The Islamic University–Gaza

Research and Postgraduate Affairs

Faculty of Science

Master of Biological Sciences/
Medical Technology

الجامعة الإسلامية –غزة

شئون البحث العلمي والدراسات العليا

كليـــة: العلـــوم

ماجستير علوم حياتية/تحاليل طبية

Prevalence of *Streptococcus agalactiae* Colonization among Pregnant Women in Gaza city, Palestine

دراسة مدى انتشار البكتيريا العقدية القاطعة للدر بين النساء الحوامل في مدينة غزة، فلسطين

Soheer E. Esleem

Supervised by

Prof. Dr. Abdelraouf A. Elmanama
Prof. of Microbiology

Dr. Nabil A. El Aila
Assistant Prof. of Microbiology

A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of
Master of Biological Science/Medical Technology

March/ 2017

إقـــــــــــرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

Prevalence of Group B Streptococcus Colonization among Pregnant Women in city strip, Palestine.

دراسة مدي انتشار البكتيريا العقدية القاطعة للدر بين النساء الحوامل في مدينة غزة، فلسطين.

أقر بأن ما اشتملت عليه هذه الرسالة إنما هو نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل أو أي جزء منها لم يقدم من قبل الآخرين لنيل درجة أو لقب علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

I understand the nature of plagiarism, and I am aware of the University's policy on this.

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted by others elsewhere for any other degree or qualification.

Student's name:	سهير عليان اسليم	اسم الطالب:
Signature:	Soheer	التوقيع:
Date:	5/3/2017	التاريخ:

بسم الله الرحمن الرحيم

الجامعة الإسلامية غزة
The Islamic University of Gaza

مكتب نائب الرئيس للبحث العلمي والدراسات العليا هاتف داخلي: 1150

الرقم: Ref: ج س غ/35/

التاريخ: Date: 2017/04/1

نتيجة الحكم على أطروحة ماجستير

بناءً على موافقة شئون البحث العلمي والدراسات العليا بالجامعة الإسلامية بغزة على تشكيل لجنة الحكم على أطروحة الباحثة/ **سهير عليان اسليم** لنيل درجة الماجستير في **كلية *العلوم*** قسم **العلوم الحياتية- تحاليل طبية** وموضوعها:

دراسة مدى انتشار البكتيريا العقدية القاطعة للدر بين النساء الحوامل في مدينة غزة، فلسطين

Prevalence of Streptococcus agalactiae Colonization among Pregnant Women in Gaza city, Palestine

وبعد المناقشة التي تمت اليوم السبت 02 رجب 1438هـ، الموافق 2017/04/1 الساعة العاشرة صباحاً في قاعة مؤتمرات مبنى اللحيدان، اجتمعت لجنة الحكم على الأطروحة والمكونة من:

أ.د. عبد الرؤوف علي المناعمة	مشرفاً و رئيساً	.....
د. نبيل عبد الله العيلة	مشرفاً	.....
أ.د. محمد عيد شبير	مناقشاً داخلياً	.....
د. باسم محمد عايش	مناقشاً خارجياً	.....

وبعد المداولة أوصت اللجنة بمنح الباحثة درجة الماجستير في كلية *العلوم*/ قسم **العلوم الحياتية - تحاليل طبية**.

واللجنة إذ تمنحها هذه الدرجة فإنها توصيها بتقوى الله ولزوم طاعته وأن يسخر علمها في خدمة دينها ووطنها.

والله ولي التوفيق،،،

نائب الرئيس لشئون البحث العلمي والدراسات العليا

أ.د. عبدالرؤوف علي المناعمة

Abstract

Background: *Streptococcus agalactiae* is one of the most important causal agents of serious neonatal infections. Early detection of perinatal rectovaginal carriage of Group B *Streptococcus* (GBS) is important in the prevention of newborn infections.

Objectives: To evaluate the prevalence of group B Streptococcal (GBS) colonization among pregnant women in Gaza city. Also to determine the susceptibility pattern of GBS isolates against different antimicrobial agents. In addition, to investigate possible risk factors for GBS colonization.

Methods: Two hundreds rectovaginal swabs collected from pregnant women from Al Shifa hospital were screened for GBS colonization. Standard microbiological methods according to the Atlanta Centers for Disease Control and Prevention (CDC) recommendations were used to isolate and identify GBS from rectovaginal swabs. Selective and chromogenic culture in addition only for 100 sample PCR was employed for the detection of GBS. Antimicrobial susceptibility testing (AST) was performed according to CLSI guidelines on 42 GBS isolates using clindamycin, erythromycin, penicillin G, tetracycline and vancomycin.

Results: Out of 200 pregnant women, 42 (21%) were colonized by GBS. The Sensitivity, Specificity, Positive predictive value and Negative predictive value of PCR was 54%, 88%, 76%, 72%, respectively. Of the 42 examined GBS isolates, (76%) were susceptible to vancomycin, (57%) isolates were sensitive to penicillin, (50%) to erythromycin. (48%), (31%) to tetracycline and clindamycin respectively.
There was no statistically significant association observed for GBS colonization with chronic disease, complications like (Previous abortion – delivery at <37weeks gestation-premature birth- intrauterine death – endometrtitis**)**, and previous antibiotic intake
($p > 0.05$).

Conclusion: Our results showed high prevalence of GBS in Gaza City, Palestine. Despite the fact that PCR is well known for its high sensitivity, low sensitivity was obtained in this study. Vancomycin was the most effective antibiotic against GBS isolates. We, recommend a screening-based strategy to detect GBS in Palestinian pregnant women.

Keywords: *Streptococcus agalactiae*, polymerase chain reaction, culture, pregnancy, Gaza city.

ملخص عربي

Arabic Abstract

المقدمة: تعتبر بكتيريا ستربتوكوكس أجلاكتيا (**العقدية القاطعة للدر**) أحد أهم العوامل المسببة للمرض عند الأطفال. حيث أن الاكتشاف المبكر لهذا النوع من البكتيريا عند النساء الحوامل له أهمية في منع الالتهابات لدى الأطفال حديثي الولادة.

الهدف: تقدير نسبة مدى انتشار بكتيريا ستربتوكوكس أجلاكتيا بين الحوامل في مدينة غزة. وكذلك معرفة عوامل الخطر المتعلقة بمدى انتشار هذا النوع من البكتيريا. وقد تم فحص استجابة هذه البكتيريا للعديد من المضادات الحيوية.

الطرق والأدوات: لقد تم جمع 200 مسحة مهبلية من الحوامل ما بين 35-37 أسبوع من الحمل من النساء الحوامل في مستشفى الشفاء وتشخيص البكتيريا باستخدام مزرعة الكروم وفحص تفاعل البوليميريز المتسلسل. كما تم إجراء الفحوصات التأكيدية منها صبغة جرام وفحص إنزيم الكتاليز, وفحص الكامب, وفحص الهيبورييت. كما تم عمل اختبارات الحساسية باستخدام خمسة أنواع مختلفة من المضادات الحيوية وهي الفانكوميسين, البنسلين, التتراسيكلين, الارثروميسين والكلنداميسين.

النتائج: كانت نسبة انتشار البكتيريا بين الحوامل باستخدام طريقة الزراعة على الكروم أجار 21%، وقد كانت نسبة الحساسية والتخصصية والقيمة التنبؤية الإيجابية والقيمة التنبؤية السلبية لفحص تفاعل البوليميريز المتسلسل هي 54%، 88%, 76%، 72% على التوالي. وكان أفضل المضادات الحيوية فعالية ضد هذا النوع من البكتيريا هو الفانكوميسين حيث كانت حساسيته 76%. ولم نجد أي دلالة إحصائية بين معدل انتشار البكتيريا والعوامل المتعلقة بالنساء الحوامل المصابة بالبكتيريا (إجهاض سابق، ولادة مبكرة، جنين غير كامل النضوج، موت الجنين داخل الرحم، موت الجنين بعد الولادة مباشرة، وجود أمراض مزمنة، وعوامل أخرى). (P>0.05)

الاستنتاج: بينت نتائج هذه الدراسة أن نسبة انتشار ستربتوكوكس أجلاكتيا مرتفعة بين النساء الحوامل في مدينة غزة، وأنه على الرغم من أن فحص تفاعل البوليميريز المتسلسل معروف بحساسيته العالية، فإنه قد تم الحصول على نسبة منخفضة في هذه الدراسة، وتبين أن أقوى أنواع المضادات الحيوية فعالية هو الفانكوميسين. ومن خلال هذه الدراسة نوصي باستخدام هذا الفحص بشكل روتيني للحوامل في العيادات والمستشفيات في مدينة غزة.

الكلمات المفتاحية: ستربتوكوكس أجلاكتيا, تفاعل البوليميريز المتسلسل, الحمل, مزرعة البكتيريا, مدينة غزة.

Dedication

To my parents

My mother and my father

My son Barra

My brothers Samy, Sameer, Nabile, Hassan, Osama and Mohammed

My sisters Somia, Namia, Nabila, Naela

My friends

All researchers who are working to improve the quality of life

Dedication is most expressed to the Palestinian people who have suffered and will be struggling with the persistence to have a free Palestine

My beloved Islamic university-Gaza

and to all I love.

Soheer E. Esleem

Acknowledgment

All praises and glory are due to Allah for all the bounty and support granted me. It is impossible to convey, in a couple of sentences, my gratitude to many people for helping me to learn and whose cooperation made this work possible.

I would like to express my gratitude to my supervisors **Prof. Dr. Abdelraouf Elmanama**, Professor of Microbiology, Faculty of medical Science, The Islamic University of Gaza, for his planning and initiating this work and for his continuous support, encouragement and close supervision that lead to the emergence of this work in its current form.

Special thanks go to **Dr. Nabil A. El Aila**, Molecular Microbiology, Faculty of Science, Al-Aqsa University of Gaza for his kind assistance, and for his scientific advices and valuable discussion throughout reading of thesis.

Special thanks for the dearest persons to me my **mother**, my **father**, my brothers and my **sisters** for their support and encouragements.

I am very grateful for **Mr. Mohammed El-Madhoon** for helping me in the practical part of research.

My special thanks to **Mr. Abed EL- Rahman Hamad** for his help in statistical analysis.

I wish to extend my thanks for all the doctors of the Delivery Department for helping in sample collection.

Finally, I am in deepest gratitude to all individuals who contributed in some way or another to this work.

List of content

Declaration II
Arabic Abstract IV
Dedication V
Acknowledgment VI
List of content VII
List of Tables X
List of Figures XI
List of Abbreviations XII
Chapter 1 2
Introduction 2
1.1 Overview 2
1.2 Objectives of the Study 4
1.3 Significance of the Study 4
Chapter 2 7
Literature Review 7
2.1 GBS classification and identification 7
2.2. Epidemiology of Group B Streptococci 8
2.3. Pathogenesis of Group B Streptococcus infection 12
2.4 Clinical features 15
2.4.1 Early-onset GBS 15
2.4.2 Late-onset GBS infection 16
2.5. Laboratory diagnosis of Group B streptococci: 17
2.5.1. Isolation of GBS by chrome agar 17
2.5.2 Immunological assay 18
2.5.3 Nucleic Acid Testing Assays 19
2.6. Management of GBS colonization and infections 21
2.6.1. Intrapartum antibiotic chemoprophylaxis (IAP) 21
2.6.2. Asymptomatic preterm neonates 23
2.6.3. Asymptomatic term and late preterm neonates 23
2.7. Prevention and control 24

Chapter 3 27
Material and methods 27
3.1 Materials 27
3.1.1 Apparatus 27
3.1.2 Equipments 27
3.1.3 Reagents and Stain 27
3.1.4 Antibiotic used in the study 28
3.1.5 Culture media 28
3.2 Methodology 28
3.2.1 Study design 28
3.2.2 Ethical considerations 29
3.2.3 Questionnaire 29
3.2.4 Sample collection 29
3.2.5 Culturing procedure of rectovaginal samples 30
3.2.6 Confirmatory tests for GBS detection: 30
3.2.6.1 Gram stain 30
3.2.6.2 Catalase test 31
3.2.6.3 CAMP test 31
3.2.6.4 Hippurate Hydrolysis test 32
3.2.7 Molecular techniques 32
3.2.7.1 DNA extraction of Bacterial isolates: 32
3.2.7.2 DNA extraction of clinical isolates 33
3.2.7.3 PCR primers 33
3.2.7.4 Conventional PCR Assay 33
3.2.8 Antibiotic susceptibility testing 34
3.2.9 Data analysis 34
Chapter 4 36
Results 36
4.1 Prevalence of GBS using conventional methods: 36
4.2 Detection of Group B Streptococci using PCR from alkaline DNA extracts: 36
4.3 Detection of Group B Streptococci using PCR directly from clinical samples: 37
4.4 Antimicrobial susceptibilities of GBS 39
4.5 Risk factors: 41
Chapter 5 44
Discussion 44

5.1 Prevalence of Group B Streptococci using conventional methods:..... 44
5.2 Detection of GBS carriage rate by PCR: 46
5.3 Antimicrobial Susceptibilities of GBS: 47
5.4 Risk factors for GBS carriage pregnant women 49
Chapter 6..... 51
Conclusion and Recommendation 51
6.1 Conclusion 51
6.2 Recommendations..... 51
References:..... 54
Annexes 65

List of Tables

Table (4.1): Prevalence of GBS colonization in 200 pregnant women at 35 - 37 weeks. **.....** 36

Table (4.2): Comparison between culture and PCR results. **.....** 38

Table (4.3): Antibiotic susceptibilities of 42 GBS isolates from pregnant women.....40

Table (4.4): Phenotypic resistance patterns amongst 42 GBS isolates. **.....** 40

Table (4.5): Variables associated/not associated with Group B Streptococcus colonization in pregnant women (35-37) weeks of gestation. **.....** 42

List of Figures

Figure (2.1) GBS colonization in pregnant women…… …….....13
Figure (3.1) GBS and non-GBS on chromogenic agar……..... 30
Figure (3.2) Gram stain of *Streptococcus agalactiae*……..31
Figure (3.3) Catalase test for *S. agalactiae*…… …….…….31
Figure (3.4) CAMP test for *S. agalactiae*……..……32
Figure (3.5) Hippurate hydrolysis test for *S. agalactiae*……32
Figure (4.1) A representative result of GBS PCR ……...……37
Figure (4.2) Result of *GBS* PCR directly from clinical sample……...… 38
Figure (4.3) Appearance of antimicrobial susceptibility pattern……..……..39

List of Abbreviations

AST	Antimicrobial susceptibilities test
CA	Chrome agar
CBC	Complete blood cell count
CDC	Centers for Diseases Control and Prevention
CFU	Colony Forming Unit
CLSI	Clinical and Laboratory Standards Institute CLSI
CNS	Central nervous system
CPS	Capsular poly saccharide
CRP	C-reactive protein
DNA	Deoxyribonucleic Acid
EOGBSD	Early-onset GBS disease
GBS	Group B Streptococcus
IL-1	Interleukin-1.
IAP	Intrapartum Antibiotic Prophylaxis
LOGBSD	Late-onset GBS disease
Mg	Milligram
NICHD	National Institute of Child Health and Human Development
PCR	Polymerase chain reaction
PROM	Premature Rupture of Membranes
THB	Todd Hewitt broth
TNF	Tumor necrosis factor
US	United State
WHO	World Health Organization
µg	Micro gram

CHAPTER 1
INTRODUCTION

Chapter 1

Introduction

1.1 Overview

Since the mid-1960s, group B Streptococcus (GBS) has become the major cause of bacterial infections in the perinatal period, including bacteremia, amnionitis, endometritis, and urinary tract infection in pregnant women as well as focal and systemic infections in newborns (Samuel, 2002).

It is a relatively rare cause of infection in older children and non-pregnant adults (Apgar, Greenberg & Yen., 2002). Initial case series reported case fatality rates as high as 50%. In the early 1980s, clinical trials demonstrated that administering antibiotics during labor to women at risk of transmitting GBS to their newborns could prevent invasive disease in the first week of life (Verani, McGee & Schrag, 2010).

Since the 1970s, GBS has been recognized as the most important infectious cause of morbidity and mortality in newborn infants. Despite decrease in mortality during the last decades, early onset GBS disease remains a serious neonatal condition, which may cause severe neurological damage (Valkenburg-van et al., 2006).

Group B *Streptococcus* is encapsulated Gram-positive cocci that usually produce a narrow zone of beta-hemolysis on blood agar. It belongs to Lancefield group B **(**Mohammed, Asrat, Woldeamanuel & Demissie, 2012; Okon, Usman, Umar & Balogun, 2013). There are 10 GBS serotypes (Ia, Ib, and II to IX) based on variations in the capsular polysaccharide (CPS), a major virulence factor that helps the microorganism to evade the host's defense mechanisms (Melin, 2011; Ueno et al., 2012).

