UNIVERSITY OF CAPE COAST

DIAGNOSTIC EVALUATION AND RISK FACTORS OF *Streptococcus agalactiae* INFECTION AMONG PREGNANT WOMEN.

BY

GRACE BIMPONG

Thesis submitted to the Department of Microbiology and Immunology of the School of Medical Sciences, College of Health and Allied Sciences, University of Cape Coast, in partial fulfillment of the requirements for the award of Master of Philosophy degree in Infection and Immunity

MAY 2023

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research work and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature: …… Date: ……..

Name: Grace Bimpong

Supervisors' Declaration

I hereby declare that the preparation and presentation of this thesis was supervised in accordance with the guidelines on supervision of dissertation laid down by the University of Cape Coast.

Supervisor's Signature: ……..….. Date:……

Name:

Co-supervisor's Signature: ……..…..

Date:……

Name:

ABSTRACT

Group B streptococcus (GBS) or *Streptococcus agalactiae,* a Gram-positive bacterium in the gastrointestinal and gastro-urinary tract, is a significant culprit in bacterial infections among parturient mothers and their neonates. It leads to life-threatening situations among the infected despite the current advances in medical care. This study aimed to determine the prevalence and risk factors associated with *Group B streptococcus* colonization and compared different diagnostic approaches among pregnant women attending the Cape Coast Teaching Hospital. The research employed a cross-sectional comparative study of 150 pregnant women between the ages of 15 to 45 years, with a mean of 25.1 (±4.7) each for every trimester. Vaginal swab samples were collected from each participant and sent for microscopy, biochemical testing, culture, and polymerase chain reaction (PCR) using the multiplex primer approach. The study reported a prevalence of 34.7% of GBS infection among the participants using the 16s rRNA PCR sequencing method as the standard. A comparison of the other PCR techniques and the culture method reported that the culture method was insensitive but specific. In all, no significant association was established between the prevalence of infection and the clinical information of participants. GBS was highly prevalent among the participants, and established protocol for diagnosis is urgent to curtail this disease.

ACKNOWLEDGEMENT

I want to acknowledge the faculty for their significant support and insight that helped me complete this work.

Also, I am grateful to my supervisor Dr Samuel Essien -Baidoo for his mentorship and continuous encouragement, as well as his humble approach throughout this write-up,

I am forever thankful for the unconditional love and support throughout the entire thesis process.

DEDICATION

To my late parents, Mr. John Yaw Bimpong and Madam Elizabeth Gyamfuaa, my siblings, and my children.

TABLE OF CONTENTS

	Page
DECLARATION	ii
ABSTRACT	iii
ACKNOWLEDGEMENT	iv
DEDICATION	v
TABLE OF CONTENTS	vi
LIST OF TABLES	ix
LIST OF FIGURES	x
CHAPTER ONE: INTRODUCTION	
Background	1
Problem Statement	3
Justification	4
Aim	5
Specific objectives	5
Organization of study	5
Chapter Summary	6
CHAPTER TWO: LITERATURE REVIEW	
Introduction	7
Brief History of GBS	7
Bacteriology of GBS	9
GBS Serotypes	10
Pathogenesis of GBS	11
Epidemiology of Group B Streptococci	12
Group B Streptococcus Disease	15

Maternal colonization 15

Neonatal colonization 16

Early-onset GBS disease 16

Late-onset GBS disease 17

Treatment of GBS Infection 19

Prevention of GBS Infection: Vaccination and Prophylaxis. 20

Laboratory Detection, Typing and Antimicrobial Susceptibility Testing of GBS 21

Sampling, Culture and Identification 21

Sampling 21

Culture 21

Identification 22

Serotyping 22

Polymerase Chain Reaction (PCR) 23

Antimicrobial susceptibility testing 25

Chapter Summary 26

CHAPTER THREE: MATERIALS AND METHOD

Introduction 28

Study Design 28

Study Site 28

Sample Size 29

Inclusion and Exclusion Criteria 29

Ethical Clearance 30

Laboratory Procedure 30

Specimen Collection and Processing 30

Gram staining technique 30

Detection by culture technique 31

Catalase test 31

CAMP test 31

DNA extraction for Group B *Streptococcus* 32

Detection of GBS by PCR technique 33

Storage 34

Quality control 34

Data Analysis 34

Chapter summary 34

CHAPTER FOUR: RESULTS AND DISCUSSION

Introduction 35

Results 35

Discussion 51

Chapter Summary 57

CHAPTER FIVE: SUMMARY, CONCLUSION AND RECOMMENDATION

Introduction 58

Summary 58

Conclusion 59

Recommendation 60

REFERENCES 61

LIST OF TABLES

Table Page

1. Primer sets synthesized for *Streptococcus agalactiae* identification in the PCR 33
2. Characterization of the Demographic Information of the Participants Across Their Age Groups 35
3. Characterization of the Clinical Information of the Participants Across Age 37
4. Characterization of the Clinical Information of the Participants Across Age 38
5. Sensitivity, Specificity, PPV, NPV and Likelihood Ratio of the various test using 16s rRNA PCR Test as the Standard 43
6. Prevalence and Multivariate Regression Analysis (OR) of GBS Infection Across the 16s RNA PCR Test of the Participants 45
7. Prevalence and Multivariate Regression Analysis (OR) of GBS Infection Across the 16s RNA PCR Test of the Participants 46

LIST OF FIGURES

Figure Page

1. Neonatal GBS pathogenesis 12
2. Prevalence of GBS Observed from Culture and the Various PCR Techniques 39
3. Occurrence of Group B *Streptococcus* at Different Trimesters Under Different Diagnostic Tests 40
4. Comparing Diagnostic Performance of Culture and Other PCR Tests to the 16s rRNA PCR Test 41

CHAPTER ONE

INTRODUCTION

Improving maternal and health care is one of the Global Sustainable Development Goals, as there are currently 5.6 million deaths attributed to vertical transmission from infected mothers around delivery time (Lawn et al., 2017).

Neonatal sepsis, pneumonia, bacteremia, and meningitis have all been linked to Streptococcus agalactiae, often known as Group B streptococcus (GBS). Data on GBS are few in Ghana, despite efforts to reduce the prevalence of infections in pregnant women and neonates.

This chapter consists of the background, problem statement, justification, aim and organisation of the study determined to look into the prevalence and risk factors associated with GBS colonization among pregnant women and comparing the diagnostic value of culture and PCR assays at the Cape Coast Teaching Hospital.

Background

Bacterial infections continue to be a major cause of morbidity and death in pregnant women and their newborns. A major cause of bacterial infections in expectant women and their newborns is Group B streptococcus (GBS), also known as Streptococcus agalactiae, a Gram-positive bacterium that lives in the gastrointestinal and urinary tracts (Ahmadzia & Heine, 2014). Although often brief, maternal GBS occurs frequently throughout pregnancy.

One in five pregnant women worldwide is presumed to carry GBS, a major yet preventable cause of maternal and infant illness (Lawn, Bianchi-Jassir, Russell, Kohli-Lynch, Tann, Hall, et al., 2017). The range in maternal

colonisation can be associated with the variation in populations such as age, ethnicity, socioeconomic status (SES) and geography (Karnabi, 2017). Manifestations of infection during pregnancy or the postpartum period include urinary tract infections, chorioamnionitis, endometritis, puerperal sepsis and other complications (Karnabi, 2017; Melin, 2011).

