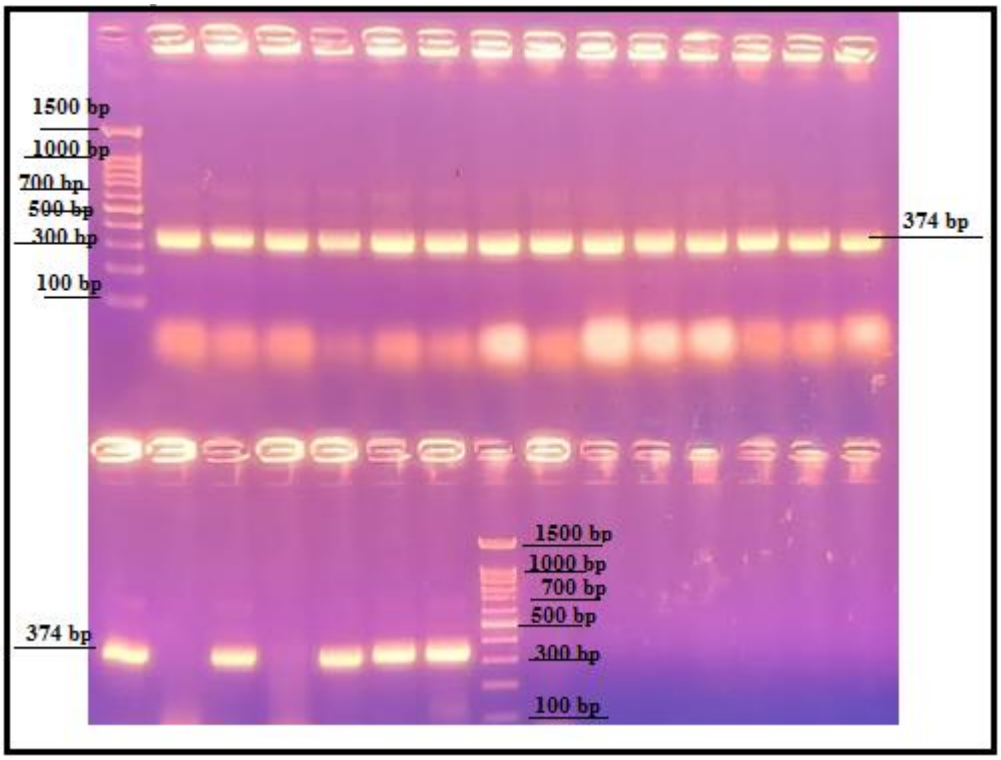
Illustration figure of isolates with ermB and mefA, resistance genes



Note. Positive GBS isolates for the gene *erm*B performed a band of DNA illustrating to 640 base pair DNA band size.
Positive GBS isolates for the gene *mef*A performed a band of DNA illustrating to 348 base pair DNA band size.

Figure 7

Illustration figure of isolates with tetM resistance



Note. Positive GBS isolates for the gene *tet*M performed a band of DNA illustrating to 374 base pair DNA band size.

3.6.1 Analysis of the relationship between antibiotics resistance genes and erythromycin, clindamycin, and tetracycline

6 isolates were resistant to erythromycin and 5 of them were resistant to clindamycin, meaning that 1 sample expressed the M phenotype and it was the same isolate expressing the gene *mef*A. Of the 5 resistant isolates, 3 were expressing the *erm*B gene.

However, one isolate was resistant to both clindamycin and erythromycin and it lacked the *erm*B gene. In contrast, one isolate possessed the *erm*B gene while exhibiting a sensitive pattern against both antibiotics. Whereas *lin*B and *erm*TR were not expressed at any isolate.

90% of isolates had the *tet*M gene, and 71.4% were resistant to tetracycline, meaning that some sensitive isolates may possess the resistance gene, and that occurred in 5 isolates, whereas 1 isolate where resistant to tetracycline but lacking the gene. Only one isolate was sensitive to tetracycline and lacked the *tet*M gene.

Tet O was not expressed at any isolate.

Analyzing the relationships statistically between, erythromycin susceptibility and *erm*B, *mef*A, and *tet*M were not significantly correlated, with P-values: of 0.053, 0.286, and 1.000 respectively. However, it seemed that there is an association between erythromycin resistance and the presence of the *erm*B gene

However, there was a significant relationship between *erm*B and clindamycin susceptibility, P-value: 0.012, but there were no relationships with *tet*M and *mef*A, P-value: 1.000 for both variables.

Also, there was no statistical relationship between tetracycline susceptibility and *tet*M, *erm* B and *mef* A, P-value: 0.5, 1.000, and 0.286 respectively.

Also, there was no statistical relationship between *erm* B and *mef* A with the D-test.

Table 7

Antibiotic susceptibility, antibiotics-resistant genes, and serotype profiles of 21 GBS isolates from pregnant women

Sample and its serotype	tetracycline	erythromycin	clindamycin	D-test phenotype	*erm*B	*mef*A	*Tet*M
V	R	S	S	N	N	N	P
V	R	R	R	N	P	N	P
III	R	R	R	N	N	N	P
III	S	S	S	N	N	N	P
III	R	S	S	N	N	N	P
IV	R	S	S	N	N	N	P
II	S	R	S	N	N	P	P
III	S	R	R	P	N	N	P
V	R	S	S	N	N	N	P
II	R	S	S	N	N	N	P
Nontypeable	R	S	S	N	N	N	P
III	S	S	S	N	N	N	P
V	S	R	R	N	P	N	P
V	R	R	R	N	P	N	P
III	R	S	S	N	N	N	P
Ib	S	S	S	N	N	N	N
III	R	S	S	N	N	N	P
II	R	S	S	N	P	N	N
III	R	S	S	N	N	N	P
Ib	R	S	S	N	N	N	P
III	R	S	S	N	N	N	P

Note. N: negative, P: positive, R: resistant S: sensitive

3.7 Distribution of GBS Serotypes

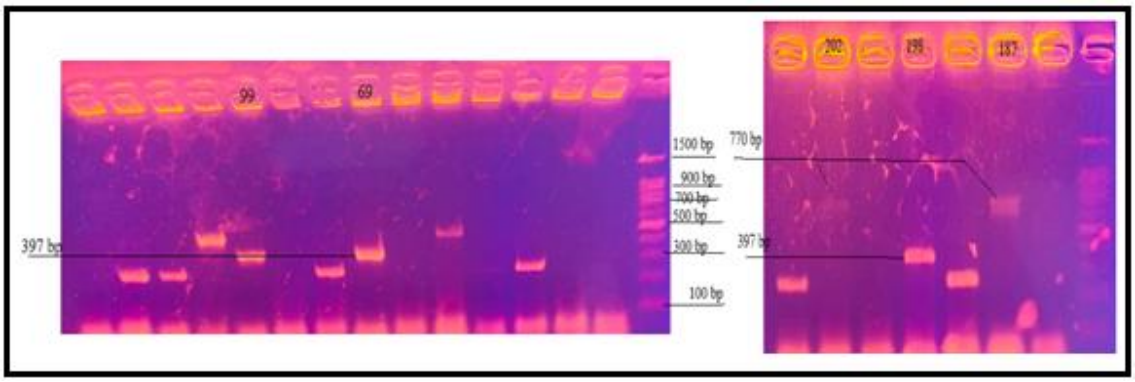
Confirmed positive GBS isolates were further tested and classified according to their polysaccharide antigens that appear on their capsular surface into serotypes of GBS.