Group B *Streptococcus* is the most frequent pathogen isolated from neonates with invasive bacterial disease and responsible for serious infections in newborns such as

pneumonia, septicemia and meningitis (Fashina, 2008; Mohammed et al., 2012). GBS neonatal infection is divided into two categories:

Early-onset GBS disease (EOGBSD), which occurs within the first week of life, and late-onset GBS disease GBS (LOGBSD), which occurs between one week to 3 months of age (Maisey, Doran & Nizet, 2008; Faro et al., 2010; Dagnew et al., 2012).

The most likely reservoir of GBS is the gastrointestinal tract, and the most frequent site of secondary spread is the genitourinary tract. The neonates become colonized with GBS by the aspiration of infected amniotic fluid or by vertical transmission during the passage through the colonized vaginal canal. It is also a cause of cystitis, amnionitis, endometritis, and stillbirth in the pregnant women. The infants who survive are often left with developmental disabilities, including mental retardation, hearing or vision loss and speech problems (Chaudhry, Akhtar & Balouch, 2010).

In pregnancy, GBS can infect the amniotic fluid, and during labor, vertical transmission may infect the newborn leading to neonatal sepsis and meningitis. Approximately 10-30 % of women are colonized with GBS in vagina during pregnancy and 50-75 % of their infants acquire this organism through birth canal (Fatemi et al., 2009 and Fatemi, Zeraati, Talebi & Asgari, 2010).

Colonization during pregnancy may be transient, chronic or intermittent, and is asymptomatic in the majority of cases (Busetti, D'Agaro & Campello, 2007; Ulett et al., 2009).

Maternal colonization with GBS in the genitourinary or gastrointestinal tract and transmission to the infant during the labor and delivery process is the principal risk factor for early-onset invasive GBS disease (Baker & Edwards, 1995; Apostol et al., 2009; El Aila et al., 2010; Madzivhandila et al., 2011). Moreover, urinary tract infection sustained by GBS, either symptomatic or asymptomatic, is considered a risk factor for neonatal infections (Busetti et al., 2007).

Palestinian Ministry of Health reported a death rates among infant less than one year of 12 per 1000 live birth in 2015 and 13 per 1000 live birth in 2016. GBS infection might be one of the causes of mortality among infant.

1.2 Objectives of the Study

General Objective

The general objective of the present study is to determine the prevalence of group B Streptococcus colonization among pregnant women (35-37) week in Gaza city, Palestine.

Specific objective

- To detect and identify *S. agalactiae* from rectovaginal swabs among pregnant women using chromogenic selective medium.
- To compare the diagnostic value of PCR and Chrom agar for the detection of GBS.

- To determine the susceptibility pattern of GBS isolates to common antimicrobial agents.
- To investigate possible risk factors for GBS colonization.

1.3 Significance of the Study

Group B *Streptococcus* (GBS) is now recognized to be an important cause of maternal and neonatal morbidity and mortality in many parts of the world (Shet, Ferrieri, 2004; Joachim, Matee, Massawe & Lyamuya, 2009; Veit et al., 2011; Ezeonu & Agbo, 2014).

To the best of our knowledge, there are no previous published studies conducted in Gaza that had addressed the prevalence of GBS colonization among pregnant women. Lack of data in this area hinders the implementation of prophylaxis program in pregnant women. Therefore, this study was conducted to determine the prevalence

of GBS colonization, and to identify risk factors related to GBS among pregnant women attending antenatal care at Al Shifa hospital, Gaza, Palestine.

The results of this study will provide original data about the prevalence of colonization of GBS and risk factors related to GBS among pregnant women in the current study area. Moreover, it may provide baseline information to formulate a policy and program for treatment, prevention and control efforts regarding perinatal GBS diseases in the country at large.

CHAPTER 2

LITERATURE REVIEW

Chapter 2

Literature Review

2.1 GBS classification and identification

S. agalactiae is a gram positive cocci belonging to the phylum Firmicutes (Ryan & Ray., 2004). Cell division in this genus occurs along a single axis in these bacteria, thus they grow in chains or pairs, hence the name from Greek στρεπτός streptos, meaning easily bent or twisted, like a chain (twisted chain).

GBS is a Gram-positive, non-motile, non-spore-forming, catalase-negative, it is usually β-hemolytic, positive Bile-Esculin test (Kim et al., 2011; Munari et al., 2012). The organism has a polysaccharide capsule that defines the individual serotypes of GBS. There was 10 immunologically unique capsular polysaccharides (Ia, Ib, II-IX) of GBS have been identified. The most common serotypes causing neonatal infections are Ia, III and V. whose prevalence varies according to geographical location, time of study and ethnicity (Slotved, Kong, Lambertsen, Sauer & Gilbert, 2007). Thus, the continuous monitoring of circulating GBS isolates is important in assessing changes in GBS serotype distribution, which is essential for the development of polysaccharide-based vaccines suitable for different geographical areas (Johri et al., 2006; Rodriguez-Granger et al., 2012; Baker al., 2014).

S. agalactiae is CAMP test positive, It is based on the fact that GBS produce an extracellular protein called the CAMP factor. This protein acts synergistically with *Staphylococcus aureus* β-toxin to hydrolyse red blood cells, (Michael & Burton, 2011). A positive test for CAMP factor appears as "arrowhead" hemolysis between the junction of growth of *S. aureus* and GBS. No enhanced or "arrowhead" hemolysis was seen when the test isolate was not GBS.

GBS harbors an important number of virulence factors, the most important being the capsular polysaccharide rich in sialic acid (Edwards,Nizet .,2011; Rajagopal .,2009) and a pore-forming toxin, β-hemolysin, that today is considered identical to the GBS pigment (Rosa-Fraile, Dramsi, Spellerberg., 2014; Whidbey et al., 2015; Leclercq et al., 2016).

2.2. Epidemiology of Group B Streptococci

GBS can be colonized in the lower gastrointestinal and genital tract for both men and women, which serve as the natural reservoir for GBS and is most likely source of vaginal colonization and the bacteria can be transmitted sexually., most studies reporting colonization rates in sexually active women over 20% (Pignanelli et al., 2015). *Streptococcus agalactiae* or GBS is widely distributed in nature and as a normal flora of the gastrointestinal tract and may colonize the vagina permanently or transiently in about one/third of women (Veit et al., 2011; Okon et al., 2013). GBS colonize the vagina and the rectum, and the colonization of these regions is a risk factor for subsequent infection in pregnant women and newborns.

An estimated 20-30% of all pregnant women are GBS carriers and the colonization can be permanent but also intermittent/transient and 50–70% of infants born to these women will themselves become colonized with the bacterium. Selective culture methods are used to optimize the isolation of GBS from a complex microflora in the vagina and rectum (Gibbs, Schrag & Schuchat, 2004; Puopolo, Madoff & Eichenwald, 2005).

Culture status can vary between pregnancies, Because of colonization can be intermittent or transient, and therefore screening during each subsequent pregnancy is advised. The predictive value of prenatal screening improves with shorter intervals between culture and delivery (Verani et al., 2010; Sri-Budayanthi & Hariyasa-Sanjaya, 2013). So that prenatal screening at 35–37 weeks of gestation is currently recommended. Chemoprophylaxis with penicillin at delivery should be based upon the 35–37 week culture even if earlier cultures were obtained (Gibbs et al., 2004).

Globally, asymptomatic colonization with GBS is common in pregnant women. Maternal GBS colonization varies according to population characteristics (e.g., age, parity, socioeconomic status, presence of sexually transmitted diseases, sexual behavior, ethnic group and geographic area) (Kim et al., 2011 and Sharmila, Joseph, Arun Babu, Chaturvedula & Sistla, 2011).

Prevalence of maternal carriage of GBS in developing countries, including populations in tropical Africa, is almost similar to that identified in populations in the United States. Different Studies from Kenya, South Africa, Zimbabwe and Malawi suggested that GBS is emerging as an important cause of neonatal sepsis in Africa (Quiroga, Pegels, Oviedo, Pereyra & Vergara, 2008).

GBS emerged as a significant neonatal and maternal pathogen in the United States (US) and Western Europe with reported mortality rates of 15 to 50% during the 1970s and 1980s. In the US, 10 to 35 % of pregnant women at the time of delivery are asymptomatic carriers of GBS in the genital and gastrointestinal tract (Shet & Ferrieri, 2004).

GBS can be isolated in 4% to 30% of pregnant women; worldwide, (Simoes et al., 2007; Mohammed et al., 2012). Neonates who are born to the colonized mothers GBS carriage rate is 40–70 % (Chaudhry et al., 2010). The prevalence of maternal colonization by GBS vary widely throughout the world depends on culture methods, including the number and type of sites cultured and type of medium used, time of pregnancy, origin (region) and race (Quiroga et al., 2008; Mohammed et al., 2012).

Colonization of GBS among 5020 pregnant women in North-Eastern Italy (2007) is reported to occur in 901 (17.9 %) were positive for GBS. On 728 positive samples, the results of selective direct plating and selective broth enrichment were compared. A total of 561 (77.1 % of positive samples, corresponding to 13.9 % of patients) were positive on direct selective agar; an additional 167 isolates (22.9 % of samples,

4.1 % of patients) were recovered from the Lim broth (Todd-Hewitt broth supplemented with colistin (10 μg/ml) and nalidixic acid (15 μg/ml) subculture.

India/Pakistan 12%, America 14 %, Asia-Pacific 19 %, Sub-Saharan Africa 19 %, Middle-East/North Africa 22 % (Chaudhry et al., 2010). Cross-sectional studies in the North East region of Brazil (2008) have found GBS colonization in the mothers rates was 20.4 %. In that study, no association was indicated between the sociodemographic variables or gynecological-obstetrical antecedents and a larger presence of GBS colonization (Costa et al., 2008). The incidence of GBS infection In Italy (2008) among pregnant women was 18% (Savoia, Gottimer, Crocilla & Zucca, 2008).

Regarding neonatal colonization by GBS, it was found that neonates born from the colonized mothers with a complicated pregnancy were more often colonized with GBS than those from the mothers with a normal pregnancy (35 % versus 26.7 %) (Strus et al., 2009). The study conducted in Switzerland (2009) reported that among 1316 pregnant women, the prevalence of GBS colonization was 21 % (Rausch, Gross, Droz, Bodmer, & Surbek, 2009).

Research applied in Bali-Indonesia, revealed that the prevalence of GBS colonization in pregnant women detected with culture method using Blood agar (BA) plates and ChromAgar (CA) plates without Todd Hewitt broth (THB) was 9.4 %, whereas the prevalence with culture method using BA and CA enriched by THB was 31.3 %. Moreover, GBS showed resistance to penicillin (10%), ampicillin (20%), erythromycin (20%), and cefazolin (20%). All GBS isolates were sensitive to chloramphenicol and ceftriaxone. It is indicated that THB enrichment medium seems to be promising as a screening method for GBS colonization in pregnant women in Bali **(**Sri-Budayanthi & Hariyasa-Sanjaya, 2013).

Screening studies performed at two tertiary care hospitals in Karachi, Pakistan (2013) reported that the overall prevalence of colonization was 69 (17%) among 405 pregnant women. The colonization was found to be significantly associated inversely

with the body mass index of the carrier of GBS (Najmi, Jehan, Sikandar & Zuberi, 2013).

Another study conducted at Hedayat hospital, Tehran, Iran revealed that among the 330 women, 68 (20.6%) were positive for GBS. In that study, statistical analyses showed no significant relationship between demographics, reproductive histories and obstetric characteristics such as history of abortion, Premature Rupture of Membranes and gestational age of subjects with the test results. Solely the antibiotic therapy was associated with GBS colonization (Fatemi et al., 2009).

The broad-range bacterial studies in Ethiopia identified, 200 postpartum women and 80 newborn infants were investigated for GBS carriage at Gondar College of Medical Sciences in 1987, using swabs from the vagina and rectum and from the throat and external ear, respectively. The rate of Colonization 5 % in the neonates and of 9% was found in the mothers (Schmidt, Halle, Halle, Mohammed & Gunther, 1989).

Health Center in Ethiopia, Hawassa documented a prevalence of GBS colonization was 21%. No statistically significant association was observed for GBS colonization with any of socio-demographic characteristics of the study subjects including age, occupation, type of contraceptives used, types of gravida, and number of antenatal clinic visits. All GBS strains were susceptible to penicillin, ampicillin, vancomycin and gentamicin. Resistance was observed against erythromycin (6.9%), tetracycline (48.2%), ceftriaxone (10.3%), chloramphenicol (51.7%), ciprofloxacin (13.8%) and norfloxacin (10.3%) (Mohammed et al., 2012).

In a study done on 113 sample taken from recto-vaginal swab in Maputo, Mozambique (2008), the prevalence showed that, 2 (1.8%) was positive for GBS (De Steenwinkel et al., 2008). Mother colonization rate was found to be significantly higher in the rural areas (60%) as compared to the urban areas (46 %) in the study conducted in Zimbabwe in 2006. GBS colonization persistence was shown to be more in rural (48%) than in urban women (12%). Similarly, baby colonization was

also shown to be more in the rural (23%) than in urban area (5%) (Mavenyengwa et al., 2010).

Screening study in AlFayom University Hospital, Egypt conducted among 95 pregnant women, and revealed that 17 (17.89%) were GBS positive (Elbaradie, Mahmoud & Farid, 2009). An antenatal clinic of a tertiary hospital study in Northeastern, Nigeria showed that of the 133 pregnant women, 13 (9.8%) was GBS positive. In that study, statistical significance difference was observed between the age groups and GBS isolation ($p<0.05$). However, other sociodemographic factors such as occupation and education level and obstetric factors did not show any statistically significant association with GBS colonization ($P>0.05$) (Okon et al., 2013).

There are also variations in early onset GBS (EOGBS) infection rates among racial groups and geographic region (Pulver et al., 2009). The incidence among black American infants increased 70% during the years 2003-2005, whereas incidence rates decreased among white Americans infants. In 2005, among black infants in the United States, the rate of EOGBS infection was 0.84 per 1000 live births compared with 0.24 per 1000 deliveries in white infants (Pulver et al., 2009). Geographic variations in EOGBS infection rates have also been noted, ranging from 0.53 cases per 1000 live births in Tennessee to 0.14 in Oregon (CDC, 2002). Generally in the United States prior to the introduction of intrapartum prophylaxis the overall incidence of neonatal GBS infections were approximately 2 per 1000 live births (Baker & Edwards, 2001).

2.3. Pathogenesis of Group B Streptococcus infection

GBS are found in the genital tract, urinary tract, throat, and respiratory tract. Genital tract colonization poses the most important threat to the newborn, because of exposure during the birth process, and to the mother, because of ascending infection after the membranes rupture. Colonization is found in the rectum more often than the vagina, in the vagina more than the urethra, and in the urethra more than the cervix (Figure: 2.1).

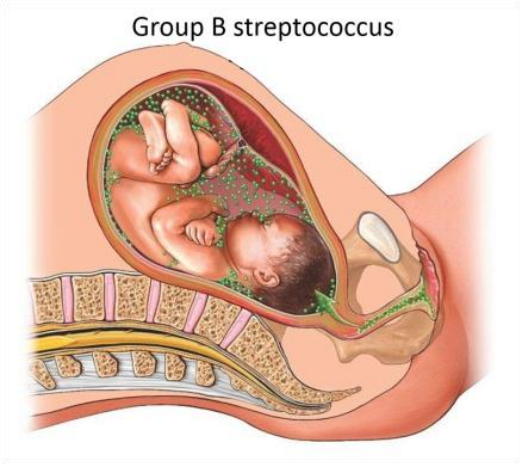


Figure (2.1): GBS colonization in pregnant women.

http://www.fullcirclehealthcareinc.com/group-b-strep-testing.html

GBS pathogenesis can be grouped systematically into: a) adherence to epithelial surfaces, b) penetration of host cellular barrier, c) avoidance of immunologic clearance mechanisms and d) inflammatory activation.

a) Adherence to epithelial surfaces

GBS adheres to a variety of human cells including vaginal epithelium, placental membranes, and respiratory tract epithelium and blood brain barrier endothelium. Optimum adherence occurs at the acidic pH of vaginal mucosa, allowing GBS to occupy a niche that places infants at risk of vertical transmission.