Despite advancements in medical procedures, studies continue to demonstrate that GBS infection is still fatal, necessitating antenatal screening of expectant mothers between weeks 35 and 37 and subsequent antibiotic prophylaxis for carriers to prevent GBS diseases (Slotved, Dayie, Banini, & Frimodt-Mller, 2017). Despite improvements in healthcare delivery, it is a major contributor to maternal deaths linked to bacterial infections (Vinnemeier, Brust, Owusu-Dabo, Sarpong, Sarfo, Bio et al., 2015). The maternal mortality rate fell from 987 to 546 per 100,000 newborns in sub-Saharan Africa between 1990 and 2015, whereas it rose to 12 in developed nations.

Recognising GBS infection as an important disease in Africa has been a problem due to the lack of data on the prevalence and serotypes among pregnant women and neonates in most countries. Although recently, some countries such as Gabon, Ethiopia, the Democratic Republic of the Congo, and Zimbabwe have done studies on the serotype distribution of GBS (Belard, Toepfner, Capan-Melser, Mombo-Ngoma, Zoleko-Manego, Groger, et al., 2015; Mitima, Ntamako, Birindwa, Mukanire, Kivukuto, Tsongo, et al., 2014; Woldu, Teklehaimanot, Waji, & Gebremariam, 2014).

In Ghana, recent publications have found the prevalence of GBS to be between 19-27% (Slotved, Dayie, Banini, & Frimodt-Møller, 2017; Vinnemeier, Brust, Owusu-Dabo, Sarpong, Sarfo, Bio, et al., 2015; Völker,

Cooper, Bader, Uy, Zimmermann, Lugert, et al., 2017). All serotypes of GBS have been isolated in previous studies conducted in Ghana. The prevalence within serotypes varied from study to study. However, serotypes Ib, V, VII and IX were the most predominant when studies in Ghana were compared (Slotved, Dayie, Banini, & Frimodt-Møller, 2017; Vinnemeier et al., 2015).

This study, therefore, aimed to add up to the existing data by determining the prevalence and risk factors associated with *Group B streptococcus* colonization and comparing different diagnostic approaches among pregnant women attending the Cape Coast Teaching Hospital in the Central Region of Ghana. This will be a useful information on the GBS situation in Cape Coast and contribute to developing a comprehensive diagnostic and management protocol for GBS among pregnant women in the country.

Problem Statement

In Sub-Saharan Africa, information on the prevalence and serotype distribution of Group B streptococcus infection is scanty. Recent studies have shown that GBS is a prevalent and crucial risk factor for both maternal and neonatal morbidity and mortality in Africa (Clouse, Shehabi, Suleimat, Faouri, Khuri-Bulos, Al Jammal et al., 2019; Mavenyengwa, Afset, Schei, Berg, Caspersen, Bergseng, et al., 2010; Said, Dangor, Mbelle, Sihlabela, Lekalakala, & Ismail, 2018). Research shows that GBS infection remains dangerous, with 5.6 million fatalities documented as a burden, mostly due to vertical transmission from infected women around delivery time. Urinary tract infections, chorioamnionitis, endometritis, puerperal sepsis, and other problems are only some of the ways in which infection may manifest itself throughout pregnancy and the postpartum period (Karnabi, 2017; Melin, 2011; Slotved,

Dayie, Banini, & Frimodt-Mller, 2017). Although GBS is a significant health issue in Africa, a paucity of data on its frequency and serotypes among pregnant women and neonates has made it difficult to recognise it as such in most countries.

In Ghana, data on the epidemiology of GBS is limited, making it hard to make informed decisions on maternal and neonatal complications resulting from GBS. About 32% of neonatal deaths have been associated with maternal vertical transmission. Surprisingly, knowledge of GBS among medical practitioners, such as diagnosis, laboratory detection, treatment, and prevention, is scanty (Enweronu-Laryea, Damale, & Newman, 2011). Additionally, no comprehensive national surveillance programs or designed national testing protocols are in place for detecting GBS.

Justification

The significance of a study like this cannot be underestimated as this study will provide information to add up to the existing data on the prevalence of GBS and contribute to the provision of information on the laboratory diagnostic protocol of GBS colonization among pregnant women and neonates. This will also contribute to the mapping out the antimicrobial sensitivity and resistivity of trends of GBS, the prevalence concerning geography, race, and economic and educational status of pregnant women and neonates in Ghana. In all, this study will contribute to providing the necessary information for comprehensive national surveillance programs and treatment protocols for GBS colonisation.

Aim

This study aimed to determine the prevalence, and risk factors associated with *Group B streptococcus* colonization and to compare different diagnostic approaches among pregnant women attending the Cape Coast Teaching Hospital.

Specific objectives

The study sought to;

I. determine the prevalence of *Group B Streptococcus* among pregnant women using PCR.
II. determine the distribution of GBS by clinical and sociodemographic characteristics.
III. compare diagnostic reliability of different assays in the diagnosis of GBS.
IV. To determine the prevalence of GBS at different trimesters using the various diagnostic techniques employed.
V. determine risk factors associated with GBS colonization among pregnant women.

Organization of study

This study is organized into five chapters. Chapter one introduces the background as well as provides the problem, justification and aim of the study. Chapter two reviews the literature on the microorganisms under study; its history, bacteriology, serotype distribution, pathogenesis, epidemiology, risk factors for colonisation of GBS in women and neonates, signs, treatment and outcomes, laboratory detection, antimicrobial susceptibility pattern of GBS and prevention. Chapter three describes the research methodology, including the

study design, study sites, sampling, biochemical tests for phenotypic and genotypic identification of GBS isolates, and the data analysis. Chapter four consists of the results from the study and a discussion of the key findings. Lastly, chapter five consists of the summary, conclusion and recommendations made from the study.

Chapter Summary

GBS, an asymptomatic bacterium colonizing healthy women's gastrointestinal and genitourinary tracts, is implicated in invasive neonatal infections. Maternal GBS colonisation is a prerequisite for neonatal early-onset disease and other complications. Yet, the lack of data and sensitive clinical diagnosis of GBS continues to negatively impact surveillance, treatment and prevention of the disease, especially in developing countries is troubling. Hence, this study looked at GBS infection among pregnant women, evaluating the prevalence and risk factors and comparing the diagnostic value of the culture method (gold standard) and gene-targeting PCR assays.

CHAPTER TWO

LITERATURE REVIEW

Introduction

This section reviewed the literature on microbiology, the study of disease transmission, serotype appropriation of Group B streptococcus, pathogenesis, risk factors for colonization of GBS in parturient women, signs, treatment and results, laboratory diagnosis and antimicrobial susceptibility pattern of GBS.

Brief History of GBS

GBS was initially isolated from bovine mastitis in 1887 (Brigtsen, 2018). It was discovered as a microorganism often occurring in mastitis, hence the term Streptococcus agalactiae, which means “no milk.” 1938 the initially recorded causes of human infection were published, describing 3 cases of fatal birth sepsis (Brigtsen, 2018). Sporadic cases were later recorded before a dramatic increase in the incidence of septicemia and meningitis in infants documented within the 70s (Baker, Barrett, Gordon, & Yow, 1973; Brigtsen, 2018; McCracken, 1973). Infections in pregnant women had a decent outcome with antimicrobial medication, however, the nonpregnant adults typically had underlying conditions, and the infection usually resulted in death Farley, Harvey, Stull, Smith, Schuchat, Wenger, et al., 1993).

In infants, case-fatality rates declined from as high as 50% in the 1970s to 4-11% in most Western countries in recent years Edmond, Kortsalioudaki, Scott, Schrag, Zaidi, Cousens, et al., 2012; Joubrel, Tazi, Six, Dmytruk, Touak, Bidet, et al., 2015). Statistics currently report the next case fatality rate in preterm newborns. Within the study by Phares et al., the case-mortality rate was

20%, with a risk of death nearly eight times that of term cases, whereas case mortality as high as 30% was reported in premature infants between twenty-five- and twenty-eight-weeks ago by Stoll et al. 2002, (Phares, Lynfield, Farley, Mohle-Boetani, Harrison, Petit, et al., 2008; Stoll, Hansen, Fanaroff, Wright, Carlo, Ehrenkranz, et al., 2002).