In our study we tested GBS isolates against 9 serotypes out of 10 identified: (Ia, Ib, II, III, IV, V, VI, VII, and VIII) from the 21 samples 20 (95.2%) were typeable and 1(4.7%) was non-typeable.

The predominant type was serotype III accounting for 42.8% of the isolated GBS 9 isolates, then 5 (23.8%) were serotype V, 3 (14.2%) were serotype II, 2 (9.5%) were serotype Ib, 1(4.7%) serotype IV, 1 was non-typeable and non were of Ia, VI, VII and VIII serotypes.

Figure 8

Illustration figure of serotypes Ib and II isolates

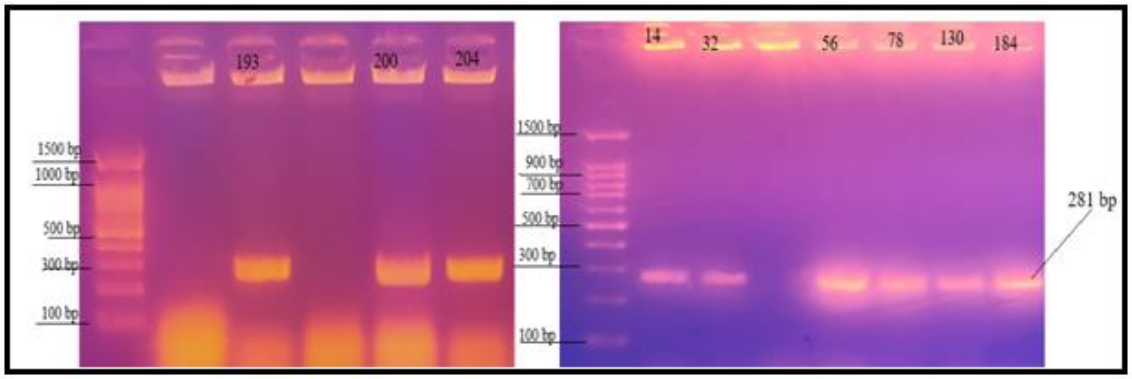


Note. GBS isolates of the serotype Ib performed a band of DNA illustrating to 770 base pair DNA band size.

GBS isolates of the serotype II performed a band of DNA illustrating to 397 base pair DNA band size.

Figure 9

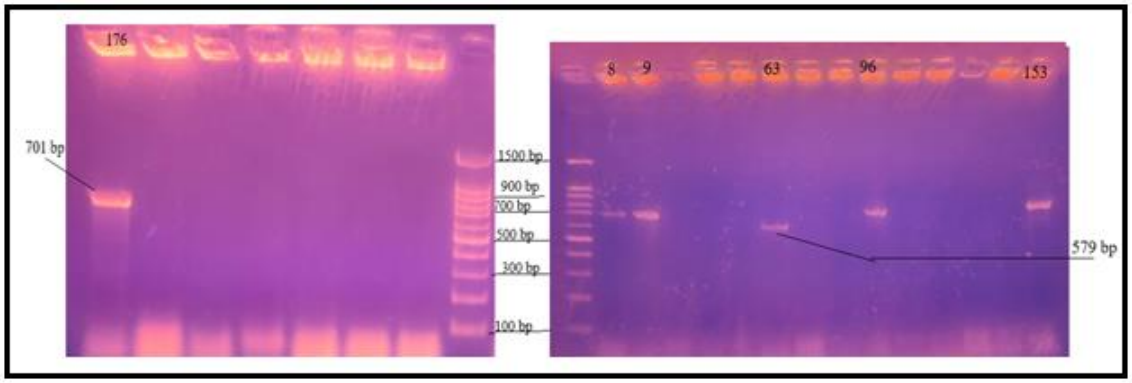
Illustration figure of serotype III isolates



Note. GBS isolates of the serotype III performed a band of DNA illustrating to 281 base pair DNA band size.

Figure 10

Illustration figure of serotypes IV and V isolates



Note. GBS isolates of the serotype IV performed a band of DNA illustrating to 579 base pair DNA band size.
GBS isolates of the serotype V performed a band of DNA illustrating to 701 base pair DNA band size.

3.7.1 Analysis of the relationship between antibiotics resistance genes and capsular polysaccharides

The relationship between capsular polysaccharides and the expression of antibiotic resistance genes was also measured and there was no statistical relationship between the capsular polysaccharides (serotypes) and the expression of antibiotic resistance genes according to the results of this study. P-value was: 0.278, 0.189, and 0.113 for *mef*A, *tet*M, and *erm*B respectively.

Chapter Four

Discussions and Conclusions

4.1 Prevalence of GBS

This study aimed to find the prevalence of GBS colonization in pregnant women at 33 weeks and above of gestation, at the hospitals of Rafidia Governmental Surgical Hospital and Al Itihad Hospital in the city of Nablus, their antibiotic susceptibility pattern, serotype distribution, antibiotic resistance genes on isolated strains and the associated risk factors with GBS colonization.

The prevalence of GBS colonization among the study population was 11.4% (21 women out of 184), These findings are comparable to the previous study held in Nablus, Palestine where the prevalence was 12% (26), however, it was lower than the prevalence found in nearby areas, Nazareth and Gaza, 31% and 21%, respectively (64) (67). Also, our prevalence finding was comparable to the estimated prevalence in East Asia 11% (15), and close to the prevalence in a Yemini study 10.95% (55), and similar to the prevalence of GBS in Iran, Egypt, South Israel, Zimbabwe, and UAE (11%, 11.3%, 11%, 12.3%, 10.2% and 10.1% respectively) (27, 29, 41, 56, 59, 65). However, some studies reported a much less prevalence rate like a study in UAE in 2021 where the prevalence was only 6.9% (51) and 9.8% in Turkey (29). On the other hand, the prevalence we found was less than similar studies conducted in Jordan where the prevalence was 19.5% (29), 24% in Morocco (29), 15% in Saudi Arabia (53), 18.4% in Lebanon (60) and 17.2% in Brazil (47).