Low affinity GBS attachment to epithelial cells is mediated by its amphiphilic cell wall associated lipotechoic acid, while higher affinity interactions with host cells are mediated by a series of size-variable, pronase-sensitive; hydrophobic GBS surface proteins. GBS effectively binds the extracellular matrix components fibronectin, fibrinogen and laminin **(**Schwartz-Linek, Hook & Potts, 2004). Unusually well adapted GBS binds to immobilized fibronectin to facilitate mucosal colonization, but not to soluble fibronectin that may serve as an opsonin for phagocyte recognition (Schwartz-Linek, Hook & Potts, 2004).

b) Penetration of host cellular barriers

The organism can pass through placental membranes and weaken its strength. As a result of this process, GBS may access the fetus within the amniotic cavity, cause placental membrane rupture or trigger premature delivery. After aspiration of infected amniotic or vaginal fluid, the newborn lung is the primary focus of GBS infection. From lung, the organism can enter into the bloodstream and then spreads to various organs and tissues (Doran & Nizt, 2004).

c) Avoidance of immunologic clearance:

Once GBS damage cellular barriers to reach deeper tissues, an immunologic response is activated to clear the organism. The most important response includes host phagocytic cells such as neutrophils and macrophages. The effective uptake and killing of GBS by these cells requires opsonization of the bacterium by specific antibodies and serum complement (Doran & Nizt, 2004).

Neonates are susceptible to GBS invasive disease because of quantitative or qualitative deficiencies in phagocytic cell function, specific anti-GBS immunoglobulin, or the classic and alternate complement pathways. In addition to these newborn host susceptibilities, GBS possess several virulence factors that seek to thwart each of the key components of effective opsonophagocytic killing (Doran & Nizt, 2004).

Majority of GBS associated with human disease are encapsulated. The serotype specific epitopes of each polysaccharide are created by different arrangements of four component sugars (glucose, galactose, N-acetylglucosamine and sialic acid) into a unique repeating unit, but all these structures contain a terminal sialic acid (Neu5Ac) bound to galactose in an α2—3-linkage. GBS capsule biosynthesis is encoded in the single long transcript of a 16-gene operon now it is fully sequenced in type Ia, III and V strains (Chaffin, Beres, Yim & Rubens, 2000).

The GBS terminal α2—3 Neu5Ac capsular components are identical to a sugar epitope widely present on the surface of all mammalian cells. The terminal α2—3

linked Neu5Ac is over expressed in humans who in evolution have lost the genes to produce the alternative sialic acid Neu5Gc. The sialylated GBS surface capsule protects GBS by interference with opsonophagocytosis **(**Doran, Liu & Nizet, 2003).

d) Activation of inflammatory responses

Resisting phagocytic clearance in the bloodstream, GBS may disseminate to reach end organs such as bones, joints and the central nervous system (CNS). As the result of this, the host inflammatory response to invasive infection mounts, and development of the sepsis syndrome and multi-organ dysfunction often occurs.

Peptidoglycan and other GBS components associated with the cell wall without the surface polysaccharide capsule, appear to be the most provocative agents in triggering host cytokine cascades, in particular the proximal mediators, tumor necrosis factor-(TNF) and interleukin-1 (IL-1) (Doran and Nizt, 2004). In neonates two syndromes exist: early-onset (< 7 days old) and late-onset (7-90 days old). Both include sepsis, pneumonia and meningitis.

2.4 Clinical features

2.4.1 Early-onset GBS

Early-onset GBS infection is caused by vertical transmission whereby the organism ascends into the chorioamnionic space prior to birth. Between 75% and 80% of newborns with early-onset GBS infection are born at term. However, premature newborns are more likely to develop early-onset GBS infection than full-term newborns (McDonald & Chambers, 2000).

2.4.1.1 Manifestation of early-onset GBS

Respiratory distress is the most common sign of newborns with early-onset GBS infection. Their chest x-rays are often indistinguishable from those of newborns with respiratory distress syndrome. Only about one third of newborns with congenital pneumonia will have infiltrates apparent on chest x-rays; Pleural effusions also may be present.

In addition, infected newborns may have other nonspecific symptoms such as lethargy, poor feeding, temperature instability, and glucose instability. More ominous signs of severe invasive infection include; hypotension, which may occur in up to 25% of all cases, fetal asphyxia, related to being infected in utero, rapidly worsening respiratory distress and persistent pulmonary hypertension. Newborns with meningitis may not have signs different from those of newborns with GBS septicemia or pneumonia, although seizures can occur in the first 24 hours of life **(**Chaudhry et al., 2010).

2.4.1.2. Risk factors for early-onset GBS

Early-onset GBS disease risk factors have been well characterized and include maternal GBS colonization, prolonged rupture of membranes (i.e. more than 18 hours), frequent vaginal examination during labor, preterm delivery, GBS bacteriuria during pregnancy, delivery of a previous infant with invasive GBS disease, maternal chorioamnionitis as evidenced by intrapartum fever, young maternal age, African or Hispanic ethnicity, and low levels of antibody to type-specific capsular polysaccharide antigens (Yancey, Schuchat, Brown, Ventura & Markenson, 1996; Bergeron et al., 2000; Lin et al., 2004). It is possible for newborns with no known maternal predisposition for GBS to develop invasive disease. In addition, nosocomial early-onset GBS infection has been known to occur.

2.4.2 Late-onset GBS infection

Newborns who present with late-onset GBS do not have signs of infection in the first week of life. The symptoms also may be somewhat indistinct, such as lethargy, poor feeding, or irritability. Most of the isolates of late-onset GBS infection are serotype III, and approximately 30% to 40% of the newborns with late-onset GBS will have meningitis (Jordan, Hall & Davis, 2010).

Newborns with meningitis will generally have fever, and 20% to 30% will have had symptoms of an upper respiratory tract infection. Otitis media may be present.

Permanent neurologic sequelae in varying degrees are evident in a large number of newborns with GBS meningitis (Chaudhry et al., 2010).

2.4.2.1 Manifestations of late-onset GBS

A focal presentation also present in Late-onset GBS infection. Facial cellulitis is being seen with increasing frequency, more often in males than females. Septic arthritis and osteoarthritis also are seen more often in late-onset infection. Just as with early-onset GBS infection, there have been isolated reports of the infection occurring in almost every organ (Baker & Edwards, 1995).

Late-onset GBS infection has a mortality rate of 2% to 6%, and the incidence has not changed following the advent of intrapartum chemoprophylaxis (Lauer et al., 2005). However, as with early-onset disease, the initial presentation may vary markedly. Newborns with late-onset infection who present with symptoms such as apnea, seizures, leukopenia, or neutropenia are more likely to have a fatal outcome.

2.4.2.2. Risk factors for late-onset GBS

Risk factors associated with late-onset GBS infection are not well defined. Vertical transmission and nosocomial acquisition are the most likely sources (Ling et al., 2010). Prematurity and African ethnicity are recognized as a risk factors for late onset GBS. It is reported that although GBS can be acquired nosocomially, it does not appear to cause outbreaks in nurseries (Lin, Weisman, Troendle & Adams, 2003). Newborns who are colonized with GBS do not need to be isolated. Colonization has been reported to last for several months after birth, even in newborns treated for GBS infection (CDC, 2002). Newborns who present with late-onset GBS do not have signs of infection in the first week of life.

2.5. Laboratory diagnosis of Group B streptococci:

2.5.1. Isolation of GBS by chrome agar

ChromAgar is selective medium supplemented with three chromogenic substrates to optimize the identification of GBS which appears as red colonies that are round and pearly after 18-24 hour incubation in aerobic conditions. Most other bacterial species are either inhibited or the colonies produced have a different color (e.g.

violet, blue, colorless). Capable of detecting all GBS strains, including non ß-hemolytic strains.

GBS is part of the microbiologically complex vaginal and rectal human flora. Consequently, selective media have been developed to improve GBS recovery from vaginal and/or rectal swabs and to inhibit overgrowth of other organisms of the normal flora **(**Trijbels-Smeulders, Kollee, & Adriaanse, 2004).

If GBS is not identified after the incubation of 18–24 hours, the blood agar plate should be reincubated and examined at 48 hour to identify suspected organisms. Suspected colonies may be tested using various slide agglutination tests or GBS antigen detection assays for specific identification of GBS, or alternatively, the CAMP test may be used for presumptive GBS identification **(**Michael & Burton, 2011).

The developed guidelines have led to marked improvements of the screening based approach using culture methods as revealed by a study showing that the use of standard direct blood agar plating rather than selective enrichment broth leads to false negative culture results in as many as 50% of pregnant women colonized by GBS. Major factors that influence the accuracy of detecting GBS maternal colonization are the choice of bacteriological media, sample collection sites, and the time of the sampling (Valkenburg-van den Berg et al., 2006).

2.5.2 Immunological assay

GBS strains are identified reliably by the production of group B Lancefield antigen. As a result, many latex agglutination tests and immunoassays that detect this antigen for GBS identification have been developed. However, the overall sensitivity of these commercially available immunological assays is low**.** When compared with the results of selective broth culture, the sensitivity of popular rapid immunoassays to detect GBS colonization in pregnant women directly from rectovaginal swabs ranged from only 4% to 37%. Rapid antigen detection tests may only be suitable to detect

GBS in heavily colonized patients or from overnight cultures in standard selective broth (Picard & Bergeron, 2004).

2.5.3 Nucleic Acid Testing Assays

The AccuProbe Group B Streptococcus Culture Identification Test from Gen-Probe (San Diego, CA, USA) is the most popular probe hybridization system for GBS. This Assay, which targets specifically the GBS ribosomal RNA, is suitable to identify GBS from 18 to 24 hours cultures in selective enrichment broth. Compared with the standard culture method, this probe-based assay had a sensitivity of 94.7–100% and a specificity of 96.9–99.5% for screening for GBS colonization in pregnant women **(**Williams-Bouyer, Reisner & Woods, 2000).

In addition, DNA hybridization probes for identifying GBS directly have been developed, but they still cannot replace antenatal culture for the most accurate detection of GBS carriers (Verani, McGee, Schrag, 2010).

Exhaustively described in the literature, nucleic acid testing assays based on nucleic acid amplification technologies such as PCR offer a great potential for rapid, highly sensitive, and specific detection of various infectious agents directly from clinical samples (Picard & Bergeron, 2004). A number of PCR assays targeting different genes for the specific detection of GBS have been developed. However, most of them rely on complicated and time consuming procedures that are not applicable to clinical use. Only one of these PCR assays has been validated for screening for GBS colonization in pregnant women by testing rectovaginal specimens. This situation may be explained by the fact that specimens obtained from pregnant women contain potent inhibitors of PCR amplification (Picard & Bergeron, 2004).

Polymerase chain reaction (PCR) assay is based on the recently developed and widely used real-time PCR technology (Cockerill & smith, 2002). This <1 hour GBS specific real-time PCR assay, which targets the *cfb* gene encoding the CAMP factor, relies on a simple and rapid (approximately 10 minutes) rectovaginal swab sample

preparation and nucleic acid extraction and uses a rapid thermal cycling instrument that allows real-time fluorescence monitoring of the PCR reactions (Ke et al., 2000). The instrument measures, at each PCR cycle, the fluorescence signal from probes labelled with fluorophores hybridizing specifically to target DNA sequences of the GBS-specific amplicons generated during the nucleic acid amplification process. Besides its rapidity, this real-time PCR assay also offers the advantage of reducing the risks of carry-over contamination by amplicons because amplification and detection occur within a closed reaction vessel (Cockerill & smith, 2002). This assay was validated by a clinical study performed with rectovaginal swab specimens obtained from 112 pregnant women at delivery (Bergeron et al., 2000).

When compared with the standard antepartum selective broth culture method, real-time PCR assay had a sensitivity of 97.0%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 98.8% for screening for GBS colonization during delivery. One important advantage of using this test for GBS screening is its suitability for testing at the time of admission for delivery, thereby offering better sensitivity and specificity for detecting colonization. PCR assay provides a novel diagnostic tool for GBS detection, potentially allowing more accurate and effective intrapartum antibiotic prophylaxis. Incidentally, a cost–benefit analysis study suggested that for a test price of up to 32–33 US dollars, a PCR test performed in less than 1 hour at the time of delivery is more cost-effective than screening using either the selective culture method or the risk-factor approach (Haberland et al., 2002).

In a multi-site clinical trial, the IDI-Strep B test demonstrated a sensitivity of 94% and a specificity of 96% in comparison to reference intrapartum culture-based screening performed at delivery (Davies et al., 2004). In a recent comparative study, the sensitivity of the IDI-Strep B test surpassed both standard antepartum selective culture at 35–37 weeks gestation and risk-factor assessment, resulting in fewer false-negative results and potentially lower infant mortality and morbidity. In fact, 13% of women in this trial would have been treated more appropriately with screening using the IDI-Strep B as compared to the use of the standard antepartum selective culture

method, while 36% of them would have been treated more appropriately with screening using IDI-Strep B as compared to the risk-based approach (Davies et al., 2004). Therefore, the IDI-Strep B PCR test has high sensitivity for screening of GBS colonization in pregnant women than the standard antepartum selective culture methods.

2.6. Management of GBS colonization and infections

2.6.1. Intrapartum antibiotic chemoprophylaxis (IAP)

The intrapartum antibiotic prophylaxis (IAP) is indicated for all GBS carriers except for those in whom cesarean delivery is planned in the absence of labor or membrane rupture. Penicillin G remains the drug of choice for prophylaxis with ampicillin as the alternative medication. The usual recommendation for the prevention of GBS transmission from colonized women to their infants during labor is to administer intravenous penicillin every 4 h for the duration of labor (AEG, 2010). Almost all GBS isolates are highly susceptible to penicillin; there have been only a few reported instances of penicillin-resistance worldwide (Moyo, Maeland & Munemo, 2000 and Hsueh et al., 2001). Penicillin therefore remains the first-line treatment of choice (Schrag, Gorwitz, Fultz-Butts & Schuchat, 2002).

For patients who are allergic to penicillin but some experts have recommended that vancomycin should constitute the second-line intrapartum chemoprophylaxis for GBS prevention If GBS susceptibility is unknown at the time of delivery (Schrag et al., 2002 ; Pelaes, Gelber, Fox & Chasen, 2009). If the GBS is sensitive to one of these antibiotics, either erythromycin or clindamycin is recommended for IAP.

However, their efficacy in the prevention of EOD is yet to be confirmed. In the presence of maternal chorioamnionitis, early treatment of the mother with broad-spectrum antibiotics is indicated. Criteria for the diagnosis of maternal chorioamnionitis include maternal fever, plus one of the following criteria: uterine tenderness, fetal tachycardia, foul smelling amniotic fluid, prolonged rupture of membranes of equal to or more than 18 hours, or maternal leukocytosis. It is impractical to administer chemoprophylaxis to all parturient mothers and neonates.

The challenge therefore is to identify correctly high risk infants before they are born (Nandyal, 2008).

To prevent early onset disease, two preventative approaches were used; a culture screening-based and a risk-based approach. The first approach involved universal screening for GBS colonization of all pregnant women between 35 and 37 weeks of gestation using vaginal and rectal cultures to detect GBS colonization. This approach has been endorsed by multiple organizations, including the United States Centers for Disease Control (Schrag et al., 2002), and by the Royal Australian and New Zealand College of Obstetricians and Gynecologists (Ranzog, 2007). Properly obtained and processed antenatal cultures correctly identified most women colonized at the time of labor. Intrapartum antibiotics are administered to all those with a positive GBS culture regardless of risk factors (CDC, 2002).

The risk-based approach involved administration of antibiotics based solely on the presence of antenatal or intrapartum risk factors. Maternal risk factors for GBS neonatal sepsis are as follows; (CDC, 2002) preterm labor or premature rupture of membranes(<37weeks gestation); prolonged rupture of membranes (18h); intrapartum fever (38.0°C); history of a previous newborn with GBS disease; and GBS bacteriuria during pregnancy (CDC, 2002).

2.6.1.1. Management of infant born to mothers who received intrapartum antibiotic prophylaxis

The 2002 prevention of perinatal GBS disease guidelines provided recommendations for the management of infants born to mothers who have received IAP. Variations that incorporate individual circumstances or institutional preference may be appropriate. If a woman receives intrapartum antibiotics for the treatment of suspected chorioamnionitis, her new-born should have a full diagnostic evaluation, and empiric antibiotic therapy (ampicillin and gentamycin) pending culture results, regardless of clinical condition at birth, duration of maternal antibiotic therapy before delivery, or gestational age at delivery (Nandyal, 2008).

All symptomatic infants, whether preterm, late preterm, or term should have full diagnostic evaluation and empiric antibiotic therapy. A full diagnostic evaluation includes complete blood cell count (CBC) with differential, blood culture, chest radiograph with respiratory signs and spinal tap (with clinical signs of sepsis). Blood culture can be sterile in 15% to 38% of infants with culture proven meningitis. For any symptomatic infant, if the spinal tap is deferred initially because of clinical instability, and the antibiotics are continued beyond 48 hours, CSF should be obtained for cell count, biochemical analysis, and culture (Nandyal, 2008).