Mavenyengwa, Afset, Schei, Berg, Caspersen, Bergseng, et al. (2010) found a 19% prevalence of GBS colonisation over all of Sub-Saharan Africa. Twenty-three percent of pregnant mothers and 8.9 percent of neonates were verified to have GBS colonised (Joachim et al., 2009) in Tanzania. Both Zimbabwe (31.6%) and Trinidad (32.9%) have high prevalence rates (Joachim, Matee, Massawe, & Lyamuya, 2009). Nsagha, Bello, and Kandakai-Olukemi (2000) conducted a research at Jos University Teaching Hospital (JUTH) and Vom Christian Hospital and found that 7% of the Nigerian population is colonised with GBS. Overall, 60.3% of Zimbabweans have been colonised, with 38.0% of instances documented among urbanites and 62.0% among ruralites (Mavenyengwa, Afset, Schei, Berg, Caspersen, Bergseng, et al., 2010). While in Ghana, Enweronu-Laryea, Damale, and Newman (2011) found a prevalence rate of 23% in the year 2000. Prophylactic intrapartum antibiotics given to high-risk pregnant women have been linked to a decrease in both the incidence of the disease and the number of infant deaths (Schrag, Zywicki, Farley, Reingold, Harrison, Lefkowitz, et al., 2000).

Bacteriology of GBS

Group B *Streptococcus* (GBS) or *Streptococcus agalactiae* is a member of the genus Streptococcus of the family *Streptococcacea*. It is a Gram-positive diplococcus that divides in one plane and consequently occurs in chains or as pairs. Bacterial colonies are 1-3 mm in diameter and have a grey-white colour when they grow on sheep blood agar medium. The flat, mucoid colonies are surrounded by a narrow, clear zone. This is caused by the lysis of red blood cells in the agar medium, induced by bacterial hemolysins; this process is called β-hemolysis. Streptococci generating β hemolysis are called β- hemolytic streptococci. Approximately 99% of GBS strains are β-hemolytic (Brigtsen, 2018). Alpha-hemolytic strains of GBS have rarely been documented though 1-2% of GBS strains are non-haemolytic (Denny, 2000; Wessels, 1997). Rebecca Lancefield developed a classification system for β-hemolytic streptococci based on immunoprecipitation. *S. agalactiae* is the only species belonging to serogroup B in this classification, leading to the term group B *Streptococcus* for the species (Karnabi, 2017; Wessels, 1997).

GBS comprises capsular polysaccharides (CPS) and macromolecule antigens (C and R); (Davies, Miller, Faro, Gregson, Kehl, & Jordan, 2004; Karnabi, 2017; Wessels, 1997). CPS is made-up of 4-7 repeating units of monosaccharides: glucose, galactose, glucosamine and N-acetyl neuraminic acid or sialic acid; glycosidic linkage is exclusive for each serotype (Denny, 2000; Wessels, 1997). A common feature of CPS, further as a key feature of GBS pathogenesis, is that of the residue of sialic acid on the terminal side chain. This structural makeup is prime to the microorganism's virulence, allowing it

to escape host defence mechanisms (Denny, 2000; Rajagopal, 2009; Wessels, 1997).

GBS Serotypes

The Initial classification of GBS by Rebecca Lancefield was three distinct serotypes, types I, II and III. At present, ten distinct serotypes have been described: (Ia, Ib, II, III, IV, V, VI, VII, X, and IX) (Karnabi, 2017). This classification is based on their unique structural CPS makeup (Rajagopal, 2009). When a C protein is present on either an alpha or beta strain, it is indicated by "/C" (e.g., serotype Ia/C) (Wessels, 1997). The predominance of different serotypes varies with time and geographical location. A recent systematic review revealed that serotypes Ia, Ib, II, III and V comprise 85% of all serotypes among regions including Africa, the Americas, Europe, the Western Pacific and the Eastern Mediterranean. Specifically, serotype III is the most predominant (49%) in all regions. Among early-onset GBS cases, serotype I was the most common (40%), followed by serotype III (37%). Conversely, serotype III was primarily associated with late-onset GBS cases (53%), followed by serotype I (30%) (Edmond, Kortsalioudaki, Scott, Schrag, Zaidi, Cousens, et al., 2012). Among strains responsible for maternal colonization, the most common serotype distribution includes serotypes Ia (34%-41%), V (22.6%-25%) and III (19% to 24.5%) (Blumberg, Stephens, Modansky, Erwin, Elliot, Facklam, et al., 1996; Zaleznik, Rench, Hillier, Krohn, Platt, Lee, et al., 2000).

Pathogenesis of GBS

Understanding the pathogenesis of GBS is ne to develop substitute preventive approaches, including vaccines. Yet, the pathogenesis of GBS is a multifaceted and multifactorial process because both the host and the bacterial virulence contribute to the disease progression (Melin, 2011; Rajagopal, 2009). This opportunistic bacterium is found in the human gastrointestinal and genitourinary tract and can act as a pathogen or commensal organism (V. L. Chen, Avci, & Kasper, 2013; Paoletti & Kasper, 2003).

Bacteria adherence to the epithelial cells in the vagina and resistance to mucosal immune defences indicate GBS colonization in pregnant women (Karnabi, 2017; Nizet, Ferrieri, & Rubens, 2000). If the bacteria ascend into the amniotic cavity and colonizes the fetus' skin or mucous membranes, it can cause neonatal infection. GBS can also enter a fetus' lungs through the aspiration of the infected amniotic fluid. Spread may occur through the infant's passage through the birth canal. Neonatal infections are mainly determined by Maternal genital tract colonization at the time of delivery (Karnabi, 2017).

After neonatal colonization, replication in the alveoli, adhesion to the epithelium and evasion of host defence mechanism in the respiratory tract are essential for developing the disease. Bacteria, upon evading the epithelial and endothelial cells to enter the bloodstream, leads to septicaemia among the infected. Once disseminated in the bloodstream, this can lead to meningitis, osteomyelitis, sepsis, and death (Melin, 2011; Nizet, Ferrieri, & Rubens, 2000). Neonatal GBS pathogenesis is described in figure 2.1 below:

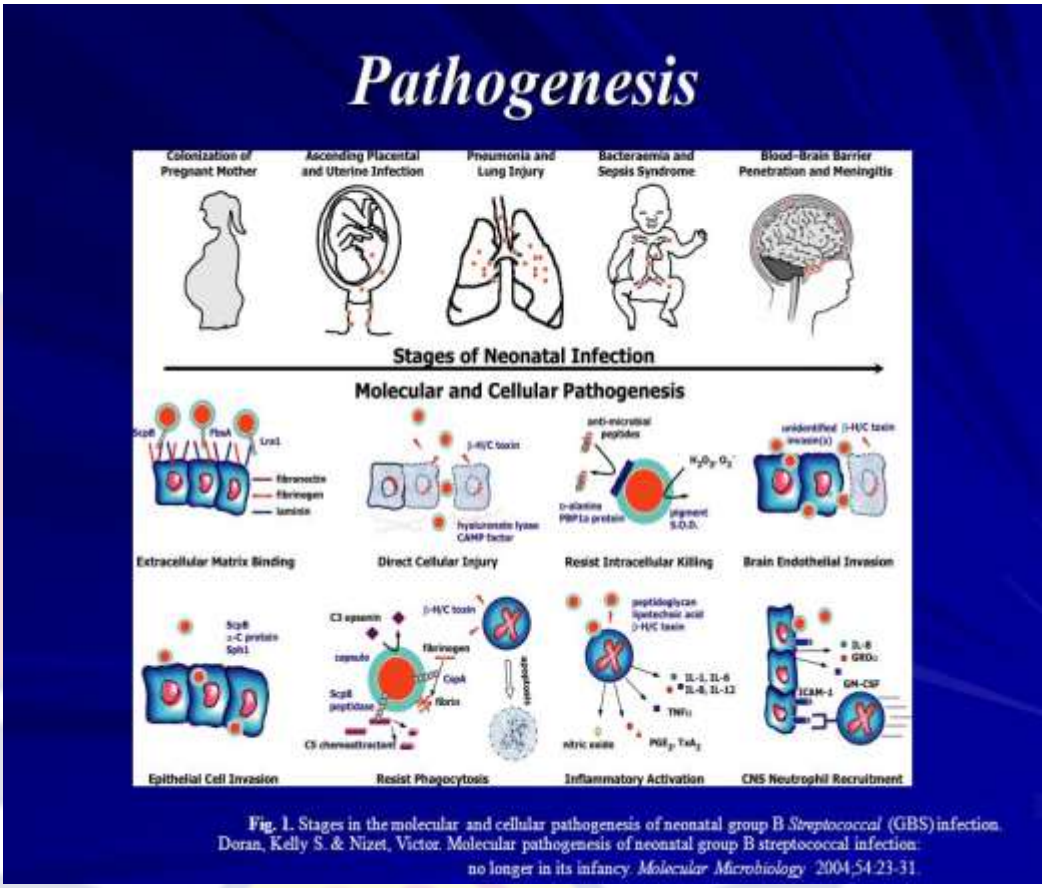

Figure 1: Neonatal GBS pathogenesis

Epidemiology of Group B Streptococci

Streptococcus agalactiae, or GBS, is cosmopolitan in nature and normal gastrointestinal tract flora. It colonises the vagina chronically or intermittently in a couple of third of the female population (Okon, Usman, Umar, & Balogun, 2013; Shabayek & Spellerberg, 2018). The reservoirs for GBS are the vaginal and the perianal regions/rectum. The colonization of those regions may serve as a risk factor subsequent to infection in the pregnant female and neonates (Martín, Cárdenas, Ocaña, Marín, Arroyo, Beltrán, et al., 2019). Worldwide, there is a common asymptomatic infection of GBS among pregnant women. Age, parity, socio-economic standing, presence of sexually transmitted diseases, sexual behaviour, team and region are the traits on which maternal

GBS infection population varies (Kim, Oh, Kim, Seo, Shin, Song, et al., 2011; Melo Costa, Silva, Silva, Tashima, Cardoso, et al., 2018; Sharmila, Joseph, Babu, Chaturvedula, & Sistla, 2011).

According to estimates from developing nations (Dagnew, Cunnington, Dube, Edwards, French, Heyderman, et al., 2012), the rate of neonatal GBS infection is 0-3/1000 live births, which is comparable to the 1-2/1000 live births reported in the United States before the intrapartum antibiotic bar (IAP) was put in place as a defence (Fultz-Butts, Gorwitz, Schuchat, & Schrag, 2002). Studies from Kenya, South Africa, and other nations indicate that GBS is increasingly contributing to sepsis in newborns in Africa (Medugu, Iregbu, Iroh Tam, & Obaro, 2018).

Between 4 and 30 percent of pregnant women are affected with GBS over the world (Mohammed, Asrat, Woldeamanuel, & Demissie, 2012; Simoes, Alves, Fracalanzza, Camargo, Mathias, Milanez, et al., 2007). Forty-seven percent to seventy-five percent of newborns born to GBS-positive women are colonised, and 1% to 2% of these instances progress to invasive illnesses (Chan, Lee, Baqui, Tan, & Black, 2015; Patras & Nizet, 2018). Time of pregnancy, racial background, and the number and variety of sites cultured all play significant roles in the prevalence of GBS infections in mothers (Mohammed, Asrat, Woldeamanuel, and Demissie, 2012; Quiroga, Pegels, Oviedo, Pereyra, & Vergara, 2008; Santhanam, Jose, Sahni, Thomas, & Beck, 2017).

The infection rate was 14.6% among 281 pregnant women surveyed in a randomly selected public antepartum care centre in New Flower, Ethiopia (Assefa, Desta, & Lema, 2018). In a cross-sectional investigation of 300 pregnant women in Ethiopia between 35 and 37 weeks of gestation in 2014, the

prevalence of GBS was found to be 7.2% (positive rectovaginal isolates were found in 22 of the 300 participants; Woldu, Teklehaimanot, Waji, & Gebremariam, 2014).

An observational cross-sectional study conducted amongst one hundred and fifty pregnant ladies at 35-40 weeks of gestation attending an antepartum hospital in Obafemi Awolowo University clinic in Nigeria quantified GBS carriage at 11.3% (Onipede, Adefusi, Adeyemi, Adejuyigbe, Oyelese, & Ogunniyi, 2012) whereas every other cross-sectional study about with 200 participants at 24-35 weeks gestation in Enugu state, Nigeria suggested GBS occurrence of 18% (Ezeonu & Agbo, 2014).

In Ghana, research performed within the capital of Ghana by Enweronu-Laryea et al. (2011) tertiary facility indicated a prevalence of 19%. Slotved et al. (2017), in two facilities, established a prevalence of 25.5% and 28.0%. Another study by Vinnemeier et al. (2015) in the Ashanti region detected a general prevalence of 19.1% (18.1% in rural Pramso and 23.1% in urban Kumasi, respectively). The prevalence of GBS in low- and middle-income countries is parallel to that of developed countries (Enweronu-Laryea, Damale, & Newman, 2011; Slotved, Dayie, Banini, & Frimodt-Møller, 2017; Vinnemeier, Brust, Owusu-Dabo, Sarpong, Sarfo, Bio, et al., 2015).

Group B Streptococcus Disease

Maternal colonization

According to the first global research of Group B Streptococcus, estimated 21.7 million pregnant women are now undiagnosed and untreated carriers. It is estimated that one in five pregnant women worldwide gets Group B Streptococcus (GBS) each year (Lawn, Bianchi-Jassir, Russell, Kohli-Lynch, Tann, Hall, et al., 2017). Differences in maternal colonisation may contribute to demographic variations. Different factors contribute to this, such as age, race/ethnicity, socioeconomic standing (SES), and geographical location (Karnabi, 2017).

The colonisation of the genitourinary system by GBS is most likely to have originated there. Colonisation rates in Western nations range from roughly 10-30% among pregnant women (Kwatra, Cunnington, Merrall, Adrian, Ip, Klugman, et al., 2016); however, these numbers vary widely depending on the country. Colonisation might be temporary, ongoing, or spotty (Hansen et al., 2004). Women who have been colonised during pregnancy are more likely to be colonised again during future pregnancies than women who have not been colonised during pregnancy. There is an increased chance of having a child with early-onset disease (EOD) if the mother has GBS bacteriuria during pregnancy, a proxy for high colonisation (Heath, 2011). Black people, Hispanics, elderly women, and the illiterate are disproportionately affected by this (Turrentine & Ramirez, 2008).