The variation of the GBS rate among countries and within the same country could be attributed to the variation of several factors that affect the GBS colonization rate, these factors include residence, living standard, maternal age, maternal hygiene, race of the study population, different gestational age among different studies, the sample size, variation of samples source either vaginal or recto-vaginal (92),(26),(36). Moreover, the variable laboratory cultural technique and confirmatory tests used, PCR detection, and confirmatory methods showed a higher GBS colonization rate than the cultural technique (93).

4.1.1 Prevalence according to sample size

The prevalence of 11.4 % of the sample size of 184 participants, close to the previous study in Nablus with 200 participants where the prevalence was 12% (26), also comparable to similar studies: in Yemen 210 (55), 186 in Brazil (47), 188 in Nazareth. However, a larger sample size gives a better estimation of GBS prevalence. Our result was comparable to studies that included larger sample sizes in Saudi Arabia 400, 1328 participants, 15% and 13.4% respectively (53),(54), and 420 participants in Zimbabwe 10.2% (41). However, 2295 participants were in the UAE study the prevalence was 6.9% (51).

4.1.2 Prevalence according to gestational age

In this study, pregnant women with a gestational age of 33 weeks and above were included, and it is approved for GBS screening of pregnant women inconsistent with Japan Society (31), however, it was recommended to start from the week 36th of gestation by the American Society (33) it was challenging in our study due to increasing rejection rate among the population so we accepted pregnant from the week 33rd of gestation, in a similar study conducted before in Palestine, the gestational age was 35 weeks and above (26), In Zimbabwe they included pregnant with a gestational age of 13-35 weeks (41) and the prevalence rate was comparable. In Brazil, they included pregnant with gestational age 32-40 weeks (47), similar to this study, 33-42 weeks however, the prevalence in Brazil was higher.

On the other hand, the Yemeni study included women with a gestational age of 35-38 weeks (55). In Iran, and Egypt study samples were between 35-37 gestational weeks (27),(59), and the prevalence was comparable. Also in UAE, Lebanon, and Kuwait studies gestational age was between 35-37 weeks (60),(51, 56).

In Jordan, Saudi Arabia, and Gaza where higher prevalences have been found, pregnant with gestational age above 35 weeks were included (29),(53),(67).

4.1.3 Prevalence according to culture media

The culture media UriSelect 4 chromogenic deferential media was used in this study, and according to the study results, the UriSelect 4 chromogenic had good specificity and sensitivity yield compared to that in Qadi's study where they used the CHROM agar StrepB media, mauve were suspected to be GBS (26).

In Gaza, selective and chromogenic media was used (67) and a higher prevalence was observed.

In the comparable studies of Yemeni study, UAE study, Saudi Arabia, and the Iranian study, they cultured samples initially on the selective enrichment media, Todd-Hewitt broth supplemented with gentamicin (8µg/ml) and nalidixic acid (15µg/ml) before subculture on blood agar for GBS identification (55),(53, 54, 58, 59).

In Egypt, they used another type of GBS selective enrichment media, Granada broth media which showed 100% specificity in GBS detection in their study (27).

4.1.4 Prevalence according to confirmation test

The CAMP test was used for confirmation of bale blue colonies on UriSelect agar followed by the PCR assay method for CAMP-positive isolates, these tools strengthen the accuracy of GBS prevalence detection and conformation, similar to that used in the Gaza Strip study where they used PCR method only for GBS confirmation (67), however, the prevalence was higher. And in contrast to Qadi's study where they used the CAMP test only for confirmation of mauve colonies (26). In Jordan, CHROMagar culture and positive CAMP test for B-hemolysis colonies on blood agar were used as confirmatory of GBS growth (29). However, the result was comparable to Qadi's study prevalence but less than that in Gaza and Jordan.

Table 8

Studies describing GBS prevalence in adjacent countries

First author	Year	Location	Pregnant gestational age	Sample size	collection method	Conformation method	Colonization rate %
This study	2023	Nablus, Palestine	≥ 33 weeks gestation	184	Vaginal	CAMP test And PCR	11.4
Qadi (26)	2020	Nablus, Palestine	≥ 35 weeks gestation	200	Vaginal	CAMP test	12
Nabil et al. (67)	2017	Gaza, Palestine	≥ 35 weeks gestation	200	Recto-vaginal	PCR test	21
Clouse et al. (29)	2019	Jordan	≥ 35 weeks gestation	200	Recto-vaginal	Agglutination test	19.5
Hakim et al. (64)	2018	Nazareth, Arab-Israel	≥ 35 weeks gestation	188	Recto-vaginal	AmpliVue GBS assay, atoB gene	31
Ghaddar et al. (60)	2014	Lebanon	35-37	168	Vaginal	Latex agglutination	18.4
Darabi et al. (58)	2017	Iran	≥ 35 weeks gestation	186	Recto-vaginal	Catalase and CAMP test	11.8
Al-Subol et al. (55)	2022	Yemen	35-39 weeks	210	Vaginal	CAMP test	10.95

4.2 Risk factors

A significant relationship between GBS colonization and the place of residency was found in this study; city populations were more vulnerable to GBS colonization. These findings were in contrast to findings in Zimbabwe in 2010, where the prevalence was significantly higher in rural areas compared to urban (94).

In a comparable study in Israel, they found GBS colonization was significantly higher among the population in poor areas (95).

There was no significant relationship between GBS colonization and parity, gravidity, age, gestational age, amniotic fluid status, UTI, vaginitis, presence of chronic diseases, smoking, education, occupation, weight, family income, history of preterm birth, miscarriages, and stillbirth. These findings were similar to the previous study held in Palestine by Qadi, however, there was an association between preterm birth and GBS colonization (26) in contrast to the Iranian study that didn't show a significant association between preterm birth and GBS colonization (58). Here in this study, only one risk factor has been figured, this may be attributed to a small sample size.

There was a significant relationship between maternal age and GBS colonization in Saudi Arabia (54). In comparison to the findings of this study, GBS colonization was higher among women with gestational age above 37 weeks of gestation in this study and above 42 weeks in the Saudi study but without being statistically related.

Our findings also are similar to Namibia study findings, where it also didn't detect any significant relationship between GBS colonization and educational level, occupation, age, parity, history of miscarriages, or stillbirths, also there was no significant relationship between GBS colonization and marital status in Namibia (68) However, we didn't ask about this status because all pregnant women in our community are supposed to be married.