2.6.2. Asymptomatic preterm neonates

For preterm neonates of less than 35 weeks gestation, limited evaluation should be obtained (because of their increased risk for sepsis and rapid deterioration) in all asymptomatic infants of GBS colonized mothers, regardless of their IAP status (Nandyal, 2008). Limited evaluation includes CBC with differential and blood culture.

They need close observation, including frequent vital signs (at least every 2 to 4 hours), frequent diligent assessment of the infant by the staff. Any significant change in vital signs including temperature imbalance, any change in the clinical status including poor feeding, respiratory distress, apnea, abdominal distension, frequent emesis, or lethargy deserves and requires immediate notification to the physician team and reassessment, which may necessitate repeating laboratory tests. Full evaluation including spinal tap and empiric antibiotic therapy seem appropriate, if the infant becomes symptomatic, or in the presence of maternal chorioamnionitis (suspected or proven), or other high-risk factors such as preterm premature rupture of membranes (Nandyal, 2008).

2.6.3. Asymptomatic term and late preterm neonates

Asymptomatic neonates born at 35 weeks gestational age or later and whose mothers received inadequate IAP may require limited evaluation and observation for 48 hours. There is some evidence suggesting that limited evaluation does not add any benefit over close clinical observation. Based on newly available data, it is

recommended that the neonate should be observed closely for 48 hours without laboratory evaluation, or two serial CBCs obtained within the first 24 hours after birth (Nandyal, 2008).

Blood culture is not necessary. Serial C-Reactive protein (CRP) measurements (because of CRP's high sensitivity) are noted to be useful along with serial CBCs (total white blood cell count, band count, and immature/total polymorphonuclear cell ratio or I/T ratio) to evaluate for sepsis in healthy/asymptomatic term and late preterm neonates (Nandyal, 2008).

2.7. Prevention and control

While different strategies for identification of high-risk mothers and infants and the provision of intrapartum prophylaxis may reduce the rate of neonatal sepsis, they are unlikely to eliminate the problem (Shet & Ferrieri, 2004). Maternal immunization against GBS appears to be a promising and potentially lasting approach for preventing neonatal sepsis, preterm deliveries and low birth weight infants (Shet and Ferrieri, 2004). Multivalent polysaccharide-protein conjugate vaccines based on serotype-specific capsular polysaccharides are in development for prevention of neonatal GBS disease (Shet & Ferrieri, 2004).

Although the high risk adult groups for invasive GBS disease have been identified, the role of capsular polysaccharide antibodies in the prevention of either localized or invasive GBS disease in non-pregnant adults has not been adequately evaluated (Shet & Ferrieri, 2004).

GBS capsular polysaccharide protein conjugate vaccines for types Ia, Ib and III have been shown to be safe and efficient in inducing type specific antibody levels in healthy vaccinated individuals**.** A vaccine formulation of GBS types Ia, II, III and V was expected to provide protection against over 90% of infections. Therefore, epidemiological surveillance of serotype distribution in the population is critical for vaccine studies (Schuchat, 2000).

The cost of developing and implementing a vaccine strategy is high, but is considerably less than that of treating infections resulting from GBS, and for some infants, prevents their life-long complications**.** Among experts in this field, there is considerable discussion regarding whom to immunize non-pregnant adolescents or pregnant women in the first trimester. In addition, an argument can be made for vaccinating "'at risk" non-pregnant adults **(**Shet & Ferrieri, 2004). A major difficulty in developing GBS vaccines is the existence of a multiple serotypes in different geographic locations (Johri et al., 2006).

CHAPTER 3

MATERIAL AND METHOD

Chapter 3

Material and methods

3.1 Materials

3.1.1 Apparatus

Apparatus	Manufacturer
Autoclave	Tuttnauer (USA)
Incubator	Memmert (Oxford)
Light microscope	Olympus (USA)
Digital camera	Hp (China)
Refrigerator	LG (Turkey)
Centrifuge	
Computer	Lenovo
Frazer(-20°C)	

3.1.2 Equipments

Automatic pipettes
Eppendorf tubes
Cotton swab
Glass tube
Inoculating plastic loops
Plastic Petri plates
Cotton
Culture swab
Tips
Microcentrifuge tube
Plastic loop
Plastic droppers
Gas
Slide

3.1.3 Reagents and Stain

Reagents and Stain	Manufacturer
3% Hydrogen peroxide	
Gram-stain kit	HiMedia (India)
Glycerol	

Distilled water
Phosphate buffer saline
Ninhydrin reagent
Genomic DNA kit Bioline.UK

3.1.4 Antibiotic used in the study

Antibiotic	Potency	Abbreviation	Manufacturer
Vancomycin	30µg	VA	Liofilchem, Roseto-Italy
Penicillin G	10IU	P	Liofilchem, Roseto-Italy
Clindamycin	2µg	CD	Liofilchem, Roseto-Italy
Erythromycin	15µg	E	Liofilchem, Roseto-Italy
Tetracycline	30µg	TE	Liofilchem, Roseto-Italy

3.1.5 Culture media

Culture media	Manufacturer
Blood agar	Himedia
Chrome agar	Himedia

3.2 Methodology

3.2.1 Study design

A descriptive cross sectional study was conducted to determine the prevalence of GBS colonization and susceptibility pattern among pregnant women attending antenatal clinic of Al Shifa hospital. Four hundreds recto-vaginal swab samples were collected from 200 pregnant women at 35-37 weeks of gestation from Al Shifa hospital. Two samples from each pregnant, one for culture and the other for PCR. This screening approach was based on universal screening of all pregnant women for GBS colonization at 35 to 37 weeks of gestation (CDC, 2002).

All participants provided informed consents. Pregnant women on any antibiotic treatment and those who were not within the range of 35 through 37 weeks of gestation were excluded. The age of the study participants ranged from 15 to 45

years with a mean of 25.1 (±4.7).The study was conducted from May to August 2016 in Gaza strip, Palestine.

3.2.2 Ethical considerations

The necessary approval to conduct the study was obtained from Helsinki committee in Gaza strip. Helsinki committee is an authorized professional body for giving permission to researchers to conduct their studies with ethical concern in the area. The necessary permission for the clinics was also obtained from antenatal clinic of Al Shifa hospital. All participating women were asked to sign a consent form after explaining the research nature and objectives.

3.2.3 Questionnaire

A questionnaire was used to collect data with the aim of determining possible risk factor associated with women who had GBS infections/colonization. A structured questionnaire was used and included questions about age, education level, medical history of patients, the number of births and the number of previous miscarriages, materials used for cleaning, and other question regarding possible risk factors of GBS colonization. The questionnaire was completed through face-to-face interview.

3.2.4 Sample collection

Two rectovaginal swabs were collected from each participating pregnant women in their third trimester (35-37 weeks of gestation). One of the swab contain Amies transport medium for culturing procedure and the second one contain 1ml of phosphate buffer saline for PCR analysis. The swabs were labeled properly and transported to the microbiology laboratory within two hours of collection.

Rectovaginal sampling was carried out by rotating a swab against the vaginal wall at the mid-portion of the vault. Subsequently, the swab was carefully withdrawn to prevent contamination with microflora from the vulva and introitus and the swab was inserted 1.5 to 2 cm beyond the anal sphincter and gently rotated to touch the anal crypts (El Aila et al., 2010).

3.2.5 Culturing procedure of rectovaginal samples

Direct plating was carried out by inoculating the swab that was inoculated Amies transport medium onto chromID Stepto B agar. The ChromAgar plates were incubated at 37°C for 18-24 h in aerobic conditions. The chromogenic selective medium is supplemented with three chromogenic substrates to optimize the identification of GBS which appears red colonies that are round and pearly after 18-24 hour incubation (Figure: 3.1).

Figure (3.1): Appearance after 24 h incubation of (A): *Streptococcus agalactiae* and (B) *Enterococcus faecalis* on Strepto B ID® chromogenic agar.

3.2.6 Confirmatory tests for GBS detection:

3.2.6.1 Gram stain

S. agalactiae is Gram-positive *streptococcus* (GBS given the color of crystal violet), spherical or ovoid and less than 2 µm in diameter and can grow in pairs or short chains as shown in Gram stained film (Figure 3.2).

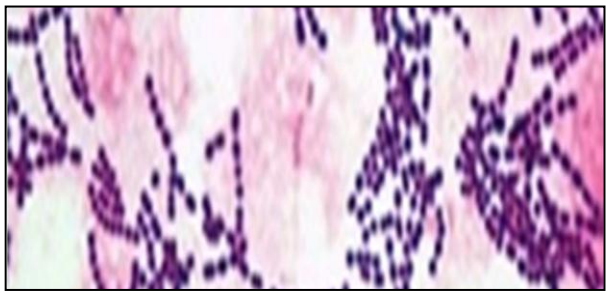


Figure (3.2): Gram stain of *Streptococcus agalactiae*

3.2.6.2 Catalase test

S. agalactiae was catalase-negative. When GBS colonies were mixed with 3% hydrogen peroxide, they did not produce air bubbles. Catalase mediates the breakdown of hydrogen peroxide H_2O_2 into oxygen and water (Figure: 3.3).

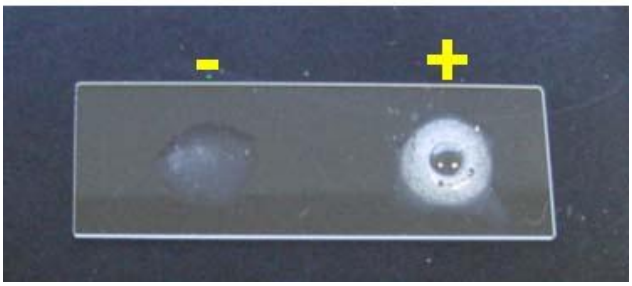


Figure (3.3)*:* GBS Catalase negative **(**No bubble formation) (left) and Catalase positive (bubble formation) *Staphylococcus aureus* (right).

3.2.6.3 CAMP test

The CAMP test (named after Christie, Atkins and Munch-Petersen) is used for the presumptive identification of GBS. It is based on the fact that GBS produces an extracellular protein called the CAMP factor. This protein acts synergistically with *Staphylococcus aureus* β-toxin to hydrolyze red blood cells.

Staphylococcus aureus was inoculated onto sheep Blood Agar plates by making a narrow streak down the center of the plate using a loop. The test organisms (suspected GBS) were streaked in a straight-line inoculum at right angles to the *S. aureus* as shown in (Figure 3.4). The *Streptococcus* streak was within 2 mm without touching the *S. aureus* streak. The plates were then incubated at 37°C for 24 hours. A positive test for CAMP factor appears as "arrow head" hemolysis between the junction of growth of *S. aureus* and GBS. No enhanced or "arrowhead" hemolysis was seen when the test isolate was not GBS (Figure 3.4).

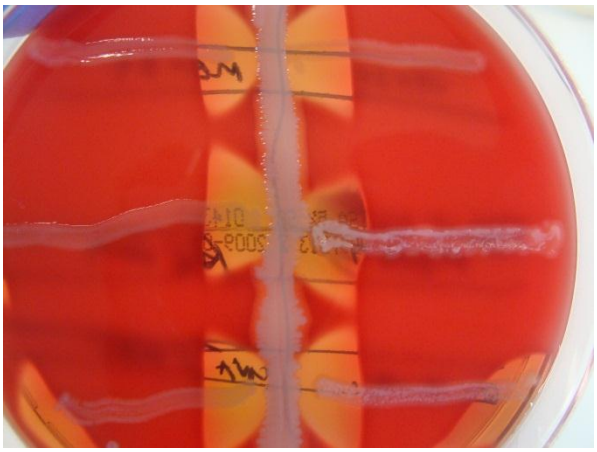


Figure (3.4): GBS isolates showing CAMP positive test

3.2.6.4 Hippurate Hydrolysis test

Streptococcus agalactiae is hippuricase positive. The glycine product resulting from the action of hippuricase can be detected by the addition of ninhydrin reagent, which produces purple color (Figure 3.5).

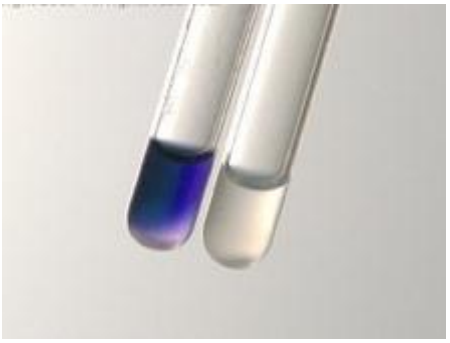


Figure (3.5): Hippurate hydrolysis test. Left (positive tube: purple), Right (Negative tube: colorless)**.**

3.2.7 Molecular techniques

3.2.7.1 DNA extraction of Bacterial isolates:

DNA was extracted from cultured isolates by alkaline lysis as previously described. Briefly, one bacterial colony was suspended in 20 µl of lysis buffer (0.25% sodium dodecyl sulfate, 0.05 N NaOH) and heated at 95 °C for 15 min. The cell lysate was diluted by 180 µl of distilled water. The cell debris was pelted by centrifugation at 16000 xg for 5 min. and the supernatants were used for PCR or frozen at -20 °C until further use (El Aila et al; 2009).

3.2.7.2 DNA extraction of clinical isolates

DNA extraction from the samples was performed using the Genomic DNA Kit according to the manufacturer's instructions. Briefly, 200 µl of transport medium (phosphate buffer saline) from each the rectovaginal swab is placed into microcentrifuge tube. 180 µL lysis buffer and 25 µL Proteinase K solution were added and incubated at 56°C for 1-3 hr. Sample tube was vortex and 200 µL lysis Buffer G3 were added and incubated at 70°C for 10 min. To adjust DNA binding condition, 210 µL of ethanol were added. 500 µL wash buffer GW1 and 600 µL wash buffer GW2 were used for washing. To elute DNA 100 µL preheated Elution buffer G are added directly onto silica membrane (Boline, UK).

3.2.7.3 PCR primers

GBS nucleic acid detection is based on targeting the *cfb* gene, which encodes the CAMP, factor. The PCR reaction mixture contained primers specific for group B streptococci. The forward and reverse sequences of the primers are Sag 059 (5´-TCACCAGCTGTATTAGAAGTA-3´) (369–391) and Sag 190, (5´-GTTCCCTGAACATTATCTTTGAT-3´) (500–522). The GBS-specific primers amplify a fragment of 153 bp (Ke et al., 2000).

3.2.7.4 Conventional PCR Assay

For the conventional PCR assay, 2 µl of the DNA extract were added to the PCR reaction mixture. In addition, purified group B streptococcal genomic DNA was used as a positive control. Multiple blanks were also included as negative controls, and other streptococcus not group B were used as negative control to verify that there is no cross-contamination between samples. For the amplification, the reaction mixtures was as follows; denaturation at 94°C for 3 minutes, followed by 40 cycles of 1 second at 95°C and 30 seconds at 55°C for the hybridization step, with a final period of extension at 72°C for two minutes.

After amplification, 10 µl of the amplified reaction mixture was analyzed by electrophoresis on 2% (w/v) agarose gel. The 100-bp molecular size marker will run

concurrently. The electrophoresis will carried out in 1x Tris, boric acid, EDTA (TBE) at 80 V for one hour. The band pattern of the amplified products on the agarose gels were visualized using gel documentation system.

3.2.8 Antibiotic susceptibility testing

GBS isolates were subcultured onto sheep Blood Agar plates and incubated aerobically for 24 hours at 37°C. Antimicrobial susceptibility testing (AST) was performed according to CLSI guidelines (CLSI, 2013). Sensi-Discs (Liofilchem, Roseto-Italy) of vancomycin (30µg), penicillin G (10IU), clindamycin (2µg), erythromycin (15µg), and tetracycline (30µg) were placed 12 mm apart to detect antimicrobial susceptibilities of 42 GBS isolates.

3.2.9 Data analysis

The results were tabulated and analyzed using version 20 of the Statistical Package for the Social Sciences software (SPSS). Frequencies cross tabulation and appropriate statistical tests as Chi-square test was performed. A P-value of less than 0.05 was considered significant.

CHAPTER 4

RESULTS

Chapter 4

Results

4.1 Prevalence of GBS using conventional methods:

A total of two hundred pregnant women (from 35-37 weeks of gestation) from antenatal clinic of Al shifa hospital - Gaza, Palestine were enrolled in this study from May to August, 2016. The overall prevalence of GBS colonization as determined by chromogenic culture was 21%. The prevalence of GBS colonization among participants with age equal or less than 25 years was 19%, while participants that age more than 25 years was 24%. The frequency of GBS colonization among different age groups was not statistically significant by culture (p> 0.05) (Table: 4.1).

Table (4.1): Prevalence of GBS colonization in 200 pregnant women at 35-37 weeks.