Most women colonized with GBS are asymptomatic. The disease is seldomly fatal in pregnant women; however, it can lead to sepsis, pneumonia, meningitis, chorioamnionitis, endometritis, mastitis, urinary tract infections and

postpartum wound infection (Acosta, Kurinczuk, Lucas, Tuffnell, Sellers, & Knight, 2014; Kalin, Acosta, Kurinczuk, Brocklehurst, & Knight, 2015; Phares, Lynfield, Farley, Mohle-Boetani, Harrison, Petit, et al., 2008). It has been suggested that infection of the amniotic fluid with GBS might lead to a miscarriage or stillbirth (Nan, Dangor, Cutland, Edwards, Madhi, & Cunnington, 2015; Seale, Koech, Sheppard, Barsosio, Langat, Anyango, et al., 2016). Another risk of GBS is preterm delivery due to premature membrane rupture (PROM) (Brigtsen, 2018).

Neonatal colonization

Infants aged 0-89 days have the highest incidence and severity rate (Le Doare & Heath, 2013). Neonatal GBS disease is grouped into two categories depending on the infant's age when the disease manifested (Karnabi, 2017). Diseases with an early onset manifest during the first six days of life, whereas those with a late onset manifest between the seventh and eighty-ninth days. Here is a comprehensive rundown of the early and late-stage disease's clinical manifestations:

Early-onset GBS disease

Early-onset (EO) GBS illness is characterised by the presence of neonatal infection during the first week of life (7 days) (Karnabi, 2017). Sixty percent to seventy percent of all cases of newborn GBS (Le Doare & Heath, 2013) are due to this strain. Transmission from a mother to her child happens vertically after membrane rip during delivery (Karnabi, 2017; Le Doare & Heath, 2013; Melin, 2011) in mothers who have been colonised. Le Doare and Heath (2013) found that without maternal prophylaxis, between 1% and 2% of newborns get infected with an invasive illness.

Ninety percent of babies born with EO GBS disease show symptoms during the first 24 hours of life (Melin, 2011). Neonatal group B streptococcus (GBS) has been linked to complications such as septic arthritis, septic meningitis, septic cellulitis, and septic osteomyelitis (Karnabi, 2017). Respiratory distress, grunting, retractions, hypoxemia, and tachypnea are all clinical hallmarks of pneumonia. Infants with newborn sepsis or meningitis may exhibit apnea, poor feeding, and irritability (Ahmadzia & Heine, 2014; Porta & Rizzolo, 2015). In up to 25% of instances, severe newborn infections might cause hypotension. Foetal hypoxia has been linked to serious intrauterine infection (infected pregnancies) (Karnabi, 2017).

Without treatment, infants with EO GBS disease will have a speedy decline in health. Administering penicillin G, cefazolin, clindamycin or vancomycin as intrapartum antibiotics during labour to the mothers as prophylaxis can prevent the disease (Karnabi, 2017; Money, Allen, Yudin, Bouchard, Boucher, Caddy, et al., 2013). Treatment is provided to infants who show signs of infection or are considered “at-risk.” Asymptomatic infants are typically not treated if their mothers receive adequate prophylaxis (Jefferies, 2017).

Late-onset GBS disease

Late-onset (LO) GBS disease is characterized by the beginning of an indication of neonatal disease at 7-89 days of life (Karnabi, 2017). Serotype III is the most frequent strain linked to late-onset disease (Edmond, Kortsalioudaki, Scott, Schrag, Zaidi, Cousens, et al., 2012; Weisner, Johnson, Lamagni, Arnold, Warner, Heath, et al., 2004). LO GBS aetiology is not comprehended but has been assumed to be multifactorial. Infections can be attained through vertical

transmission (Karnabi, 2017). Horizontal transmission has also been a concern in late-onset disease. Such transmission can involve the faecal-oral route or direct person-to-person contact. Horizontal transmission has been detected in hospitals and communities (Ahmadzia & Heine, 2014; Pintye, Saltzman, Wolf, & Crowell, 2016).

Previously, the hands of health care workers were a frequent source of infection (Berardi, Rossi, Lugli, Creti, Reggiani, Lanari, et al., 2013). However, with advancements in infection control in neonatal care, this source is now considered infrequent. Case reports have also suggested GBS in breast milk as a possible transmission pathway (Karnabi, 2017). Finally, a recent case report has revealed maternal ingestion of infected placenta as a possible transmission pathway in late-onset manifestation (Buser, Mató, Zhang, Metcalf, Beall, & Thomas, 2017). The reoccurrence of GBS disease in the same infant has been documented in 1% of cases, despite appropriate neonatal treatment (Karnabi, 2017).

Many newborns initially seem unwell, with symptoms including fever, poor feeding, and irritability. A positive blood culture that lacks any significant clinical significance. Meningitis, pneumonia, cellulitis, and osteoarthritis are the next most prevalent diagnoses for people who get them (Phares, Lynfield, Farley, Mohle-Boetani, Harrison, Petit, et al., 2008). Meningitis symptoms include agitation, convulsions, and fatigue (Porta & Rizzolo, 2015). Compared to early-onset GBS illness, IAP usage during childbirth did not affect late-onset symptoms (Berardi, Rossi, Lugli, Creti, Reggiani, Lanari, et al., 2013).

Failure to initiate antimicrobial treatment for bacteremia promptly may result in progression to septic shock, particularly in premature infants, and extension of infection to distant sites such as the central nervous system.

Treatment of GBS Infection

Penicillin Ampicillin, vancomycin, clindamycin, erythromycin, cotrimoxazole, and ceftriaxone are antimicrobial agents used to treat invasive GBS infections. According to research conducted by Joachim et al., all GBS isolates were completely susceptible to ampicillin, 90% to 100% susceptible to penicillin G and ciprofloxacin, and 80% to 90% susceptible to clindamycin, erythromycin, cotrimoxazole, and ceftriaxone (Joachim, Matee, Massawe, & Lyamuya, 2009). Penicillin is the preferred medication for intrapartum antimicrobial prophylaxis (API) and treatment; however, women who are allergic to penicillin or who are at high risk for anaphylaxis may be treated with erythromycin or clindamycin (Florindo, Viegas, Paulino, Rodrigues, Gomes, & Borrego, 2010; McGee, Schrag, & Verani, 2010).

Moreover, performing antimicrobial susceptibility testing on GBS isolates is important to select appropriate antibiotic prophylaxis. Since GBS is now developing resistance to clindamycin, D-zone testing using the double-disk diffusion method has been used to identify isolates that are erythromycin-resistant and clindamycin-susceptible yet have inducible resistance to clindamycin (McGee, Schrag, & Verani, 2010). Isolates that are D-zone positive are considered to have inducible clindamycin resistance. Newborns with early-onset are treated the same as the mothers through intravenous antibiotics.

Prevention of GBS Infection: Vaccination and Prophylaxis.

The use of intrapartum antimicrobial prophylaxis during labour has provided substantial prevention of EO GBS infection. Yet, the consistent usage of antimicrobial prophylaxis strategies may lead to the emergence of resistant strains of GBS or other organisms during pregnancy (Banini, 2015). Also, antimicrobial prophylaxis does not prevent most late-onset infections, GBS-related stillbirths, or prematurity. It also does not address GBS disease in non-pregnant adults (Banini, 2015).

Creating a vaccine is a better option for combating all types of GBS illnesses. The success of maternal immunisation in preventing young infant morbidity and mortality is best characterised by the accomplishment of the tetanus vaccination program and inactivated influenza vaccine studies during pregnancy (Madzivhandila, Adrian, Cutland, Kuwanda, Schrag, & Madhi, 2011). Capsular polysaccharide antigens define the serotypes of GBS (Banini, 2015), which majorly present as the antigenic and virulence factor and the main target for antibody-mediated killing (Martins, Melo-Cristino, & Ramirez, 2010).