4.3 Serotype distribution of GBS

Serotype III was the predominant one among our study sample accounting for 42.8% of detected GBS strains, similar findings have been reported in Jordan where serotype III accounted for 48% (29) and serotype V was 23.8%, in contrast to findings in Israel where serotype VI was the predominant accounted for 40.8% followed by serotype III 17.5% (28). In a Palestinian study where the sample population differs from ours', serotype III accounted for 35% of the isolated GBS strains. Also similar to ours', followed by serotype V which accounted for 19% of Awwad's study close to our study findings (71).

Serotype II accounted for 14.2% of our study isolates, comparable to the finding in Jordan 20% (29).

The prevalence of Ib serotype in our study was 9.5% similar to 5.5% in Namibia (68), while serotype IV was 4.7% in our study whereas, a study in Israel revealed that serotype IV accounted for 11.7% (66), however in Awwad's this serotype wasn't tested at all, it could present in the non-typeable strains in that study, in our study there was only one sample that is non-typeable however, in her study, there were 10 isolates.

4.4 Antibiotics susceptibility pattern

GBS isolated strains in this study were 100% susceptible to ampicillin, penicillin, amoxicillin /clavulanic acid, cefepime, cefotaxime, ceftriaxone, vancomycin, and linezolid. This correlates with the finding of a similar study held in Namibia where GBS isolates were 100% sensitive to penicillin, ampicillin, linezolid, vancomycin, chloramphenicol, and ceftriaxone. In this study, GBS isolates showed 71.4% resistance to tetracycline whereas in Namibia's study isolates were 100% resistant to tetracycline (68). In contrast to our study, in Namibia, 100% of isolates were sensitive to erythromycin and clindamycin whereas in this study 28.5% and 23.8 % were resistant to erythromycin and clindamycin respectively, only one isolate was positive towards D-test, harboring the iMLSB phenotype whereas the other resistance isolates were either cMLSB or M phenotype. Also in this study, 4.7% were resistant to levofloxacin.

Erythromycin and clindamycin resistance patterns were similar to the previous study conducted in Palestine by Qadi, in which the resistance in his study was 29.2% and 25% respectively, and 8.4% resistance to levofloxacin, comparable to our findings, however,

a significant variation from our study was reported, 91.7%, 54.2% and 45.8% resistance to ampicillin, vancomycin, and cefotaxime respectively (96) and this is may be attributed to different causes including, variation of cultural media he used CHROM agar StrepB media deferential media whereas we used UriSelect 4 agar media, in addition to the confirmatory tests he used only CAMP test whereas we used it in addition to PCR assay method, and the majority of his population were from the city in contrast to ours' where the majority were from the village. Interestingly, in our study, all isolates were sensitive to the mentioned antibiotics. In the Gaza study, 43% were resistant to penicillin also different than our findings however 69% were resistant to tetracycline, variation between the Gaza study and this study could be attributed to the variations in the population (67).

In Awwad's study conducted in Palestine, 16% were resistant to ampicillin and similarly, 26% were resistant to clindamycin, variation in this study could be attributed to the variation of population type, in which we select only vaginal pregnant women samples whereas their study included GBS isolates collected from various sources not only vaginal swabs (71).

Similar studies in Yemen and Saudi Arabia showed a 100% susceptibility pattern against penicillin, ampicillin, and vancomycin (54),(55).

4.5 Antibiotics resistance genes

GBS isolates that possess the tetracycline resistance gene *tet*M were 90% of the isolates corresponding to the high resistance of isolated GBS strains against tetracycline. Isolates that had the *erm*B gene were 19% (3 isolates) and isolates that had the *mef*A gene were 4.7% (1 isolate). A similar study in Zimbabwe conducted 97.6% presence of the *tet*M gene and the *tet*O was present in only 2.4% of isolates however it was not present among any of the isolates of our study. Similarly, they didn’t find the *lin*B gene among any of the isolates similar (41), also the resistance genes, *erm*TR, *lin*B, and *tet*O were not detected in any of the isolates in our study.

Resistant 6 isolates to erythromycin of which 2 had the *erm*B gene and one the *mef*A gene. There was one isolate possessing the *erm*B gene but was sensitive to both clindamycin and erythromycin these findings were similar to findings in a study held in South Africa in which there were 2 isolates sensitive to erythromycin but possessing the

gene, this was attributed to having the gene without being expressed or due to presence of mutated genes that encode the 23S rRNA (48). Two isolates were resistant to clindamycin and erythromycin but they lacked genetic antibiotic resistance mechanisms similar findings were found in an Irish study, in which there were 8 isolates with resistance phenotypes but lacking genetic resistance mechanisms (83).

A similar scenario was with tetracycline, 5 sensitive isolates to tetracycline appeared to possess the *tet*M gene similar to a study in Zimbabwe (48), whereas 1 isolate was resistant to tetracycline but lacked a genetic resistance mechanism, the *tet*M gene neither *tet*O gene. This also supposes that resistance phenotype is not always predicted by genotype (41).

The *mef*A gene was identified in one sample 4.7% presented to be resistant to erythromycin only (M phenotype), similar findings were in Zimbabwe and South Africa in which 3.4% possessed the *mef*A gene (41) also similar to the South African study none of isolates harbored both of *mef*A and *erm*B together (48).

4.6 Limitations

A larger and more representative study sample of pregnant women could not be achieved easily. In addition, we lowered the accepted gestational age from 35 weeks and above of gestation to 33 weeks and above due to the high rate of rejection among women.

In this study it was planned to test vaginal swabs by PCR directly in parallel to the cultural technique to detect small quantities of GBS bacteria and to compare molecular results with cultural however, this was unachievable due to the presence of contaminants that impedes the work and low budget thus couldn't buy a DNA extraction kit to enhance the DNA yield, extraction was performed using simple boiling method.

In this study, only vaginal swabs were used, while the recommended screening sample is a recto-vaginal swab which enhances results and provides more representative results.

4.7 Conclusion

In this study, we found the prevalence of GBS colonization among pregnant women who attended the delivery department at Rafidia Surgical Governmental Hospital and Al-Itihad Hospital to be 11.4%, (57.2%) were in Rafidia Hospital and (42.8%) were in Al-Itihad Hospital. A significant relationship was found between GBS colonization and living in the city.

In this study, there wasn't any significant relationship between GBS colonization and maternal age, gestational age, gravidity, parity, amniotic fluid status, UTI, vaginitis, history of stillbirth, preterm birth, and miscarriages.

Antibiotic susceptibility of GBS isolates has reported 100% susceptibility against penicillin, amoxicillin, ampicillin, cefepime, cefotaxime, ceftriaxone, vancomycin, and linezolid. With 71.4% resistance to tetracycline, 4.7% resistance to levofloxacin, 28.5% resistance to erythromycin, and 23.8% resistance to clindamycin.