Age group	Culture results				p-value
	Positive	%	Negative	%	
≤ 25 years	25	19	104	81	0.551
>25 years	17	24	54	76	
Total	42	21	158	79	

4.2 Detection of Group B Streptococci using PCR from alkaline DNA extracts:

Alkaline DNA extraction was done for all 42 GBS isolates that were detected by conventional methods (Chromagar). The DNA extracts were subjected to PCR testing. Positive and negative controls were included in each run. All DNA extracts of 42 GBS isolates were positive by PCR (Figure: 4.1). This emphasizes our results by conventional method.

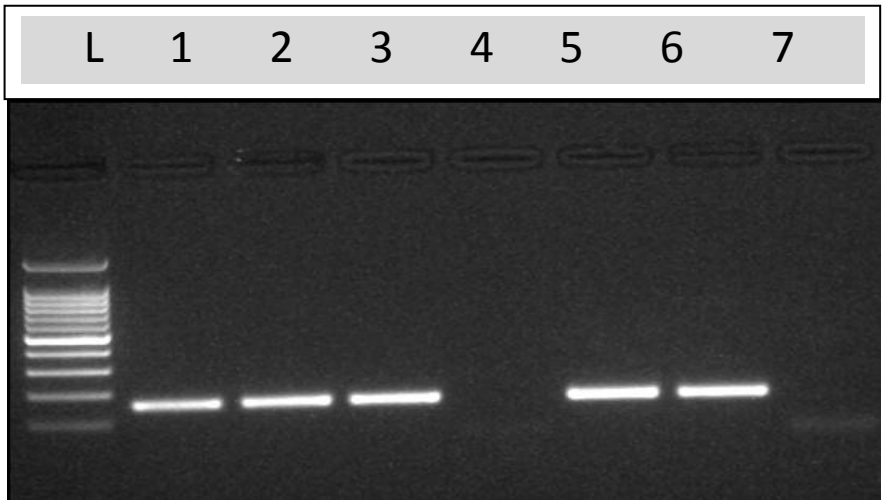


Figure (4.1): A representative result of GBS PCR.

Lane L: 100 bp DNA ladder; lane 1: positive control; lanes 2, 3, 5 and 6 are tested isolates with positively amplified GBS genes; lane 4 is negative control and lane 7 is a blank.

4.3 Detection of Group B Streptococci using PCR directly from clinical samples:

Among 200 women included, 42 (21%) were identified as GBS positive based upon the culture results of rectovaginal swabs. Only 100 rectovaginal samples were subjected to PCR analysis. They included the 42 samples that were positive by conventional methods. Another, 58 samples were selected randomly.

Out of the 42 samples which were positive by conventional methods, only 23 were positive by PCR (54%), but 19 (45%) were positive by culture and negative by PCR (Figure: 4.2). Of the 58 randomly selected samples, seven (12%) were positive using PCR and negative by phenotype (culture methods).

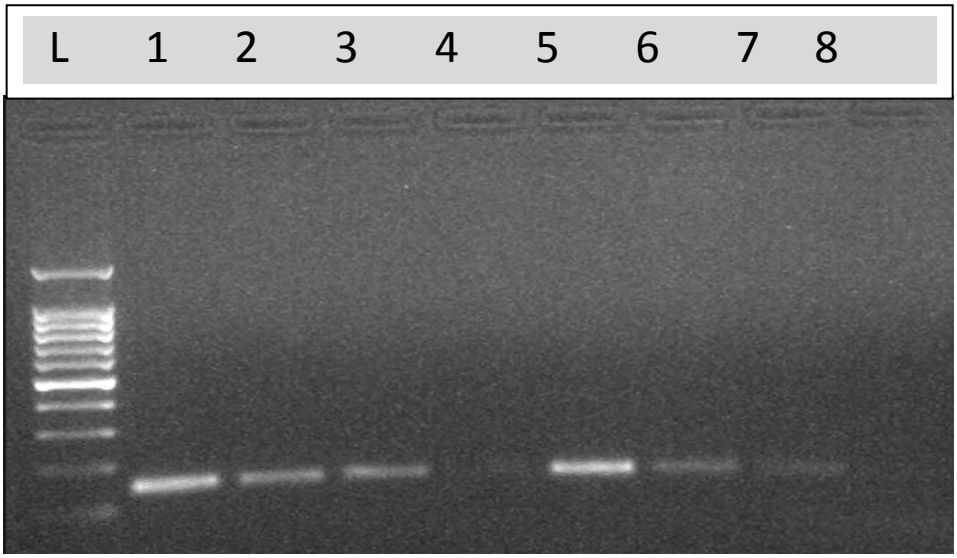


Figure (4.2): A representative result of GBS PCR directly from clinical sample.

Lane L: 100 bp DNA ladder; lane 1: positive control; lanes 2, 3, 5,6 and 7 are tested isolates with positively amplified GBS genes; lane 4 is negative control and lane 8 is a blank.

Table (4.2): Comparison between culture and PCR results.

PCR	Culture		Total
	Positive	Negative	
Positive	**23**	**7**	**30**
Negative	**19**	**51**	**70**
Total	**42**	**58**	**100**

Sensitivity = 23/42 = 54%

Specificity = 51/58= 88%

Positive predictive value= 23/30 = 76%

Negative predictive value= 51/70= 72%

Not all culture-positive samples were also positive with the PCR technique therefore resulting in 54% PCR sensitivity. Of 58 culture negative samples for GBS, 7 were positive with PCR and 51 were negative with both methods, which indicate a specificity of 88%. The positive and negative predictive value was 76%, 72% respectively.

4.4 Antimicrobial susceptibilities of GBS

Intrapartum chemoprophylaxis for pregnant GBS carriers reduces vertical transmission, with a resultant decrease in early onset disease as well as maternal morbidity from invasive GBS infection. Isolates were tested by agar disc diffusion method, with manual reading of zone diameters analyzed according to the interpretive criteria recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2013). (Figure: 4.3).

Figure (4.3): Appearance of antimicrobial susceptibility pattern for GBS isolates.

The antimicrobial susceptibility patterns of GBS isolated from pregnant women are summarized in table 4.3. Of the 42 examined GBS isolates, 32 (76%) were susceptible to vancomycin, 24 isolates (57%) were sensitive to penicillin, and 21 (50%) erythromycin. The overall susceptibilities of GBS isolates to tetracycline were 20 (48%), and to clindamycin, 13 (31%) (Table: 4.3). The intermediate susceptibilities of GBS isolates to clindamycin, vancomycin, penicillin, tetracycline and erythromycin was found to be 0 %, 2 %, 5 %, 7%, and 7 % respectively.

The antimicrobial agent that GBS isolates were most resistant to was Clindamycin 29 (69%). Resistance to tetracycline, erythromycin, penicillin, and vancomycin was found to be 45%, 43%, 38%, and 21% respectively. Of the erythromycin-resistant isolates, 18/47 (38%) showed cross-resistance to clindamycin. However, tetracycline resistant isolates showed 14/48 (29 %) cross-resistance to clindamycin (Table: 4.4).

Table (4.3): Antibiotic susceptibilities of 42 GBS isolates from pregnant women.

No. (%) of isolates			
Agent	**Susceptible**	**Intermediate**	**Resistant**
Clindamycin	13 (31%)	0 (0%)	29 (69%)
Erythromycin	21 (50%)	3 (7%)	18 (43%)
Penicillin	24 (57%)	2 (5%)	16 (38%)
Tetracycline	20 (48%)	3 (7%)	19 (45%)
Vancomycin	32 (76%)	1 (2%)	9 (21%)

Table (4.4): Phenotypic resistance patterns amongst 42 GBS isolates.

Colonizing GBS	**Total**	**Percentage**
Erythromycin resistant only	18	43
Clindamycin resistant only	29	69
Resistant to both erythromycin and clindamycin	13	28
Tetracycline resistant only	19	45
Resistant to both tetracycline and clindamycin	14	29

4.5 Risk factors:

Different variables associated with GBS colonization are outlined in Table (4.5). There was no statistically significant difference among groups based on education level (university education, level 21% by PCR and 30% by culture; secondary school level, 20% by PCR and 23% by culture and the Preparatory education level, 22% by PCR and 25% by culture)

Of women with GBS colonization, those with genital tract inflammation were 29% ($p=0.59$) by PCR, and 25% ($p=0.65$) for the culture assay. However, GBS colonization and inflammation was not statistically significant. Among GBS isolated, 33% were isolated from pregnant women had chronic disease by culture assay (0% for the PCR assay). (Table: 4.5).

No statistically significant difference was observed in GBS colonization rate and chronic disease ($p>0.05$). Of the pregnant women who had previous antibiotic intake, 10 (24%) were positive for GBS by culture and 5 (17%) by PCR ($p >0.05$). In addition, no statistically significant correlation was observed in GBS colonization rate and previous antibiotic intake.

Out of GBS positive (by culture and PCR), 24% were from pregnant women who had complication (Previous abortion – delivery at <37weeks gestation- premature birth- intrauterine death – endometritis**)**. No statistically significant difference was observed in GBS colonization rate and chronic disease ($p >0.05$).

Overall, no statistically significant association was observed for GBS colonization in the study subjects with any of the characteristics mentioned above.

Table (4.5): Variables associated/not associated with Group B Streptococcus colonization in pregnant women (35-37) weeks of gestation.

Education level	Culture results				p-value	PCR results				p-value
	Positive	%	Negative	%		Positive	%	Negative	%	
University	7	21	27	79	0.979	6	30	14	70	0.834
Secondary	25	20	97	80		13	23	44	77	
Preparatory	8	22	28	78		6	25	17	75	
Second Wife Yes	0	0	8	100	0.299	0	0	5	100	0.297
No	40	22	145	78		28	30	67	70	
Chronic disease Yes	2	33	4	67	0.557	0	0	6	100	0.267
No	38	20	150	80		28	30	66	70	
Inflammation Yes	21	25	62	75	0.645	12	29	29	71	0.589
No	19	17	91	83		16	27	43	73	
Antiseptic material Water and soap	40	21	152	79	0.777	28	15	164	85	0.419
No	2	25	6	75		2	25	6	75	
Using of antibiotic Yes	10	24	32	76	0.832	5	17	24	83	0.620
No	30	20	120	80		23	32	48	68	
Complication Yes	11	24	34	76	0.798	7	24	22	76	0.888
No	28	19	116	81		21	30	50	70	

CHAPTER 5

DISCUSSION

Chapter 5
Discussion

To prevent group B streptococcal disease in neonates, the current recommendation is to screen pregnant women by culturing recto-vaginal swabs at 35 to 37 weeks gestation. This is to improve the sensitivity and specificity of the identification of women who are colonized at the time of delivery and to treat those with positive cultures or to treat women with risk factors for disease transmission empirically (Daniels et al., 2009). A more rapid and sensitive method would be beneficial, and cost-effective approach especially in dealing with patients who present at term with unknown GBS colonization status and preterm labor conditions (Block, Munson, Culver, Vaughan & Hryciuk, 2008; CDC, 2010).

5.1 Prevalence of Group B Streptococci using conventional methods:

Since vaginal and in particular rectal flora contains numerous microorganisms, the use of selective culture medium is recommended to maximize the isolation of GBS and to avoid the overgrowth of other organisms. In this study, a selective chromogenic medium was used, which enables the recognition of *S. agalactiae* as pink to red, round and pearly colonies, without the need of anaerobic incubation. This medium has excellent performance for the GBS prenatal screening in terms of nutrient capacity and sensitivity of detection. Capable of detecting all GBS strains, including non ß-hemolytic strains. Most other bacterial species are either inhibited or the colonies produced have a different color (e.g. violet, blue, and colorless) (Perry, Oliver, Nicholson, Wright & Gould, 2006; Tazi, Reglier-Poupet, Dautezac, Raymond & Poyart, 2008; Tazi et al., 2009).

In our study, the prevalence rate of group B Streptococci among pregnant women was 21%. Our results were consistent with findings in the developing and developed countries Germany (16%) (Brimil et al., 2006), Blantyre, Malawi, (16.5%) (Dzowela, Komolafe & Lgbigbia, 2005), Lebanon (17.7%) (Seoud et al., 2010), Egypt,

(17.89%) (Elbaradie et al., 2009), (16.4%) Kuwait (Al-Sweih et al., 2003), Zimbabwe, (21%) (Mavenyengwa et al., 2010), and Dare salaam, Tanzania, (23%) (Joachim et al., 2009), (22%) Belgium (El Aila et al., 2010), Sweden (17.3%) (Ekström et al., 2013), However, the finding of this study is higher when compared to colonization rate from other countries like Mozambique which reported colonization rate of 1.8% (Steenwinkel et al., 2008), 4.8% Irane (Shirazi et al., 2014), the Philippines (7.5%) (Ippolito et al., 2010).

Lower GBS colonization rate have been found in some Mediterranean countries, e.g. studies from Istanbul, Turkey and Elazig, Turkey found a colonization rate 8% (Barbaros et al., 2005) and 8.7% (Ayata et al., 1994) respectively. Study conducted in a city of Northern Greece also found a low colonization rate of GBS among pregnant women 6.6% (Tsolia et al., 2003). However, findings from different studies conducted in Trinidad found a higher colonization rate of pregnant women with GBS, 32.9% (Orrett, 2003).

The different prevalence rates may be explained by gestational age at culturing, differences in culture sites and culture techniques, a change of prevalence with time, or real differences of prevalence in different populations or ethnic groups (Valkenburg-van den Berg et al., 2006). Personal hygiene, the extensive use of antiseptics/antibiotics, standard of living, and other socioeconomic factors may contribute to the variation in colonization rates.

Numerous studies have documented that the accuracy of prenatal screening cultures in identifying intrapartum colonization status can be enhanced by careful attention to the timing of cultures, the anatomic sites swabbed and the laboratory procedures used for culture and detection of the organisms (Schrag et al., 2002). Swabbing both the vagina and the rectum substantially increases the yield compared with sampling only the cervix or sampling the vagina without swabbing the rectum. Several studies that find rectovaginal sampling more appropriate than vaginal sampling only (CDC, 2002; Diaz & Nieves, 2008; El Aila et al., 2010).

5.2 Detection of GBS carriage rate by PCR:

Discrepancy was observed between culture and PCR results. Out of 100 rectovaginal samples were subjected to PCR, 30 were positive for GBS. Twenty-three of them were culture positive and PCR positive whereas the remaining seven samples were positive PCR and culture negative. In addition, there was 19 samples were positive by culture method but negative by PCR.

The discrepancy of the results may be explained by the lower sample number tested by PCR technique and rectovaginal sample contain many inhibitor for PCR. The collection of these swabs although at the same time may not represent identical microbial content and may indicate poor specimen collection.

In contrast to our results, several studies have reported an increased GBS detection rates by PCR diagnostic tests over standard culture method (Espy et al., 2000; Sloan, et al., 2002; Shabayek, Abdalla & Aboueid, 2010; El Aila et al., 2011; Bakhtiari, Dallal, Mehrabadi, Heidarzadeh & Pourmand, 2012).

Nineteen of the culture-positive recto-vaginal samples were PCR negative when DNA extracted directly from the rectovaginal swab rectovaginal sample contain many inhibitor for PCR, loss of internal control and Inappropriate sample collection may explain the false negative PCR results. In addition, it can be explained by the fact that we used two different swabs collected from each pregnant women (one for PCR and one for culture). This may result in having unequal sample in each swab. Other studies performed culture and PCR from the same swab collected (Rallu, Barriga, Scrivo, Martel – Laferriere, Laferriere, 2006; El Aila et al, 2011).

Considering culture as a gold standard, Sensitivity, Specificity, Positive predictive value and Negative predictive value of PCR was 54%, 88%, 76%, 72%, respectively.

Low sensitivity of PCR was reported in our study which is consistent with PCR results obtained by Chan et al, who reported that PCR sensitivity was lower than that of standard conventional culture. The sensitivity and specificity of the PCR assay were 45% and 99%, respectively (Chan et al., 2006). He claimed that the problem with their test was at the sample preparation stage and the swabs they used for collection contain charcoal in the transport medium, which has been shown to reduce the sensitivity of PCR (Cloud, Hymas & Carroll, 2002; Chan et al., 2006).

Other studies reported the rate of sensitivity of PCR 100% (de Paris et al. 2011). Similar results with PCR specificity were obtained by de Paris et al., (2011) who reported a specificity of 86.88% and it was greater than 64.5% found by Gavino & Wang (2007). Out of 58 samples were negative for GBS detection by culture, seven (12%) were positive by PCR. False negative culture results may be explained by the presence of antagonistic microorganisms, such as enterococci, which may inhibit the growth of GBS on the selective media (Dunne & Holland-Staley, 1998). In addition, antibiotics and feminine hygiene products have been shown to inhibit the growth of GBS and scanty colonization, which would be difficult to obtain in culture (Rallu et al., 2006; Werneke et al., 2009). Furthermore, PCR contamination, inappropriate storage and transport conditions of the samples could give false-negative culture results (Rosa-Fraile et al., 2001).