Maternal vaccinations with capsular polysaccharide type-specific serum IgG will possibly diminish maternal colonisation and vertical transmission and improve the transplacental transfer of anti-GBS antibodies to the fetus hence protect against invasive disease in their newborns (Madzivhandila, Adrian, Cutland, Kuwanda, Schrag, & Madhi, 2011; McGee, Schrag, & Verani, 2010).

A recent, double-blind, randomized trial of a conjugate vaccine against GBS serotype III among non-pregnant women of reproductive age found a significant delay in the acquisition of colonization with the vaccine-serotype

among vaccine recipients. Although an effective GBS vaccine would be a powerful tool against GBS disease, no licensed vaccine has yet been available (McGee, Schrag, & Verani, 2010).

Laboratory Detection, Typing and Antimicrobial Susceptibility Testing of GBS Sampling, Culture and Identification.

Sampling

When detection of GBS in colonized women is done by swabbing the lower vagina and rectum, it increases the culture yield substantially compared with sampling the vagina or the cervix without swabbing the rectum (Verani, McGee, & Schrag, 2010). The use of proper transport media is essential, and the sensitivity of culture is greatest when the specimen is stored at 4°C before culture and processed within 24 hours of collection (Brigtsen, 2018; Håkansson, Axemo, Bremme, Bryngelsson, Carlsson Wallin, Ekström, et al., 2008).

Culture

The use of a selective broth medium is significant for increasing GBS isolation. Via a selective broth, bacteria growth can increase by as much as 50% (Baker et al., 1973b). Selective enrichment broths include Todd-Hewitt broth supplemented either with gentamicin and nalidixin acid (TransVag broth) or with colistin and nalidixin acid (LIM broth) (Brigtsen, 2018; Fenton & Harper, 1979). GBS grows well on blood agar and chocolate agar plates. As GBS generally shows ß-hemolysis, chromogenic agars detecting ß-hemolysis are available (Tazi, Réglier-Poupet, Dautezac, Raymond, & Poyart, 2008). Since 1-4% of the strains are nonhemolytic, these agars are not optimal for detecting GBS and are, therefore, a supplement to other tests.

Identification

Regular laboratory microbiological testing may be used to diagnose GBS. Colony morphology, beta-hemolysis, and the catalase test may be used for identification. A drop of 3 percent hydrogen peroxide is placed on a microscope slide and examined for signs of catalase activity. If the enzyme catalase is present in the bacterial colony, the presence of oxygen bubbles may be determined. As with other streptococci, GBS is "catalase-negative" because it lacks this enzyme. GBS may also be identified by the use of a CAMP test (Brigtsen, 2018). Christie, Atkinson, and Munch Peterson (CAMP) are the scientists who described the test. Unlike most other hemolytic streptococci, GBS may create the CAMP factor, which can be used in the CAMP test. Hemolytic and non-hemolytic GBS both get good results on the CAMP test.

Serotyping

Different forms of capsular antigens are used to categorise streptococci into different groups. Banini (2015), Brigtsen (2018), and Lancefield (1933) classify these antigens by group, naming them A, B, C, etc. The best technique to identify GBS is the standard method, which classifies it as Lancefield Group B based on its surface antigen. Group *B*-specific antibodies will cause a tiny bacterial culture to agglutinate or clump together, suggesting a positive response and the detection of GBS.

The CPS also allows for the differentiation of GBS strains. Antibodies developed in rabbits or mice may recognise the CPS antigens. The first immunoprecipitation technique was reported by Lancefield in the 1930s (Brigtsen, 2018). Polyclonal antibodies are specific for the capsular polysaccharides and are employed in the most common method for

phenotypically serotyping GBS, which is a latex agglutination test (Afshar, Broughton, Creti, Decheva, Hufnagel, Kriz, et al., 2011). These tests are convenient, trustworthy, and widely accessible in the marketplace.

Serotyping has its limitations. Due to a lack of or low expression of the CPS under experimental conditions, an isolate may not be typeable. Also, serotyping is highly dependent on the quality of the diagnostic kit used and on the experience of the laboratory personnel (Afshar, Broughton, Creti, Decheva, Hufnagel, Kriz, et al., 2011).

Polymerase Chain Reaction (PCR)

Since GBS colonisation occurs transiently, chronically, or intermittently in a sizable percentage of pregnant women, a faster, more sensitive, and more specific test is required to detect GBS-related infections in mothers prior to or during childbirth (Mousavi, Hosseini, Mashouf, & Arabestani, 2016; Rosa-Fraile & Spellerberg, 2017).

The detection of a segment of DNA specific to GBS allows for its identification. Genotyping, using polymerase chain reaction, is how we get at this. In the first phase of PCR, called denaturation, the temperature is raised to about 95 degrees Celsius; at this point, double-stranded DNA splits (denatures) into single-stranded DNA. The appropriate primers are subsequently annealed to the ssDNA when the temperature is at 55-58 degrees Celsius. Ultimately, the temperature is optimised for the DNA polymerase activity known as an extension to facilitate the most effective synthesis of DNA copies. Up to 40 cycles are performed to sufficiently amplify the target DNA. The amplicon, or target DNA, is often replicated in a good PCR reaction, yielding millions of copies. In traditional PCR, the accumulated amplicon is identified by gel

electrophoresis, but in real-time PCR, the amplicon is quantified at each PCR cycle using a fluorescent reporter.

The GBS CPS and its genotype may be determined using two distinct PCR techniques. Multiple polymerase chain reaction (PCR)-based diagnostics for identifying GBS are now on the market (Verani, McGee, & Schrag, 2010). When comparing tests with and without an enrichment stage, it is clear that the ones based on enrichment followed by subculture have greater sensitivity and specificity. These tests are less suitable for intrapartum screening since the enrichment phase increases the time required to achieve a final result. Studies have been conducted to counter this, and intrapartum PCR assays have been made accessible for GBS screening (El Helali et al., 2012; El Helali, Nguyen, Ly, Giovangrandi, & Trinquart, 2009). The expensive cost of these tests has prevented their widespread implementation into clinical practice, although their popularity is growing.

There are several PCR tests available for determining the GBS CPS serotypes. Using single PCRs for the various CPS types, Kong, Gowan, Martin, James, and Gilbert reported their first genotyping tests in 2002, and Borchardt published his in 2004 (Borchardt, Foxman, Chaffin, Rubens, Tallman, Manning, et al., 2004; Kong, Gowan, Martin, James, & Gilbert, 2002). Later, Poyart and Imperi created multiplex PCRs, which simultaneously screen for many serotypes in the isolate (Imperi, Pataracchia, Alfarone, Baldassarri, Orefici, & Creti, 2010; Poyart, Tazi, Réglier-Poupet, Billot, Tavares, Raymond, et al., 2007). Before identifying serotype IX, Poyart et al. established the two-set multiplex PCR test. Imperi's technique, a one-set multiplex assay, includes

serotype IX and allows for the differentiation of all 10 known serotypes in a single PCR reaction.

PCR assays are commonly considered modern, sophisticated techniques. However, molecular methods also have limitations (Yao, Poulsen, Maione, Rinaudo, Baldassarri, Telford et al., 2013). For instance, they do not reveal if the CPS gene locus detected is expressed as a CPS.

Antimicrobial susceptibility testing

Disc diffusion tests are often used to determine the antimicrobial susceptibility of GBS; however, E-tests or agar/broth dilution procedures may provide a more thorough examination (Brigtsen, 2018; Matuschek, Brown, & Kahlmeter, 2014).