Serotype III was the predominant accounting 42.8% for followed by serotype V accounting for 23.8%, serotype II 14.2%, serotype Ib 9.5%, 4.7% were serotype IV whereas 4.7% were non-typeable.

Tetracycline resistance gene (*Tet*M) was the most prevalent one, it was presented on 90% of GBS isolates. Erythromycin resistance genes (*erm*B and *mef*A) were presented in 19% and 4.7% of isolated GBS strains respectively.

4.8 Recommendations

Our study conducted a comparable prevalence with the adjacent regions, and on the findings of this study, it is recommended to insert GBS screening as a part of maternal health evaluation to improve maternal health and to decrease neonatal morbidity that could be due to GBS transmission from mothers. It is more cost-effective to screen pregnant women for GBS colonization than treating newborns with EOD.

Also, the development of vaccines that are clonal dependent which target serotype III would be effective in nearly 42% of infected mothers in the Nablus area as the majority of colonized women possessed serotype III of GBS.

According to susceptibility patterns and antibiotic resistance genes results of this study, it goes totally with the guidelines in the drug of choice for GBS prophylaxis and treatment using penicillin G and it is not recommended to use tetracycline in GBS infection treatment due to the high resistance rate present among strains. Further studies are needed to elucidate the detection protocol and antibiotic sensitivity.

List of abbreviations

Abbreviation	Meaning
ACOG	American College of Obstetricians and Gynecologists
BA	Bachelor
BMI	Body Mass Index
CAMP	Christie, Atkins, Munch-Petersen
CC17	Clonal complex 17
CDC	Centers for Disease Control and Prevention
cMLSB	consecutive MLSB
CLSI	Clinical and Laboratory Standards Institute
EOD	Early-Onset Disease
Erm	Erythromycin resistance methylase
F	Forward primer
GBC	Group B-specific Carbohydrate antigen
GBS	Group B *Streptococcus*
IAP	Intrapartum Antibiotics Prophylaxis
iMLSB	inducible MLSB
IRB	Institutional ReviewB
JSOG	Japan Society of Obstetrics and Gynecology
LB	Luria-Bertani
LOD	Late-Onset Disease
MALDI-TOF	Matrix-Assisted Laser Desorption Ionization–Time Of Flight mass spectrometry
MLSB	Macrolides, Lincosamide, and Streptogramin-B
N	Negative
P	Positive
R*	Reverse primer
R	Resistant
S	Sensitive
TAE	Tris-Acetate EDTA
UTI	Urinary Tract Infection

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Appendices

Appendix A

Informed Consent

Palestine
An-Najah National University
Faculty of graduate studies

دولـــــــــــة فلســـــــــــطين
جامعةالنـجـاح الـوطنـيـة
كليـة الدراسات العليا

اقرار واستبيان عن دراسة

الوصف الجزيئي ل البكتيريا العقدية القاطعة(Group B streptococcus) و تكرار الاصابة والحساسية ضد المضادات الحيوية

1) **الباحثة:** آلاء أحمد لطفي عواد وبإشراف الدكتور أمجد عزالدين و الدكتور محمد قادي.
2) **هدف الدراسة:** تهدف هذه الدراسة الى معرفة الوصف الجزيئي ل البكتيريا العقدية القاطعة و تكرار الاصابة و الحساسية ضد المضادات الحيوية في منطقة نابلس من خلال الاستبيانة المرفقة أدناه.
3) **سؤال الدراسة:** ما هو تكرار الإصابة بالبكتيريا القاطعة العقدية في مدينة نابلس و محيطها وما السلالات المنتشرة في نابلس و مدى حساسيتها للمضادات الحيوية.
4) **نتائج البحث:** سوف تستخدم نتائج البحث للأغراض العلمية فقط وذلك للحصول على درجة الماجستير في الكيمياء الحيوية السريرية/ جامعه النجاح الوطنية.
5) **طريقة البحث:** تعبئة الاستبيانة ادناه وأخذ عينة مهبلية او شرجية لتحليلها و جمع النتائج.
6) **المخاطر المتوقعة:** لا يوجد أي مخاطر، حيث ان التعهد والمشاركة في تعبئة الاستبيانة اختياري. المشاركة في هذه الدراسة عبارة عن عمل تطوعي ويمكن الامتناع او الانسحاب عن المشاركة في أي وقت من دون ذكر الأسباب وبدون أي التزامات أو فقدان مزايا.
7) **الاستفادة المتوقعة للمشاركين:** سيتم أخذ الاحتياطات اللازمة للمريضات التي تشخص بوجود الاصابة بالبكتيريا لديها لمنع انتقالها للطفل أثناء الولادة, و قد يساعد في إثراء البحث العلمي لمعرفة توزيع هذه البكتيريا في فلسطين و حساسيتها للمضادات الحيوية.
8) **السرية واحترام الخصوصية:** المعلومات سوف تستخدم لأغراض البحث العلمي فقط بما يضمن الحفاظ على الخصوصيه والسريه التامه بحيث لا يكون هناك أي إزعاج للمشاركين . وأي استفسار او سؤال له علاقه بهذه الدراسه يمكن للشخص المشارك مراجعة الدكتور أمجد عز الدين (0599651641) أو الدكتور محمد قادي (0599372785) كما يحق لاي مشارك رفض دخول الدراسة في اي وقت من الدراسة. **كل المعلومات التي سوف يتم الحصول عليها من هذا الاستبيان هي سريه وليست للنشر.** شاكرين لكم مشاركتكم وتعاونكم البناء لما فيه من الخير.

موافقة المشاركه: .....

تاريخ الاجابه:..... التاريخ :.....

Appendix B

Questionnaire

الاستبيانة

1. أسئلة عامة:

ضع إشارة X أو √ في المكان المخصص:

العمر (بالسنوات):				
منطقة السكن:	☐ مدينة	☐ قرية	☐ مخيّم	
مكان الإقامة:	☐ محافظة نابلس	☐ غير ذلك، ----------		
الوزن:	_____			
الطول:	_____			
مستوى التعليم:	☐اقل من توجيهي	☐ توجيهي	☐ دبلوم	☐ بكالوريوس ☐ دراسات عليا
عدد أفراد الاسرة:	_____			
المهنة:	العمل الحالي؟ ---------- منذ --------- ☐ ربة منزل (لا تعمل)			
معدل دخل الأسرة الشهري (بالشيقل):	☐ اقل من 1850	☐ 1850 – 3500	☐ 3501 – 7000	☐ 7000 <

2. الأسئلة التالية متعلقة بالحالة الصحية والسلوكية النفسية والعوامل البيئية للمتطوع/ المشارك، ضع إشارة X أو √ في المكان المخصص:

أ. الطبيعة و السلوكيات الصحية:		
1. هل تتناولين المضادات الحيوية حاليا أو خلال أسبوعين من عمل الفحص؟	☐ لا	☐ نعم, ما هي؟ _____
2. هل لديك حساسية للبنسلين؟	☐ لا	☐نعم

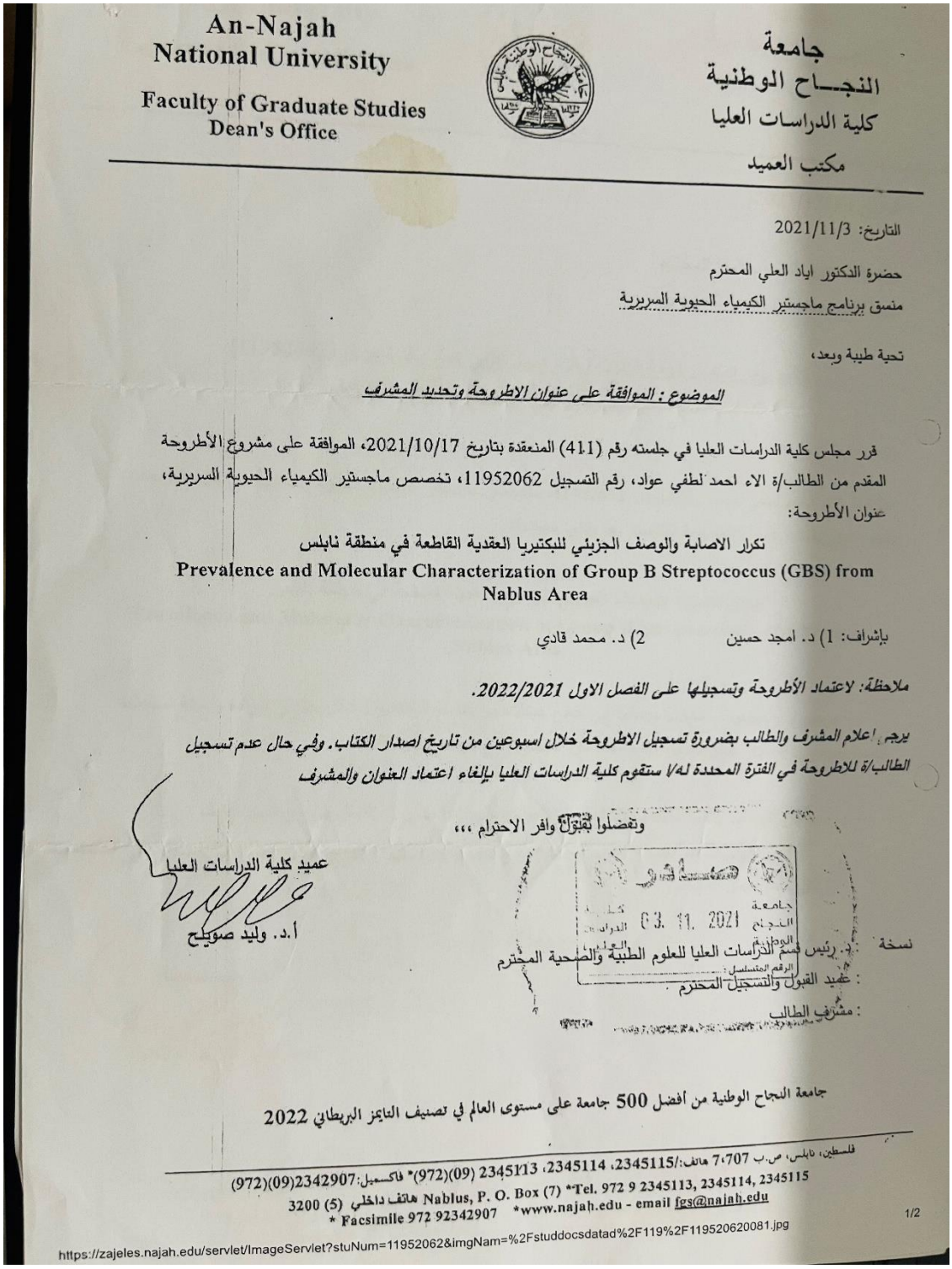
3. هل تدخنين؟	☐ لا	☐نعم
4. الامراض المزمنة؟	☐ لا يوجد	☐ الدهنيات ☐ الضغط ☐-السكري ☐ حساسية الربيع ☐غير ذلك؟ _____
5. هل اصبتي بالكورونا	☐ لا	☐ نعم, عدد المرات؟ _____
6. هل تلقيتي مطعوم الكورونا	☐ لا	☐ نعم, كم جرعة؟ _____

ج- الحالة الصحية والسلوكية النفسية:

1. عمر الحمل بالاسبوع والايام	_____ اسبوع _____ يوم
2. ما هي حالة السائل الأمنيوسي؟	☐ طبيعي ☐ أقل من الطبيعي ☐ أكثر من الطبيعي ☐ لا اعلم عن حالته
3. ما هو عدد مرات الحمل؟	_____
4. كم عدد الولادات؟	_____
5. هل سبق و أنجبت قبل الموعد الطبيعي؟	☐ لا ☐ نعم, في أي اسبوع ؟ _____
6. هل سبق و أجهضت؟	☐ لا ☐ نعم، كم عدد الحالات الإجهاض؟ _____ كم عمر الجنين في كل حالة اجهاض؟ _____
7. هل سبق وأنجبت طفل ميت؟	☐ لا ☐ نعم, في اي اسبوع؟ _____
8. هل سبق إنجاب طفل بعيوب خلقية؟	☐لا ☐نعم, حددي العيوب؟
9. هل تعانين من التهابات في الجهاز البولي أو التناسلي؟	☐ لا ☐ نعم، حددي اين؟ _____ مسبب الالتهاب _____
10.هل تعانين من أي من الاعراض التالية؟	☐ حرقة مهبلية ☐ حكة مهبلية ☐ افرازات مهبلية ☐ فطريات مهبلية ☐ التهابات مهبلية ☐ الم الجماع ☐ رائحة لافرازات المهبل ☐ حرقة التبول ☐ تقطع التبول ☐ الاضطرار للتبول ☐ عدد مرات التبول _____/يوم

Appendix C

Approval from the Faculty of Graduate Studies



An-Najah
National University
Faculty of Graduate Studies
Dean's Office

جامعة
النجـــاح الوطنية
كلية الدراسات العليا
مكتب العميد

التاريخ: 2021/11/3

حضرة الدكتور اياد العلي المحترم
منسق برنامج ماجستير الكيمياء الحيوية السريرية

تحية طيبة وبعد،

الموضوع: الموافقة على عنوان الاطروحة وتحديد المشرف

قرر مجلس كلية الدراسات العليا في جلسته رقم (411) المنعقدة بتاريخ 2021/10/17، الموافقة على مشروع الأطروحة المقدم من الطالب/ة الاء احمد لطفي عواد، رقم التسجيل 11952062، تخصص ماجستير الكيمياء الحيوية السريرية، عنوان الأطروحة:

تكرار الاصابة والوصف الجزيئي للبكتيريا العقدية القاطعة في منطقة نابلس
Prevalence and Molecular Characterization of Group B Streptococcus (GBS) from Nablus Area

بإشراف: 1) د. امجد حسين 2) د. محمد قادي

ملاحظة: لاعتماد الأطروحة وتسجيلها على الفصل الاول 2022/2021.