5.3 Antimicrobial Susceptibilities of GBS:

The prophylaxis currently recommended for prevention of neonatal disease is the intrapartum use of antibiotics only in women known to be colonized by GBS. In the present study, the susceptibility pattern of 42 GBS isolated from pregnant women against five antimicrobial agents is presented in Table 4.4. Only 76% of isolates were susceptible to vancomycin, 57% of the isolates were susceptible to penicillin. Such finding coincides with that obtained by Moyo et al. (2000); Hsueh et al., (2001); Banno et al. (2014); Gaudreau et al. (2010); Kimura, et al. (2008) and Longtin et al. (2011), who reported reduced susceptibilities to penicillin. This result is in agreement with CDC (2012) and Brandon and Dowzicky (2013) who found that that group B Streptococci (GBS) isolates were 100% susceptible to both penicillin and

vancomycin. Due to the widespread use of the antibiotic and the misuse of antibiotics, antibiotic-resistant organisms are in the rise. As indicated in the present study, 18% of GBS isolates were resistant to erythromycin. Similar results were obtained by Azavedo, McGavin & Duncan, (2001); Joachim et al., (2009), and (29%) reported by Manning et al., (2004).

Azavedo et al., (2001) and Hannoun et al., (2010) have reported resistance rate >80% for tetracycline. Which was higher than the findings reported in the present study (45%). However, the rate of resistance to clindamycin in our study was (69%), which is higher than the findings reported by Andrews et al., (2000) which was only from 3 to 15 per cent.

Verani et al., (2010), recommended Vancomycin for GBS-colonized mothers with a high risk of anaphylaxis to penicillin and if the isolate is resistant to clindamycin. In this study, we found that 21% of the isolates were resistance to vancomycin. This finding is comparable to those reported in other studies (Fashina, 2008; Onipede et al., 2012). Since vancomycin is another alternative recommended by the CDC for pregnant women who are allergic to penicillin and clindamycin resistant isolates.

Penicillin is the first choice drug, while ampicillin is an alternative, in cases of history of allergy (rash or a history of difficulty in breathing) to penicillin and at high risk for anaphylaxis; clindamycin and erythromycin were recommended (Verani et al., 2010). In GBS-colonized mothers with allergy and low risk of anaphylaxis to penicillin, the use of cefazolin is recommended. In those with a high risk of anaphylaxis to penicillin and if the isolate is resistant to clindamycin, vancomycin is recommended (Verani et al., 2010; Frohlicher et al., 2014).

However, vancomycin has not been shown to cross the placenta and achieve suitable concentrations in amniotic fluid as well as the fetal blood. This tremendous use of B-lactam antibiotics coupled with the exposure to B-lactams for other reasons can potentially induce the emergence of resistant strains among the resident vaginal

microflora. Emergence of resistance has been documented, especially to ampicillin (Simos, Aroutcheva, Heimler & Faro, 2004).

Antibiotic resistance amongst GBS is considered an increasing problem so that it was recommended to test the susceptibility of other antibiotics than those recommended as part to established control measures and that could be used as alternative choices for prophylaxis or treatment of GBS infection (Quiroga et al., 2008; Verani et al., 2010).

5.4 Risk factors for GBS carriage pregnant women

Knowledge about risk factors contributing to GBS colonization in pregnant women is relevant to minimize the morbidity, mortality associated with maternal and neonatal GBS infections. In the presents study, no statistically significant association was observed for GBS colonization in the study subjects with any of sociodemographic characteristics as outlined in (Table 4.3). Similar findings have been reported in studies conducted by Zusman, Baltimore & Fosica (2006) and Costa et al., (2008).

In the present study, history of spontaneous abortion did not influence GBS colonization in pregnant women. Similar findings have been reported in studies conducted by Joachim et al., 2009. Although the study of McDonald & Chambers (2000) demonstrated an association between the presence of group B streptococci spontaneous abortions. Therefore, further studies are needed to confirm the correlation between sociodemographic factor and GBS colonization. The lack of association with this factor can possibly be explained by the fact that the numbers of participants in this study with such risk factor were small. This finding is consistent with studies from other authors (Joachim et al., 2009).

Garland, Kelly & Ugoni (2000) did not detect an association between GBS colonization and preterm labor and premature rupture of the membranes. These results are similar to those observed in our study. Although some investigators as Feikin et al., (2001), found that women with preterm delivery have a significantly higher frequency of GBS colonization.

CHAPTER 6

CONCLUSION & RECOMMENDATION

Chapter 6

Conclusion and Recommendation

6.1 Conclusion

To the best of our knowledge, this is the first study in Gaza strip, which report the maternal GBS carriage among pregnant women. Based on the results obtained from this cross sectional prospective study, the following conclusions could be withdrawn:

1. The overall prevalence of GBS colonization was 21%.
2. Despite the fact that PCR is well known for its high sensitivity, low sensitivity was obtained in this study.
3. Resistance to clindamycin, tetracycline, erythromycin, penicillin and vancomycin was found to be 69%, 45%, 43%, 38%, and 21% respectively.
4. Vancomycin showed the highest rate of sensitivity against GBS isolates (76%).
5. No statistically significant association between the GBS colonization rate and chronic diseases, previous antibiotic intake, educational level and complications due to obstetric factors such as previous abortion, delivery at <37weeks gestation, premature birth, intrauterine death and endometritis.

6.2 Recommendations

In light of the results and discussion presented in this study, the following recommendations are made:

1. The high prevalence rate of GBS among pregnant women necessitate the screening approach for all pregnant women at 35–37 weeks' gestation in Gaza hospitals to provide antibiotic prophylaxis to GBS carrier.
2. Setting public health and governmental laboratories with necessary equipment and reagents for culturing and identifying GBS.

3. Training microbiology staff on techniques of detecting GBS from clinical specimens.
4. We recommend to use only one swab for culture and PCR to avoid the low Sensitivity of PCR which obtained in our study when we used two separate swabs.
5. Serotyping of GBS is recommended to be performed to develop and implement effective vaccine for prevention of pregnant and neonatal GBS disease. In addition, it is an effective epidemiological tool for studying GBS
6. The role of GBS in neonatal pneumonia, sepsis and meningitis should be investigated.
7. In this study, we observed resistance to clindamycin and erythromycin, the drugs of choice for penicillin-allergic women at high risk for anaphylaxis, which strongly supports the current CDC recommendation that antibiotic susceptibility test, should be performed if erythromycin and clindamycin therapy is needed to prevent neonatal GBS infection.
8. Antibiotic resistant GBS may occur as a result of misuse or overuse of antibiotics. Therefore, health care providers worldwide should be encouraged to join public health authorities, to control the inappropriate use of antibiotics and promote responsible prescribing. This will greatly help to improve prevention and control of drug resistant organisms in communities.
9. In this study, there is resistance to the commonly used antibiotics such as clindamycin, tetracycline and penicillin. Which calls for performing susceptibility testing before administration of any of these antibiotics.
10. It is recommended to conduct more studies with larger sample size covering larger number of women care centers which will impact the management of pregnant women before giving birth.
11. Formulate guidelines for the GBS screening policy in Palestine.

REFERENCES

References:

Al-Sweih, N., Maiyegun, S., Diejomaoh, M., Rotimi, V., Khodakhast, F., Hassan, N., …& Baig, S. (2003). Streptococcus agalactiae (Group B Streptococci) carriage in late pregnancy in Kuwait. *Medical Principles and Practice*, *13*(1), 10-14.

Andrews, J. I., Diekema, D. J., Hunter, S. K., Rhomberg, P. R., Pfaller, M. A., Jones, R. N., & Doern, G. V. (2000). Group B streptococci causing neonatal bloodstream infection: antimicrobial susceptibility and serotyping results from SENTRY centers in the Western Hemisphere. *American journal of obstetrics and gynecology*, *183*(4), 859-862.

Antibiotic Expert Group (AEG). (2010). *Therapeutic Guidelines: Antibiotic*. Vol. 14. North Melbourne, Victoria: Therapeutic Guidelines Limited.

Apgar, B., Greenberg, G., & Yen, G. (2002). Prevention of group B Streptococcal infection in newborns. *Can Med Assoc J*, *166*, 928-930.

Apostol, M., Gershman, K., Petit S., Arnold, K., Harrison, L., & Lynfield, R. (2009). Trends in perinatal group B streptococcal disease-United States, 2000-2006. *Morbidity and Mortality Weekly Report*, *58*(5), 109-112.

Ayata, A., Güvenç, H., Felek, S., Aygün, A. D., Kocabay, K., & Bektas, S. (1994). Maternal carriage and neonatal colonisation of group B streptococci in labour are uncommon in Turkey. Paediatric and perinatal epidemiology, 8(2), 188-192.

Azavedo, S., McGavin, M., & Duncan, C. (2001). Prevalence and mechanisms of macrolide resistance in invasive and noninvasive group B streptococcus isolates from Ontario, Canada. Antimicrob Agents and Chemother ,45, 3504-08.

Baker, CJ., Carey, VJ., Rench, MA., Edwards, MS., Hillier, SH., Kaspe, DL., Platt, R. (2014*).* Maternal Antibody at Delivery Protects Neonates From Early Onset Group B Streptococcal DiseaseMaternal Antibody at Delivery Protects Neonates From Early Onset Group B Streptococcal Disease. *J Infect Dis*, *209*,781–788. *doi*:10.1093/infdis/jit54910.1093/infdis/jit549.

Baker, C., & Edwards, M. (2001). Group B Streptococcal infections, Infectious diseases of the fetus and newborn infant. *Philadelphia: W.B. Saunders*, *10*, 91-156.

Baker, C., & Edwards. M. (1995). Group B streptococcal infections, In J. S. Remington and J. O. Klein (ed.), Infectious diseases of the fetus and newborn infant, 4th ed. W. B. Saunders Co., Philadelphia p.980–1054.

Bakhtiari, R., Dallal, M. S., Mehrabadi, J. F., Heidarzadeh, S., & Pourmand, M. R. (2012). Evaluation of culture and PCR methods for diagnosis of group B streptococcus carriage in Iranian pregnant women. *Iranian journal of public health*, *41*(3), 65.

Banno, H., Kimura, K., Tanaka, Y., Kitanaka, H., Jin, W., Wachino, J. I., ... & Arakawa, Y. (2014). Characterization of multidrug-resistant group B streptococci

with reduced penicillin susceptibility forming small non-beta-hemolytic colonies on sheep blood agar plates. *Journal of clinical microbiology*, *52*(6), 2169-2171.

Barbaros, I., Murat, C., Mehmet, V., Ismet, T. A., Can, K., Sukufe, D., ... & Yildiz, P. (2005). The colonization incidence of group B streptococcus in pregnant women and their newborns in Istanbul. *Pediatrics international*, *47*(1), 64-66.

Bergeron, M. G., Ke, D., Ménard, C., François, F. J., Gagnon, M., Bernier, M., ... & Fraser, W. D. (2000). Rapid detection of group B streptococci in pregnant women at delivery. *New England Journal of Medicine*, *343*(3), 175-179.

Block, T., Munson, E ., Culver, A., Vaughan, K., Hryciuk, J. (2008). Comparison of Carrot broth and selective Todd-Hewitt broth enhanced PCR protocols for Real-time detection of *Streptococcus agalactiae* in prenatal vaginal/anorectal specimens. *J Clin Microbiol,46,* 3615-3620.

Brandon, M., Dowzicky, M. (2013). Antimicrobial susceptibility among Gram-positive organisms collected from pediatric patients globally between 2004 and 2011: results from the Tigecycline Evaluation and Surveillance Trial. *Journal of microbiology*, 51(7), 2371-2378.

Brimil, N., Barthell, E., Heindrichs, U., Kuhn, M., Lütticken, R., & Spellerberg, B. (2006). Epidemiology of Streptococcus agalactiae colonization in Germany. *International Journal of Medical Microbiology*, *296*(1), 39-44.

Busetti, M., D'agaro, P., & Campello, C. (2007). Group B streptococcus prevalence in pregnant women from North-Eastern Italy: advantages of a screening strategy based on direct plating plus broth enrichment. *Journal of clinical pathology*, *60*(10), 1140-1143.Center for Disease Control and Prevention. (2000). Early-onset group B Streptococcal disease, United States. *MMWR*, *49*, 793-96.

Centers for Disease Control and Prevention (CDC). (2010). Prevention of perinatal group B streptococcal disease: Revised guidelines from CDC – United States. *MMWR Morb Mortal Wkly Rep, 59,* 1-32.

Centers for Disease Control and Prevention .(2002). Prevention of perinatal group B streptococcal disease. *Morbid Mortal Weekly Rep, 51,* 1-24.

Centers for Disease Control and Prevention. (2012). Antimicrobial susceptibilities among group B *Streptococcus* isolates-Active Bacterial Core Surveillance (ABCs), 2010. Centers for Disease Control and Prevention, Atlanta, GA.

Chaffin, D. O., Beres, S. B., Yim, H. H., & Rubens, C. E. (2000). The serotype of type Ia and III group B streptococci is determined by the polymerase gene within the polycistronic capsule operon. *Journal of bacteriology*, *182*(16), 4466-4477.

Chan, K. L., Levi, K., Towner, K. J., Weston, V. C., Ramsay, M. M., & Kean, L. H. (2006). Evaluation of the sensitivity of a rapid polymerase chain reaction for detection of group B streptococcus. *Journal of obstetrics and gynaecology*, *26*(5), 402-406.

Chaudhry, B. Y., Akhtar, N., & Balouch, A. H. (2010). Vaginal carriage rate of group B Streptococcus in pregnant women and its transmission to neonates. *J Ayub Med Coll Abbottabad*, *22*(4), 167-70.

Cheng, P. J., Chueh, H. Y., Liu, C. M., Hsu, J. J., & Soong, Y. K. (2008). Risk factors for recurrence of group B streptococcus colonization in a subsequent pregnancy. *Obstetrics & Gynecology*, *111*(3), 704-709.

Clinical and Laboratory Standards Institute (CLSI). (2013). *Performance standards for antimicrobial susceptibility testing*; 23rd informational supplement, M100 S23, (33), Wayne CLSI.

Cloud, J. L., Hymas, W., & Carroll, K. C. (2002). Impact of nasopharyngeal swab types on detection of Bordetella pertussis by PCR and culture. *Journal of clinical microbiology*, *40*(10), 3838-3840.

Cockerill, F. R., & Smith, T. F. (2002). Rapid-cycle real-time PCR: a revolution for clinical microbiology. *ASM News-American Society for Microbiology*, *68*(2), 77-83.

Convert, M., Martinetti Lucchini, G., Dolina, M., & Piffaretti, J. C. (2005). Comparison of LightCycler PCR and culture for detection of group B streptococci from vaginal swabs. *Clinical microbiology and infection*, *11*(12), 1022-1026.

Costa, A. L. D. R., Lamy Filho, F., Chein, M. B. D. C., Brito, L. M. O., Lamy, Z. C., & Andrade, K. L. (2008). Prevalence of colonization by group B Streptococcus in pregnant women from a public maternity of Northwest region of Brazil. *Revista Brasileira de Ginecologia e Obstetrícia*, *30*(6), 274-280.

Dagnew, A. F., Cunnington, M. C., Dube, Q., Edwards, M. S., French, N., Heyderman, R. S., ... & Clemens, S. A. C. (2012). Variation in reported neonatal group B streptococcal disease incidence in developing countries. *Clinical infectious diseases*, *55*(1), 91-102.

Daniels, J., Gray, J., Pattison, H., Edwards, E., Milner, P., Spicer, L., ... & Magill, L. (2009). Rapid testing for group B streptococcus during labour: a test accuracy study with evaluation of acceptability and cost-effectiveness. *Health technology assessment*, *13*(42), 1-178.

Davies, H., Miller, M., Faro, S., Gregson, D., Kehl, S., & Jordan, J. (2004). Multi-center study of a rapid, molecular-based assay for the diagnosis of group B streptococcal colonization in pregnant women. *Clinical Infectious Diseases*, *94*, 420-429.

Davies, H. D., Miller, M. A., Faro, S., Gregson, D., Kehl, S. C., & Jordan, J. A. (2004). Multicenter study of a rapid molecular-based assay for the diagnosis of group B Streptococcus colonization in pregnant women. *Clinical Infectious Diseases*, *39*(8), 1129-1135.

de-Paris, F., Machado, A. B. M. P., Gheno, T. C., Ascoli, B. M., de Oliveira, K. R. P., & Barth, A. L. (2011). Group B Streptococcus detection: comparison of PCR assay and culture as a screening method for pregnant women. *The Brazilian Journal of Infectious Diseases*, *15*(4), 323-327.