In general, GBS is a penicillin-resistant Gram-positive bacteria (Brigtsen, 2018; Longtin, Vermeiren, Shahinas, Tamber, McGeer, Low, et al., 2011; Seki, Kimura, Reid, Miyazaki, Banno, Jin, et al., 2015); however, decreasing sensitivity has been documented from Japan, the United States, and Canada. A history of penicillin allergy in the United States affects around 8% of the population (Brigtsen, 2018). Erythromycin and clindamycin are the antibiotics for IAP in women allergic to penicillin. However, other nations have reported less erythromycin and clindamycin susceptibility. Recent research out of China found that over three-quarters (74%) of invasive and non-invasive isolates were resistant to erythromycin, and nearly two-thirds (64%) were resistant to clindamycin.

Decreased susceptibility to erythromycin and clindamycin has also been reported from other countries, underscoring how crucial it is to know the

susceptibility of the pathogen to give appropriate antibiotic prophylaxis in penicillin-allergic women (Brigtsen, 2018).

Chapter Summary

This chapter reviewed the history, bacteriology, serotype distribution of Group B streptococcus, pathogenesis, epidemiology, risk factors for colonization of GBS in women and their neonates, signs, treatment and outcomes, laboratory detection, antimicrobial susceptibility pattern of GBS and prevention.

GBS was initially isolated from bovine mastitis 1887 (Brigtsen, 2018). Streptococcus agalactiae means “no milk was given to it because it was mostly found in mastitis. It is a Gram-positive diplococcus that divides in one plane and consequently occurs in chains or as pairs. Bacterial colonies are 1-3 mm in diameter and have a grey-white colour when they grow on sheep blood agar medium. GBS is widely distributed in nature, and a normal gastrointestinal tract flora may colonize the genitourinary tract chronically or intermittently in about a third of women. Streptococcus agalactiae can be subdivided into 10 serotypes based on the type-specific capsular polysaccharide (1a, 1b, II, III, IV, V, VI, VII, VIII, and IX). Among these, the most common serotypes that colonize pregnant women and result in neonatal infections include Ia, Ib, III, VI, and V. GBS is not limited to pregnant women. This pathogen can cause severe invasive disease in neonates. Disease occurring in the first 6 days of life is known as early-onset. The late-onset disease first manifests between 7-89 days of life. Antimicrobial therapy for invasive GBS infection involves penicillin Ampicillin, vancomycin, clindamycin, erythromycin, cotrimoxazole, and ceftriaxone. Prevention of maternal transfer of the disease to neonates can be

achieved through intrapartum prophylaxis usage. Laboratory diagnosis includes phenotypic, biochemical and molecular methods for identifying GBS.

CHAPTER THREE

MATERIALS AND METHODS

Introduction

This chapter is composed of the study design, study site and sample size, which considers how the study was designed, the location of the research and the number of participants involved in the research, respectively. The chapter further provides information on the inclusion and exclusion criteria, ethical clearance, sample collection, laboratory work and statistical analysis used in the study.

Study Design

The research employed a cross-sectional comparative study of 150 pregnant women aged 15 to 45 years with a mean of 25.1 (±4.7) each for every trimester and those who came into labour at term from December 2018 to February 2019. One hundred and fifty vaginal swab samples were collected from each category and sent for microscopy, biochemical testing, culture, and polymerase chain reaction (PCR) at the University of Cape Coast School of Medical Science Microbiology Laboratory. All pregnant women were provided with informed consent after a thorough explanation of the aim and objectives of the research. A face-to-face interview was conducted using an organised questionnaire. These included information on their demographic characteristics such as age, sex, ethnicity, religion, educational status, marital status and health insurance, medical history, parity and miscarriages, and other questions concerning the likely risk of GBS colonisation.

Study Site

The research was conducted at the Cape Coast Teaching Hospital, formerly the Cape Coast Regional Hospital. The Hospital was transformed into Cape Coast Teaching Hospital with the inception of the School of Medical Sciences at the University of Cape Coast. It is a 400-bed capacity referral Hospital on the Northern Cape Coast. It is bounded on the north by Abura Township, on the south by Peru Estate/4th Ridge, Nkanfua on the East and Abura/ Pedu Estate on the West. A facility that attends to mostly the inhabitants of Cape Coast Municipality and the Central Region.

Sample Size

A total of 150 participants were recruited out of the approximated 200 participants calculated using the sample size calculation formula below:

$$N = \frac{Z^2 (P) (1-P)}{(ERROR)^2}$$

Where Z, 1.96 is the standard normal variate (at 5% type error ($P<0.05$), it is for the confidence interval of 95%).

A 7% allowable ERROR was used.

$$\text{Minimum sample size, } N = \frac{1.96^2(0.5)(1-0.5)}{(7/100)^2}$$

$$= 196 \approx 200$$

Inclusion and Exclusion Criteria

All pregnant women who consented from the first to last trimester of pregnancy were included in the study. And women who were not pregnant were excluded from the study.

Ethical Clearance

Ethical clearance and approval were obtained from the University of Cape Coast Institutional Review Board (UCCIRB) and the Cape Coast Teaching Hospital (CCTH) Ethical Review Board. Informed consent were obtained from individuals who participated in the study willingly without coercion.

Laboratory Procedure

Specimen Collection and Processing

Vaginal swabs were collected from every participating pregnant woman. The swabs were placed into a broth containing skim milk, tryptone, glucose and glycerol (STGG), well-labelled and transported to the CCTH laboratory within an hour. Samples were vortexed to loosen bacteria from the swab into STGG solution to enable their growth (da Gloria Carvalho et al., 2010). Vaginal swabs were collected by rotating the swab against the upper 1/3rd of the vaginal wall.

Gram staining technique

Gram staining was performed by making a smear of the specimen to be stained on a slide. The slide was heat fixed for a few seconds until the bacteria were firmly mounted on the slide. The fixed smear was covered with a crystal violet stain for 30-60 seconds and rapidly washed off the stain with clean water. Gram's iodine was added for 30 seconds. The slide was decolourized rapidly with acetone-alcohol. Gram-positive bacteria retained the primary stain, while Gram-negative bacteria lost the primary stain and appeared colourless. A secondary stain, neutral red, was added for 15 seconds and washed off with water. The smear was examined microscopically, first with the X40 objective to check the staining and distribution of material and then with the oil immersion

objective. Gram-positive bacteria appeared black-violet whilst Gram-negative bacteria appeared red-pink.

Detection by culture technique

The swabs inoculated into STGG broth were directly seeded onto 10 % sheep blood agar (SBA) plates prepared under aseptic conditions and tested for sterility. SBA was supplemented with gentamicin (8 μg/ml) and nalidixic acid (Sigma Aldrich). It was incubated for 18-24 hours at 37ºC in 5% CO2. The plates that had no growth were re-incubated for another overnight. Where there were growths, the colonies were examined for their characteristic colonial morphology and beta- haemolysis. The suspected colonies were Gram-stained (Gram-positive) and tested for *catalase* production (catalase-negative).

Catalase test

The catalase test was necessary to differentiate between *Streptococcus spp*. and *Staphylococcus spp*., both of which are Gram-positive cocci that stain blue to purple. The catalase enzyme plays a key role in this assay by mediating the transformation of hydrogen peroxide into oxygen and water, and its detection is the primary objective. The Gram-positive bacterial colonies were picked out using the inoculating loop and then cultured on a glass slide with a drop of 3% hydrogen peroxide. Indicators of the catalase enzyme's existence included the development of bubbles (oxygen gas), whereas the lack of bubbles indicated catalase-negative conditions. Staphylococcus aureus was used as the positive control in the test, while Streptococcus agalactiae strain (ATCC 12386) served as the negative control.