يرجى اعلام المشرف والطالب بضرورة تسجيل الاطروحة خلال اسبوعين من تاريخ اصدار الكتاب. وفي حال عدم تسجيل الطالب/ة للاطروحة في الفترة المحددة له/ا ستقوم كلية الدراسات العليا بإلغاء اعتماد العنوان والمشرف

وتفضلوا بقبول وافر الاحترام ،،،

عميد كلية الدراسات العليا
أ.د. وليد صويلح

03. 11. 2021

نسخة: أ. رئيس قسم الدراسات العليا للعلوم الطبية والصحية المحترم
: عميد القبول والتسجيل المحترم
: مشرف الطالب

جامعة النجاح الوطنية من أفضل 500 جامعة على مستوى العالم في تصنيف التايمز البريطاني 2022

فلسطين، نابلس، ص.ب 707، هاتف: 2345115، 2345114، 2345113 (09)(972)* فاكسميل: (972)(09)2342907
Nablus, P. O. Box (7) *Tel. 972 9 2345113, 2345114, 2345115
هاتف داخلي (5) 3200 * Facsimile 972 92342907 *www.najah.edu - email fgs@najah.edu

1/2

https://zajeles.najah.edu/servlet/ImageServlet?stuNum=11952062&imgNam=%2Fstuddocsdatad%2F119%2F119520620081.jpg

Appendix D

تسهيل مهمة بحث

State of Palestine
Ministry of Health
General Directorate of Education in
Health and Scientific Research

دولة فلسطين
وزارة الصحة
الإدارة العامة للتعليم الصحي
والبحث العلمي

Ref.: .....
Date:.....

الرقم: عس/ ٢٧٤٤/ ٢٠٢١
التاريخ: ٢٠٢١/٤/٢٦

الأخ مدير عام الادارة العامة للمستشفيات المحترم،،،

تحية واحترام،،،

الموضـــوع: تسهيل مهمة بحث

يرجى التكرم بتسهيل مهمة الطالبة: **آلاء احمد لطفي عواد، تخصص ماجستير الكيمياء الحيوية السريرية– جامعة النجاح**، لعمل بحث بعنوان:

"تكرار الاصابة والوصف الجزيئي للبكتيريا العقدية القاطعة في منطقة نابلس"

مع العلم أن مشرف الدراسة: د. امجد حسين ود. محمد القادي، حيث ستقوم الطالبة بجمع عينات مهبلية عن طريق الطاقم التمريضي بالمشفى، وتعبئة استبانة من المريضات (بعد اخذ موافقتهن)، بشرط عدم التعرض للمعلومات الشخصية، وذلك في:

- **مستشفى رفيديا**

على ان يتم الالتزام بجميع تعليمات واجراءات الوقاية والسلامة الصادرة عن وزارة الصحة بخصوص جائحة كورونا، وتحت طائلة المسؤولية.
على ان يتم تزويد الوزارة بنسخة PDF من نتائج البحث، التعهد بعدم النشر الا بعد الحصول على موافقة وزارة الصحة على النتائج.

مع الاحترام،،،

د. عبد الله القواسمي
مدير التعليم الصحي والبحث العلمي

نسخة: عميد كلية الدراسات العليا المحترم/ جامعة النجاح

P.O .Box: 14
Telfax.:09-2333901
scientificresearch.dep@gmail.com
ص.ب. 14
تلفاكس: 09-2333901

An-Najah
National University
Faculty of Graduate Studies

جامعة
النجـــاح الوطنية
كلية الدراسات العليا

التاريخ : 2021/12/7م

حضرة الدكتور قاسم دغلس المحترم
مدير عام مستشفى رفيديا الحكومي

الموضوع: تسهيل مهمة الطالبة/ الاء احمد لطفي عواد رقم تسجيل (11952062)
تخصص ماجستير الكيمياء الحيوية السريرية

تحية طيبة وبعد ،،،

الطالبة/ الاء احمد لطفي عواد، رقم تسجيل 11952062، تخصص ماجستير الكيمياء الحيوية السريرية في كلية الدراسات العليا، وهي بصدد اعداد الاطروحة الخاصة بها والتي عنوانها:

تكرار الاصابة والوصف الجزيئي للبكتيريا العقدية القاطعة في منطقة نابلس
Prevalence and Molecular Characterization of Group B Streptococcus (GBS) from Nablus Area

يرجى من حضرتكم تسهيل مهمتها في جمع عينات من اقسام الولادة والأطفال حديثي الولادة وعمل استبانة خاصة بموضوع البحث لجمع بيانات ومعلومات عن المطلوب في موضوع الرسالة.

علما بأن البيانات والمعلومات سوف تستخدم لأغراض البحث العلمي واستكمال مشروع البحث فقط.

شاكرين لكم حسن تعاونكم.

مع وافر الاحترام ،،،

أ.د. وليد صويلح
عميد كلية الدراسات العليا

فلسطين، نابلس، ص.ب 7-707 هاتف /2345115، 2345114، 2345113 (09)(972)+ فاكسميل 2342907(09)(972)
Nablus, P. O. Box (7) *Tel. 972 9 2345113, 2345114, 2345115 هاتف داخلي (5) 3200

An-Najah
National University
Faculty of Graduate Studies

جامعة
النجـــاح الوطنية
كلية الدراسات العليا

التاريخ : 2021/12/7م

حضرة الدكتور ماجد ابو جيش المحترم
مدير عام مستشفى الإتحاد النسائي

الموضوع: تسهيل مهمة الطالبة/ الاء احمد لطفي عواد رقم تسجيل (11952062)
تخصص ماجستير الكيمياء الحيوية السريرية

تحية طيبة وبعد ،،،

الطالبة/ الاء احمد لطفي عواد، رقم تسجيل 11952062، تخصص ماجستير الكيمياء الحيوية السريرية في كلية الدراسات العليا، وهي بصدد اعداد الاطروحة الخاصة بها والتي عنوانها:

تكرار الاصابة والوصف الجزيئي للبكتيريا العقدية القاطعة في منطقة نابلس
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يرجى من حضرتكم تسهيل مهمتها في جمع عينات من اقسام الولادة والأطفال حديثي الولادة وتعبئة استبانة خاصة بموضوع البحث لجمع بيانات ومعلومات عن المطلوب في موضوع الرسالة.