Diaz, T. M., & Nieves, B. M. (2008). Comparison between culture media and procedures to detect Streptococcus agalactiae in pregnant women. *Revista Chilena De Infectologia*, *25*(2), 108-113.

Doran, K. S., & Nizet, V. (2004). Molecular pathogenesis of neonatal group B streptococcal infection: no longer in its infancy. *Molecular microbiology*, *54*(1), 23-31.

Doran, K. S., Liu, G. Y., & Nizet, V. (2003). Group B streptococcal β-hemolysin/cytolysin activates neutrophil signaling pathways in brain endothelium and contributes to development of meningitis. *The Journal of clinical investigation*, *112*(5), 736-744.

Dunne, W. M., & Holland-Staley, C. A. (1998). Comparison of NNA agar culture and selective broth culture for detection of group B streptococcal colonization in women. *Journal of clinical microbiology*, *36*(8), 2298-2300.

Dzowela, T., Komolafe, O. O., & Igbigbi, A. (2005). Prevalence of group b Streptococcus colonization in antenatal women at the Queen Elizabeth Central Hospital, Blantyre–a preliminary study. *Malawi Medical Journal*, *17*(3), 97-99.

Edwards, MS.,& Nizet V. (2011). Group B streptococcal infections. Infectious Diseases of the Fetus and Newborn Infant . Elsevier.(7) 419–469. .

Ekström, L., Adolfsson, A., Ericson, H., Poutakidis, G., Charonis, G., & Larsson, P. G. (2013).Vaginal flora and urinary and vaginal group B streptococci in early pregnancy. *Gynecol*,1-6.

El Aila, N. A., Tency, I., Claeys, G., Saerens, B., Cools, P., Verstraelen, H., ... & Vaneechoutte, M. (2010). Comparison of different sampling techniques and of different culture methods for detection of group B streptococcus carriage in pregnant women. *BMC infectious diseases*, *10*(1), 285.

El Aila, N. A., Tency, I., Claeys, G., Saerens, B., De Backer, E., Temmerman, M., ... & Vaneechoutte, M. (2009). Genotyping of Streptococcus agalactiae (group B streptococci) isolated from vaginal and rectal swabs of women at 35-37 weeks of pregnancy. *BMC infectious diseases*, *9*(1), 153.

El Aila, NA., Tency, I., Claeys, G., Verstraelen, H., Deschaght, P., Decat, E., Lopes dos Santos Santiago, G. et al. (2011). Comparison of culture with two different qPCR assays for detection of rectovaginal carriage of Streptococcus agalactiae (group B streptococci) in pregnant women. *Res Microbiol. 162(5)*, 499-505.

Elbaradie, S., Mahmoud, M., & Farid, M. (2009). Maternal and neonatal screening for Group B streptococci by SCP B gene based PCR: a preliminary study. Indian J Med Microbiol, 27(1), 17-2.

Espy, M. J., Uhl, J. R., Mitchell, P. S., Thorvilson, J. N., Svien, K. A., Wold, A. D., & Smith, T. F. (2000). Diagnosis of herpes simplex virus infections in the clinical laboratory by LightCycler PCR. *Journal of Clinical Microbiology*, *38*(2), 795-799.

Ezeonu, I. M., & Agbo, M. C. (2014). Incidence and anti-microbial resistance profile of Group B Streptococcus (GBS) infection in pregnant women in Nsukka, Enugu State, Nigeria. *African Journal of Microbiology Research*, *8*(1), 91-95.

Faro, S., Brehm, B., Smith, F., Mouzoon, M., Greisinger, A., Wehmanen, O., & Turrentine, M. A. (2010). Screening for group B streptococcus: a private hospital's experience. *Infectious diseases in obstetrics and gynecology*, *2010*.

Fashina, N. (2008). Isolation and characterization of Group B Streptococci and other pathogens among pregnant women in Ibadan, Southwestern Nigeria. *J Appl Biosci, 29*,1781-1792.

Fatemi, F., Chamani, L., Pakzad, P., Zeraati, H., Rabbani, H., & Asgari, S. (2009). Colonization rate of group B Streptococcus (GBS) in pregnant women using GBS agar medium. *Acta Medica Iranica*, *47*(1), 25-30.

Fatemi, F., Pakzad, P., Zeraati, H., Talebi, S., Asgari, S., Akhondi, M. M., & Chamani-Tabriz, L. (2010). Comparative molecular and microbiologic diagnosis of vaginal colonization by group b streptococcus in pregnant women during Labor. *Iranian Journal of Basic Medical Sciences*, *13*(4), 183-188.

Feikin, D. R., Thorsen, P., Zywicki, S., Arpi, M., Westergaard, J. G., & Schuchat, A. (2001). Association between colonization with group B streptococci during pregnancy and preterm delivery among Danish women. *American journal of obstetrics and gynecology*, *184*(3), 427-433.

Fröhlicher, S., Reichen-Fahrni, G., Müller, A. M., Surbek, D., Droz, S. C., Spellerberg, B., & Sendi, P. (2014). Serotype distribution and antimicrobial susceptibility of group B streptococci in pregnant women: results from a Swiss tertiary centre. *Swiss medical weekly*, *144*, w13935.

Garland, S. M., Kelly, N., & Ugoni, A. M. (2000). Is antenatal group B streptococcal carriage a predictor of adverse obstetric outcome?. *Infectious diseases in obstetrics and gynecology*, *8*(3-4), 138-142.

Gaudreau, C., Lecours, R., Ismaïl, J., Gagnon, S., Jetté, L., & Roger, M. (2010). Prosthetic hip joint infection with a Streptococcus agalactiae isolate not susceptible to penicillin G and ceftriaxone. *Journal of antimicrobial chemotherapy*, *65*(3), 594-595.

Gavino, M., & Wang, E. (2007). A comparison of a new rapid real-time polymerase chain reaction system to traditional culture in determining group B streptococcus colonization. *American journal of obstetrics and gynecology*, *197*(4), 388-e1.

Gibbs, R. S., Schrag, S., & Schuchat, A. (2004). Perinatal infections due to group B streptococci. *Obstetrics & Gynecology*, *104*(5, Part 1), 1062-1076.

Gray, K. J., Bennett, S. L., French, N., Phiri, A. J., & Graham, S. M. (2007). Invasive group B streptococcal infection in infants, Malawi. *Emerging infectious diseases*, *13*(2), 223-9.

Haberland, C. A., Benitz, W. E., Sanders, G. D., Pietzsch, J. B., Yamada, S., Nguyen, L., & Garber, A. M. (2002). Perinatal screening for group B streptococci: cost-benefit analysis of rapid polymerase chain reaction. *Pediatrics*, *110*(3), 471-480.

Hannoun, A., Shehab, M., Khairallah, M. T., Sabra, A., Abi-Rached, R., Bazi, T., ... & Matar, G. M. (2010). Correlation between group B streptococcal genotypes, their antimicrobial resistance profiles, and virulence genes among pregnant women in Lebanon. *International journal of microbiology*, *2009*.

Hsueh, P. R., Teng, L. J., Lee, L. N., Ho, S. W., Yang, P. C., & Luh, K. T. (2001). High incidence of erythromycin resistance among clinical isolates of Streptococcus agalactiae in Taiwan. *Antimicrobial agents and chemotherapy*, *45*(11), 3205-3208.

Ippolito, D. L., James, W. A., Tinnemore, D., Huang, R. R., Dehart, M. J., Williams, J., ... & Demons, S. T. (2010). Group B streptococcus serotype prevalence in reproductive-age women at a tertiary care military medical center relative to global serotype distribution. *BMC infectious diseases*, *10*(1), 336.

Joachim, A., Matee, M., Masswawe, F., & Layamuya, E. (2009). Maternal and neonatal colonization of group B *streptococcus* at Muhimbili National Hospital in Dares Salaam, Tanzania: prevalence, risk factors and antimicrobial resistance. *BMC Public Health*, *9*, 437-45.

Johri, A. K., Paoletti, L. C., Glaser, P., Dua, M., Sharma, P. K., Grandi, G., & Rappuoli, R. (2006). Group B Streptococcus: global incidence and vaccine development. *Nature Reviews Microbiology*, *4*(12), 932-942.

Jordan, J. A., Hall, G., & Davis, T. (2010). Multicenter study evaluating performance of the Smart Group B Streptococcus (GBS) assay using an enrichment protocol for detecting GBS colonization in patients in the antepartum period. *Journal of clinical microbiology*, *48*(9), 3193-3197.

Ke, D., Ménard, C., Picard, F. J., Boissinot, M., Ouellette, M., Roy, P. H., & Bergeron, M. G. (2000). Development of conventional and real-time PCR assays

for the rapid detection of group B streptococci. *Clinical chemistry*, *46*(3), 324-331.

Kim, E. J., Oh, K. Y., Kim, M. Y., Seo, Y. S., Shin, J. H., Song, Y. R., ... & Ki, M. (2011). Risk factors for group B streptococcus colonization among pregnant women in Korea. *Epidemiology and health*, *33*, e2011010.

Kimura, K., Suzuki, S., Wachino, J. I., Kurokawa, H., Yamane, K., Shibata, N., ... & Arakawa, Y. (2008). First molecular characterization of group B streptococci with reduced penicillin susceptibility. *Antimicrobial agents and chemotherapy*, *52*(8), 2890-2897.

Lauer, P., Rinaudo, C. D., Soriani, M., Margarit, I., Maione, D., Rosini, R., ... & Telford, J. L. (2005). Genome analysis reveals pili in Group B Streptococcus. *Science*, *309*(5731), 105-105.

Leclercq ,SY., Sullivan, MJ., Ipe, DS., Smith, JP., Cripps, AW., &Ulett, GC. (2016). Pathogenesis of Streptococcus urinary tract infection depends on bacterial strain and β-hemolysin/cytolysin that mediates cytotoxicity, cytokine synthesis, inflammation and virulence. Sci Rep, 6, 29000. doi:10.1038/srep29000. PMC 4935997. PMID 27383371.

Lin, F. Y. C., Weisman, L. E., Troendle, J., & Adams, K. (2003). Prematurity is the major risk factor for late-onset group B streptococcus disease. *Journal of Infectious Diseases*, *188*(2), 267-271.

Lin, F. Y. C., Weisman, L. E., Azimi, P. H., Philips, J. B., Clark, P., Regan, J., ... & Brenner, R. A. (2004). Level of maternal IgG anti-group B streptococcus type III antibody correlated with protection of neonates against early-onset disease caused by this pathogen. *Journal of Infectious Diseases*, *190*(5), 928-934.

Ling, Z., Kong, J., Liu, F., Zhu, H., Chen, X., Wang, Y., ... & Xiang, C. (2010). Molecular analysis of the diversity of vaginal microbiota associated with bacterial vaginosis. *BMC genomics*, *11*(1), 488.

Longtin, J., Vermeiren, C., Shahinas, D., Tamber, G. S., McGeer, A., Low, D. E., ... & Pillai, D. R. (2011). Novel mutations in a patient isolate of Streptococcus agalactiae with reduced penicillin susceptibility emerging after long-term oral suppressive therapy. *Antimicrobial agents and chemotherapy*, *55*(6), 2983-2985.

Madzivhandila, M., Adrian, P. V., Cutland, C. L., Kuwanda, L., Schrag, S. J., & Madhi, S. A. (2011). Serotype distribution and invasive potential of group B streptococcus isolates causing disease in infants and colonizing maternal-newborn dyads. *PloS one*, *6*(3), e17861.

Maisey, H. C., Doran, K. S., & Nizet, V. (2008). Recent advances in understanding the molecular basis of group B Streptococcus virulence. *Expert reviews in molecular medicine*, *10*, e27.

Manning, S., Neighbors, K., Tallman, P., Gillespie, B., Marrs, C., Borchardt, S., … Foxman, B. (2004). Prevalence of group B *streptococcus* colonization and potential for transmission by casual contact in healthy young men and women. *Clin infect Dis, 39,* 381-387.

Mavenyengwa, R. T., Afset, J. E., Schei, B., Berg, S., Caspersen, T., Bergseng, H., & Moyo, S. R. (2010). Group B Streptococcus colonization during pregnancy and maternal-fetal transmission in Zimbabwe. *Acta obstetricia et gynecologica Scandinavica*, *89*(2), 250-255.

McDonald, H. M., & Chambers, H. M. (2000). Intrauterine infection and spontaneous midgestation abortion: is the spectrum of microorganisms similar to

that in preterm labor?. *Infectious diseases in obstetrics and gynecology*, *8*(5-6), 220-227.

Melin, P. (2011). Neonatal group B streptococcal disease: from pathogenesis to preventive strategies. *Clinical Microbiology and Infection*, *17*(9), 1294-1303.

Michael, J., & Burton, E. (2011). *Photographic Atlas for the Microbiology Laboratory*. (4th ed.). USA: Douglas N. Morton.

Mohammed, M., Asrat, D., Woldeamanuel, Y., & Demissie, A. (2012). Prevalence of group B Streptococcus colonization among pregnant women attending antenatal clinic of Hawassa Health Center, Hawassa, Ethiopia. *Ethiopian Journal of Health Development*, *26*(1), 36-42.

Moyo, S. R., Maeland, J. A., & Munemo, E. S. (2000). Susceptibility of Zimbabwean Streptococcus agalactiae (group B Streptococcus; GBS) isolates to four different antibiotics. *The Central African journal of medicine*, *47*(9-10), 226-229.

Munari, F. M., De-Paris, F., Salton, G. D., Lora, P. S., Giovanella, P., Machado, A. B. M. P., ... & Laurino, C. C. F. C. (2012). A combined enrichment/polymerase chain reaction based method for the routine screening of Streptococcus agalactiae in pregnant women. *Brazilian Journal of Microbiology*, *43*(1), 253-260.

Najmi, N., Sikandar, R., Zuberi, N. F., & Jehan, I. (2013). Maternal genital tract colonisation by Group-B Streptococcus: a hospital based study. *Journal of Pakistan Medical Association*, *63*(9), 1103.

Nandyal, R. R. (2008). Update on group B streptococcal infections: perinatal and neonatal periods. *The Journal of perinatal & neonatal nursing*, *22*(3), 230-237.

Okon, K. O., Usman, H., Umar, Z., & Balogun, S. T. (2013). Prevalence of Group B Streptococcus (GBS) colonization among pregnant women attending antenatal clinic of a tertiary hospital in northeastern Nigeria. *American Journal of Research Communication*, *1*(6), 54-66.

Onipede, A., Adefusi, O., Adeyemi,A., Adejuyigbe, E., Oyelese, A., & Ogunniyi, T. (2012). Group B *streptococcus* carriage during late pregnancy in Ile-Ife, Nigeria. *African Journal of Clinical and Experimental Microbiology*,*13*(3),135-43.

Orrett, F. A. (2003). Colonization with Group B streptococci in pregnancy and outcome of infected neonates in Trinidad. *Pediatrics international*, *45*(3), 319-323.

Bergeron, M. G., Ke, D., Ménard, C., François, F. J., Gagnon, M., Bernier, M., ... & Fraser, W. D. (2000). Rapid detection of group B streptococci in pregnant women at delivery. *New England Journal of Medicine*, *343*(3), 175-179.

Pelaez, L. M., Gelber, S. E., Fox, N. S., & Chasen, S. T. (2009). Inappropriate use of vancomycin for preventing perinatal group B streptococcal (GBS) disease in laboring patients. *Journal of perinatal medicine*, *37*(5), 487-489.

Perry, J. D., Oliver, M., Nicholson, A., Wright, J., & Gould, F. K. (2006). Evaluation of a new chromogenic agar medium for isolation and identification of Group B streptococci. *Letters in applied microbiology*, *43*(6), 615-618.

Picard, F. J., & Bergeron, M. G. (2004). Laboratory detection of group B Streptococcus for prevention of perinatal disease. *European Journal of Clinical Microbiology and Infectious Diseases*, *23*(9), 665-671.

Pignanelli., S., Pulcrano., G., Schiavone, P., Di Santo, A., & Zaccherini., P. (2015). Selectivity evaluation of a new chromogenic medium to detect group B Streptococcus. *Indian J Pathol Microbiol, 58,* 45–7. doi:10.4103/0377-4929.151186

Pulver, L. S., Hopfenbeck, M. M., Young, P. C., Stoddard, G. J., Korgenski, K., Daly, J., & Byington, C. L. (2009). Continued early onset group B streptococcal infections in the era of intrapartum prophylaxis. *Journal of Perinatology*, *29*(1), 20-25.

Puopolo, K. M., Madoff, L. C., & Eichenwald, E. C. (2005). Early-onset group B streptococcal disease in the era of maternal screening. *Pediatrics*, *115*(5), 1240-1246.