علماً بأن البيانات والمعلومات سوف تستخدم لأغراض البحث العلمي واستكمال مشروع البحث فقط.

شاكرين لكم حسن تعاونكم،

مع وافر الاحترام ،،،

أ.د. وليد صويلح
عميد كلية الدراسات العليا

فلسطين، نابلس، ص.ب 7-707 هاتف /2345115، 2345114، 2345113 (09)(972)+ فاكسميل 2342907(09)(972)
Nablus, P. O. Box (7) 707 Tel. 972 9 2345113, 2345114, 2345115 هاتف داخلي (5) 3200
Facsimile 972 92342907 www.najah.edu - email fgs@najah.edu

Appendix E

IRB Approval

An-Najah National University
Faculty of Medicine & Health Sciences
Institutional Review Board

جامعة النجاح الوطنية
كلية الطب وعلوم الصحة
لجنة اخلاقيات البحث العلمي

Ref :Mas. Nov. *2021/25*

IRB Approval Letter

Title of Research:

Prevalence and Molecular characterization of group B streptococcus (GBS) from Nablus Area

Submitted by:
Alaa Ahmad Awwad

Supervisor:
Amjad Hussein , Mohammad Qadi

Approved:
17 th Nov. 2021

Your Study Title **"Prevalence and Molecular characterization of group B streptococcus (GBS) from Nablus Area "** reviewed by An-Najah National University IRB committee and was approved on 17th Nov.2021.

Hasan Fitian, MD

IRB Committee Chairman IRB

Nablus - P.O Box :7 or 707 | Tel (970) (09) 2342902/4/7/8/14 | Faximile (970) (09) 2342910| E-mail : hgs@najah.edu

جـــامعـــــة النجاح الوطنية

كليـــة الدراســـــات العليـــا

تكرار الاصابة والوصف الجزيئي للبكتيريا العقدية القاطعة في منطقة نابلس

إعداد

آلاء أحمد عواد

إشراف

المشرف الأول: د. أمجد حسين

المشرف الثاني: د. محمد القادي

قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في الكيمياء الحيوية السريرية، من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس- فلسطين.

2023

تكرار الاصابة والوصف الجزيئي للبكتيريا العقدية القاطعة في منطقة نابلس

إعداد

آلاء أحمد عواد

المشرفان

د. أمجد حسين

د. محمد القادي

الملخص

الخلفية: بكتيريا المكورات العقدية المجموع "ب" (GBS)، موجبة لصبغة غرام، عادة ما تستعمر الجهاز التناسلي و الهضمي للإنسان بدون أعراض، حوالي (18%) من النساء عالميا تستعمرهن هذه البكتيريا بدون أعراض، قد تنتقل البكتيريا إلى (50%) من أطفال هؤلاء النساء مسببة عدوى قد تتطور لمرض خطير.

الأهداف: تهدف الدراسة لمعرفة مدى انتشار البكتيريا (GBS) في نابلس، فلسطين، أنماطها المصلية الموجودة، حساسيتها للمضادات الحيوية، جينات مقاومة المضادات الحيوية الموجودة في عزلات (GBS)، و عوامل الخطر المرتبطة باستعمار (GBS).

المنهجية: هي دراسة وصفية مقطعية، أُجريت في الفترة الممتدة ما بين شهر أيلول 2022 و كانون الثاني 2023 في مستشفى رفيديا الجراحي الحكومي و مستشفى الاتحاد، استُخدامت تقنية أخذ العينات المناسبة لدراسة 184 امرأة من الحوامل بعمر حمل 33 اسبوع فأعلى، جُمعت البيانات الوصفية للمشاركات باستخدام الاستبيانات. جُمعت 184 مسحة مهبلية من المشاركات. زُرعت العينات على وسط (UriSelect 4)، فُحصت المستعمرات الزرقاء الفاتحة على هذا الوسط لتأكيدها على أنها (GBS) باستخدام فحص (CAMP) و تقنية ال (PCR) ثم فُحصت استجابتها للمضادات الحيوية. استُخدمت تقنية ال (PCR) لفحص جينات مقاومة المضادات الحيوية، و الأنماط المصلية.

النتائج: بلغ معدل انتشار (GBS) 11.4%. وكان هناك ارتباط احصائي ملحوظ بين استعمار (GBS) للحوامل و الإقامة في المدينة (قيمة P : 0.014). كانت (100%) من العينات حساسة ل كل من: البنسلين، امبيسيلين، فانكومايسن، سيفترياكسون، لينزوليد، سيفيبم، سيفترياكسون، (71.4%) من العزلات مضادة لتيتراسايكلين، (28.5%) مضادة ل الايريثرومايسن، (23.8%) مضادة ل الكليندامايسن، (4.7%) مضادة ل الليفوفلوكساسين. كان جين مقاومة المضادات الحيوية (*erm*B) موجود في (19%) من العزلات و جين (*mef*A) موجود في (4.7%) من العزلات. غالبية العزلات (90%) كانت تمتلك جين (*tet*M). كان النمط المصلي III موجود بنسبة (42.8%) من العينات، النمط المصلي V (23.8%)، النمط المصلي II (14.2%)، النمط المصلي Ib (9.5%)، النمط المصلي IV (4.7%) بينما كانت (4.7%) غير معروفة النمط المصلي.

الخلاصة: استخدمت الدراسة آلية مزدوجة يمكن مقارنتها بإرشادات (CLSI) الدولية لإثبات عزلات (GBS)، أنماطها المصلية، و استجابتها للمضادات الحيوية. وجدت (GBS) في (11.4%) من الحوامل اللواتي تم فحصهن. ارتبط استعمار (GBS) احصائيا ب بالعيش في المدينة (قيمة P : 0.014). وكانت (100%) من العزلات حساسة للبنسلين بينما كانت (71.4%) من العزلات مضادة للتيتراساكلين. كانت معظم العزلات تمتلك جين المقاومة (*tet*M)، كان النمط المصلي (III) هو النمط السائد (42.8%).

الكلمات المفتاحية: بكتيريا المكورات العقدية المجموعة "ب"، العقدية مجموعة ب، الانماط المصلية، الجينات المقاومة للمضادات الحيوية.