Quiroga, M., Pegels, E., Oviedo, P., Pereyra, E., & Vergara, M. (2008). Antibiotic susceptibility patterns and prevalence of group B Streptococcus isolated from pregnant women in Misiones, Argentina. *Brazilian Journal of Microbiology*, *39*(2), 245-250.

Rajagopal, L. (2009). Understanding the regulation of Group B Streptococcal virulence factors" (PDF). Future Microbiol, 4, 201–221. doi:10.2217/17460913.4.2.201. PMC 2691590. PMID 19257847

Rallu, F., Barriga, P., Scrivo, C., Martel-Laferrière, V., & Laferrière, C. (2006). Sensitivities of antigen detection and PCR assays greatly increased compared to that of the standard culture method for screening for group B streptococcus carriage in pregnant women. *Journal of clinical microbiology*, *44*(3), 725-728.

Ranzcog, (2007). Screening and Treatment for Group B Streptococcus in Pregnancy, Ranzcog statement C-Obs 19. Melbourne: *Royal Australian and New Zealand College of Obstetricians and Gynaecologists*.

Rausch, A. V., Gross, A., Droz, S., Bodmer, T., & Surbek, D. V. (2009). Group B Streptococcus colonization in pregnancy: prevalence and prevention strategies of neonatal sepsis. *Journal of perinatal medicine*, *37*(2), 124-129.

Rodriguez-Granger, J., Alvargonzalez, J. C., Berardi, A., Berner, R., Kunze, M., Hufnagel, M., ... & Telford, J. (2012). Prevention of group B streptococcal neonatal disease revisited. The DEVANI European project. *European journal of clinical microbiology & infectious diseases*, *31*(9), 2097-2104.

Rosa-Fraile, M., Dramsi, S.,& Spellerberg, B. (2014). Group B streptococcal haemolysin and pigment, a tale of twins." . *FEMS Microbiol Rev.* ***38***, 932–946. doi:10.1111/1574-6976.12071

Rosa-Fraile, M., Sampedro, A., Rodríguez-Granger, J., García-Peña, M. L., Ruiz-Bravo, A., & Haïdour, A. (2001). Pigment production by Streptococcus agalactiae in quasi-defined media. *Applied and environmental microbiology*, *67*(1), 473-474.

Samuel, P. G. (2002). Group B streptococcal infections. *Pediatr Rev*, *23*, 381-385.

Savoia, D., Gottimer, C., Crocilla, C., & Zucca, M. (2008). Streptococcus agalactiae in pregnant women: phenotypic and genotypic characters. *Journal of Infection*, *56*(2), 120-125.

Schmidt, J., Halle, E., Halle, H., Mohammed, T., & Gunther, E. (1989). Colonization of pregnant women and their newborn infants with group B streptococci in the Gondar College of Medical Sciences. *Ethiopian medical journal*, *27*(3), 115-119.

Schrag, S., Gorwitz, R., Fultz-Butts, K., & Schuchat, A. (2002). Prevention of perinatal group B streptococcal disease. *MMWR Recomm Rep*, *51*(11), 1-22.

Schuchat, A. (2000). Neonatal group B streptococcal disease-Screening and prevention. *N. Engl. J. Med*, *343*, 209-210.

Schwarz-Linek, U., Höök, M., & Potts, J. R. (2004). The molecular basis of fibronectin-mediated bacterial adherence to host cells. *Molecular microbiology*, *52*(3), 631-641.

Seoud, M., Nassar, A. H., Zalloua, P., Boghossian, N., Ezeddine, J., Fakhoury, H., ... & Sanyoura, M. (2010). Prenatal and neonatal Group B Streptococcus screening and serotyping in Lebanon: incidence and implications. *Acta obstetricia et gynecologica Scandinavica*, *89*(3), 399-403.

Shabayek, S., Abdalla, S., & Abouzeid, A. M. (2010). Comparison of scpB gene and cfb gene polymerase chain reaction assays with culture on Islam medium to detect Group B Streptococcus in pregnancy. *Indian journal of medical microbiology*, *28*(4), 320.

Sharmila, V., Joseph, N. M., Babu, T. A., Chaturvedula, L., & Sistla, S. (2011). Genital tract group B streptococcal colonization in pregnant women: a South Indian perspective. *The Journal of Infection in Developing Countries*, *5*(08), 592-595.

Shet, A., & Ferrieri, P. (2004). Neonatal & maternal group B streptococcal infections: a comprehensive review. *Indian Journal of Medical Research*, *120*(3), 141.

Shirazi, M., Abbariki, E., Hafizi, A., Shahbazi, F., Bandari, M., & Dastgerdy, E. (2014). The prevalence of group B streptococcus colonization in Iranian pregnant women and its subsequent outcome. Int J Fertil Steril, 7(4), 267-270.

Simoes, J. A., Alves, V. M. N., Fracalanzza, S. E. L., Camargo, R. P. S. D., Mathias, L., Milanez, H. M. B. P., & Brolazo, E. M. (2007). Phenotypical characteristics of group B streptococcus in parturients. *Brazilian Journal of Infectious Diseases*, *11*(2), 261-266.

Simoes, J. A., Aroutcheva, A. A., Heimler, I., & Faro, S. (2004). Antibiotic resistance patterns of group B streptococcal clinical isolates. *Infectious diseases in obstetrics and gynecology*, *12*(1), 1-8.

Sloan, L. M., Hopkins, M. K., Mitchell, P. S., Vetter, E. A., Rosenblatt, J. E., Harmsen, W. S., ... & Patel, R. (2002). Multiplex LightCycler PCR assay for detection and differentiation of Bordetella pertussis and Bordetella parapertussis in nasopharyngeal specimens. *Journal of clinical microbiology*, *40*(1), 96-100.

Slotved, H. C., Kong, F., Lambertsen, L., Sauer, S., & Gilbert, G. L. (2007). Serotype IX, a proposed new Streptococcus agalactiae serotype. *Journal of clinical microbiology*, *45*(9), 2929-2936.

Sri-Budayanthi, N., & Hariyasa-Sanjaya, N. (2013). Group B *Streptococcus* in pregnant women: Prevalence of Colonization and Sensitivity Pattern in Denpasar during June, 2007-May, 2008. *Bali Medical Journal*, *2*(1), 17-20.

Strus, M., Pawlik, D., Brzychczy-Włoch, M., Gosiewski, T., Rytlewski, K., Lauterbach, R., & Heczko, P. B. (2009). Group B streptococcus colonization of pregnant women and their children observed on obstetric and neonatal wards of the University Hospital in Krakow, Poland. *Journal of medical microbiology*, *58*(2), 228-233.

Steenwinkel, D., Florentien, D. O., Tak, H. V., Muller, A. E., Nouwen, J. L., Oostvogel, P. M., & Mocumbi, S. M. (2008). Low carriage rate of group B streptococcus in pregnant women in Maputo, Mozambique. *Tropical Medicine & International Health*, *13*(3), 427-429

Tazi, A., Doloy, A., Reglier-Poupet, H., Hemet, M. E., Raymond, J., & Poyart, C. (2009). Evaluation of the new chromogenic medium StrepB Select for screening of group B streptococcus in pregnant women. *Pathologie-biologie*, *57*(3), 225-228.

Tazi, A., Réglier-Poupet, H., Dautezac, F., Raymond, J., & Poyart, C. (2008). Comparative evaluation of Strepto B ID® chromogenic medium and Granada media for the detection of group B streptococcus from vaginal samples of pregnant women. *Journal of microbiological methods*, *73*(3), 263-265.

Trijbels-Smeulders, M. A., Kollée, L. A., Adriaanse, A. H., Kimpen, J. L., & Gerards, L. J. (2004). Neonatal group B streptococcal infection: incidence and strategies for prevention in Europe. *The Pediatric infectious disease journal*, *23*(2), 172-173.

Tsolia, M., Psoma, M., Gavrili, S., Petrochilou, V., Michalas, S., Legakis, N., & Karpathios, T. (2003). Group B streptococcus colonization of Greek pregnant women and neonates: prevalence, risk factors and serotypes. *Clinical microbiology and infection*, *9*(8), 832-838.

Ueno, H., Yamamoto, Y., Yamamichi, A., Kikuchi, K., Kobori, S., & Miyazaki, M. (2012). Characterization of group B streptococcus isolated from women in Saitama city, Japan. *Japanese journal of infectious diseases*, *65*(6), 516-521.

Ulett, K. B., Benjamin, W. H., Zhuo, F., Xiao, M., Kong, F., Gilbert, G. L., ... & Ulett, G. C. (2009). Diversity of group B streptococcus serotypes causing urinary tract infection in adults. *Journal of clinical microbiology*, *47*(7), 2055-2060.

Valkenburg-van den Berg, A. W., Sprij, A. J., Oostvogel, P. M., Mutsaers, J. A., Renes, W. B., Rosendaal, F. R., & Dörr, P. J. (2006). Prevalence of colonisation with group B Streptococci in pregnant women of a multi-ethnic population in The Netherlands. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, *124*(2), 178-183.

Veit, A. R., Roehrs, M. C. S. M., Mayer, L. E., Santos, S. O., Martini, R., Tizotti, M. K., ... & Colpo, P. R. (2011). Colonization Prevalence and Susceptibility of Streptococcus agalactiae in Pregnant Women at HUSM. *Saúde (Santa Maria)*, *36*(1), 9-14.

Verani, J. R., McGee, L., & Schrag, S. J. (2010). *Prevention of perinatal group B streptococcal disease: Revised guidelines from CDC, 2010*. Department of Health and Human Services, Centers for Disease Control and Prevention.

Wernecke, M., Mullen, C., Sharma, V., Morrison, J., Barry, T., Maher, M., & Smith, T. (2009). Evaluation of a novel real-time PCR test based on the ssrA gene for the identification of group B streptococci in vaginal swabs. *BMC infectious diseases*, *9*(1), 148.

Whidbey.,C., Vornhagen, J., Gendrin, C., Boldenow,E., Samson, JM., Doering K., ... & Rajagopal L. (2015). A streptococcal lipid toxin induces membrane permeabilization and pyroptosis leading to fetal injury. *EMBO Mol Med*, *7*, 488–505.
doi:10.15252/emmm.201404883. PMC 4403049. PMID 25750210.

Williams-Bouyer, N., Reisner, B. S., & Woods, G. L. (2000). Comparison of Gen-Probe AccuProbe Group B streptococcus culture identification test with conventional culture for the detection of Group B streptococci in broth cultures

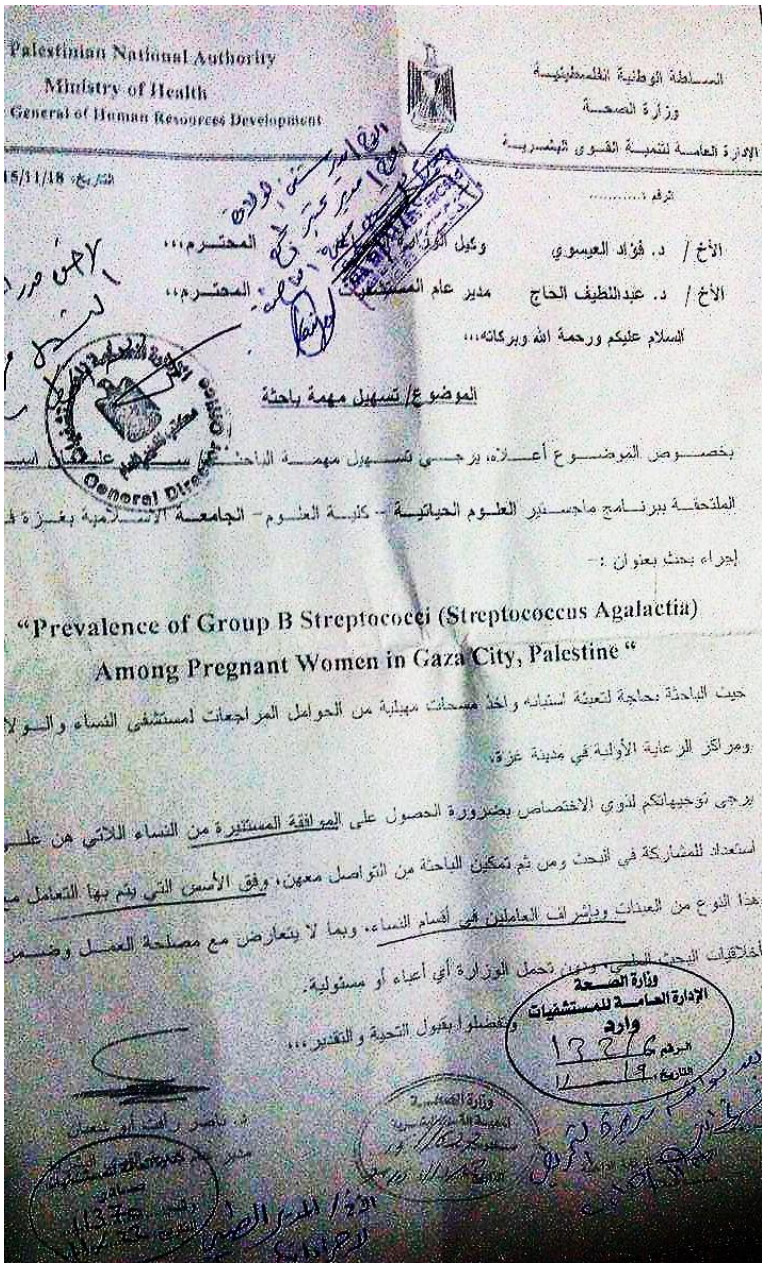
of vaginal-anorectal specimens from pregnant women☆. *Diagnostic microbiology and infectious disease*, *36*(3), 159-162.

Yancey, M. K., Schuchat, A., Brown, L. K., Ventura, V. L., & Markenson, G. R. (1996). The accuracy of late antenatal screening cultures in predicting genital group B streptococcal colonization at delivery. *Obstetrics & Gynecology*, *88*(5), 811-815.

Yang, M. J., Sun, P. L., Wen, K. C., Chao, K. C., Chang, W. H., Chen, C. Y., & Wang, P. H. (2012). Prevalence of maternal group B streptococcus colonization and vertical transmission in low-risk women in a single institute. *Journal of the Chinese Medical Association*, *75*(1), 25-28.

Zusman, A. S., Baltimore, R. S., & Fonseca, S. N. (2006). Prevalence of maternal group B streptococcal colonization and related risk factors in a Brazilian population. *Brazilian Journal of Infectious Diseases*, *10*(4), 242-246.

Annexes



Palestinian National Authority
Ministry of Health
General of Human Resources Development

السلطة الوطنية الفلسطينية
وزارة الصحة
الإدارة العامة لتنمية القوى البشرية

التاريخ: 15/11/18

الرقم: .....

الأخ / د. فؤاد العرسوي وكيل الوزارة المحترم،،،

الأخ / د. عبداللطيف الحاج مدير عام المستشفيات المحترم،،،

السلام عليكم ورحمة الله وبركاته،،،

الموضوع/ تسهيل مهمة باحثة

بخصوص الموضوع أعلاه، يرجى تسهيل مهمة الباحثة [illegible] الملتحقة ببرنامج ماجستير العلوم الحياتية - كلية العلوم - الجامعة الإسلامية بغزة [illegible] إجراء بحث بعنوان :-

"Prevalence of Group B Streptococci (Streptococcus Agalactia) Among Pregnant Women in Gaza City, Palestine "

حيث الباحثة بحاجة لتعبئة استبانه وأخذ مسحات مهبلية من الحوامل المراجعات لمستشفى النساء والولا ومراكز الرعاية الأولية في مدينة غزة.

يرجى توجيهاتكم للذوي الاختصاص بضرورة الحصول على الموافقة المستنيرة من النساء اللاتي هن على استعداد للمشاركة في البحث ومن ثم تمكين الباحثة من التواصل معهن، وفق الأسس التي يتم بها التعامل مع هذا النوع من العينات وبإشراف العاملين في أقسام النساء، وبما لا يتعارض مع مصلحة العمل وضمن أخلاقيات البحث العلمي، ودون تحمل الوزارة أي أعباء أو مسئولية.

وتفضلوا بقبول التحية والتقدير،،،

وزارة الصحة
الإدارة العامة للمستشفيات
وارد
الرقم 13276
التاريخ 19/11/18

د. ناصر رأفت أبو معمر
مدير عام الإدارة العامة للمستشفيات

Annex 2

- **Date of birth** ___/____/_____
- **Education level** ❑Preparatory ❑ Secondary ❑ University
- **Pregnancy complication** ❑ Yes ❑ No

  ❑endometritis
  ❑Previous abortion
  ❑premature birth
  ❑intrauterine death
  ❑delivery at <37weeks gestation
- **History of cystitis** ❑ Yes ❑ No
- **History of vaginitis** ❑ Yes ❑ No
- **Antibiotic used**
- **Material used for cleaning and scraping**