CAMP test

The CAMP test is presumptively done to identify group B streptococcus (GBS). This test was first described in 1944 by Christie, Atkins and Munch-Petersen hence the CAMP test is an acronym of their names. The principle is based on an extracellular protein produced by GBS called the CAMP factor that acts synergistically with *Staphylococcus aureus* β-toxin to cause enhanced lysis of red cells. The suspected GBS was streaked perpendicular to a known *Staphylococcus aureus* on sheep blood agar without both streaks directly touching each other. Plates were incubated at 37°C for 24 hours. A positive test for the 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.

DNA extraction for Group B *Streptococcus*

The vaginal swabs were intubated in Todd Hewitt selective medium at 37℃ for 15-18 hours. After the intubation, 1ml of a sample was vortexed, pipetted into 2ml Eppendorf tube, and then centrifuged at 9000rpm for 3 minutes. 700µl of the supernatant was pipetted and discarded. 500µl of the PBS was vortexed and centrifuged at 9000rpm for 3 minutes, and all the supernatant was discarded.

Exactly 200µl of 1xTE buffer was added and vortexed till pellets dissolved in the buffer. The solution was then boiled for 10 minutes at 100℃ using a heat block. After, it was then frozen at -20℃ for 10 minutes and then centrifuged at 14000rpm for 10 minutes. 100µl of the supernatant, which served as the DNA of GBS, was then pipetted

Detection of GBS by PCR technique

A primer mix was prepared to consist of the 16srRNA, cfb, atr and ScpB primers. The Multiplex PCR mix was 25µL which was prepared as follows: 12.5µL of 2x Gotaq master mix (Promega Corporation, USA), 6.3µL Nuclease free water,0.6µL of the forward primer mix, 0.6µL of the reverse primer mix, and 5µL of the DNA sample. The DNA samples, as well as a positive control (a sample including DNA from *Streptococcus agalactiae*) and negative control (a sample without template), were amplified by an initial denaturation step for five minutes at 94ºC, followed by 35 cycles of 94ºC for 45 seconds, 60ºC for 60 seconds, and 72ºC for one minute, and a final cycle of 72ºC for seven minutes in a thermal cycler. After amplification, 5µL of each amplification product was analyzed by electrophoresis on a 2% (w/v) agarose gel, stained with Gel red.

Table 1: Primer sets synthesized for *Streptococcus agalactiae* identification in the PCR.

Name	Sequence (5`to 3`)	Length
atr-F	CgATTCTCTCAgCTTTgTTA	20
atr-R	AAgAAATCTCTTgTgCggAT	20
16S-F	CgCTgAggTTTggTgTTTACA	21
16S-R	CACTCCTACCAACgTTCTTC	20
cfb-F	TTTCACCAgCTgTATTAgAAgTA	23
cfb-R	gTTCCCTgAACATTATCTTTgAT	23
scpB-F	ACAATggAAggCTCTACTgTTC	22
scpB-R	ACCTggTgTTTgACCTgAACTA	22”

Storage

The isolates were stored at -80 ºC in a broth containing skim milk, tryptone, glucose and glycerol (STGG) at the Biomedical Science Department, University of Cape Coast.

Quality control

Quality-assured materials and protocols were used and followed strictly. Stored isolates were always sub-cultured before use. ATCC 25923 isolate was used to verify the potency of the bacterial isolation, media, materials and reagents used during collecting samples, bacteriological analysis and storage.

Data Analysis

Descriptive statistics of the participants were characterized using IBM SPSS version 25. A sensitivity and specificity analysis comparing GBS infection between culture and PCR was done using GraphPad Prism version 8. Microsoft Excel was used to develop the various graphs for the study.

Chapter summary

A facility-based cross-sectional study was conducted in Cape Coast Teaching hospital between June and August, 2019. 150 Vaginal swabs were collected from pregnant women. Swabs were inoculated into STGG and seeded directly onto sheep-blood agar made selective with antibiotics. Biochemical tests were conducted to identify suspected GBS colonies. Molecular testing for the presence of GBS was conducted on all 301 samples with *atr, scpB, cfb* and 16SrRNA gene assays at the Bacteriology laboratory of Noguchi Memorial Institute for Medical Research, University of Ghana. Inferences were then made to assess the risk and prevalence of the infection and the sensitivity of the culture technique to the PCR gene assays.

CHAPTER FOUR

RESULTS AND DISCUSSION

Introduction

The main purpose of this study is to determine the prevalence and risk factors associated with group B Streptococcus colonization among pregnant women and compare the diagnostic value of culture to the PCR assays. The result section contains details of analyzed collected data in tables and figures from the demographic and clinical information on participants. In the discussion, findings from this study were compared to other previous studies and possible explanations were given on why there were some disparities or agreements found in this study to the previous studies.

Results

Table 2: Characterization of the Demographic Information of the Participants Across Their Age Groups

Variable	Age in Years			Total
	≤24yrs	25-32yrs	≥33yrs	
Ethnicity				
Akan	26(76.5)	46(64.8)	31(68.9)	103(68.7)
Ga	2(5.9)	5(7.0)	9(20.0)	16(10.7)
Ewe	2(5.9)	10(14.1)	2(4.4)	14(9.3)
Nzema	1(2.9)	4(5.6)	1(2.2)	6(4.0)
Others	3(8.8)	6(8.5)	2(4.4)	11(7.3)
Education Level				
Non-Formal	0(0.0)	5(7.0)	7(15.6)	12(8.0)
Primary	5(14.7)	3(4.2)	3(6.7)	11(7.3)
JHS	18(52.9)	14(19.7)	5(11.1)	37(24.7)
SHS	10(29.4)	19(26.8)	13(28.9)	42(28.0)
Tertiary	1(2.9)	30(42.3)	17(37.8)	48(32.0)
Occupation				
Employed	17(50.0)	65(91.5)	44(97.8)	126(84.0)
Unemployed	17)50.0)	6(8.5)	1(2.2)	24(16.0)
Trimester				
1st	9(26.5)	29(40.8)	11(24.4)	49(32.7)
2nd	8(23.5)	19(26.8)	19(42.2)	46(30.7)
3rd	17(50.0)	23(32.4)	15(33.3)	55(36.7)

The majority of the participants were Akans 103(68.7), followed by Ga 16(10.7), and the least ethnic group was the Nzema 6(4.0). Also, irrespective of the age group, the Akans predominated. The Ewe's majority of the participants were between 25-32yrs and 10/14 of the participants. Most participants had 48(32.0) tertiary education, and senior high school education, 42(28.0). Very few participants had primary and no formal education, 11(7.3) and 12(8.0), respectively. Participants within aged 25-32yrs those aged ≥33yrs were mostly educated up to the tertiary level, 30(42.3) and 17(37.8), respectively. While those aged ≤25yrs were mostly Junior High School level graduates, 18(52.9). Irrespective of the age group, participants were mostly employed. Participants were also mainly in their 3rd trimester. (Table 2)

Table 3: Characterization of the Clinical Information of the Participants Across Age

Variable	Age in Years			Total
	≤24yrs	25-32yrs	≥33yrs	
Recent Antibiotic Usage				
≤2 Weeks	0(0.0)	2(2.8)	1(2.2)	3(2.0)
>2 Weeks	3(8.8)	7(9.9)	7(15.6)	17(11.3)
None of the Above	31(91.2)	62(87.3)	37(82.2)	130(86.7)
Gravidity				
≤3	31(91.2)	51(71.8)	8(17.8)	90(60.0)
4-6	3(8.8)	20(28.2)	27(60.0)	50(33.3)
≥7	0(0.0)	0(0.0)	10(22.2)	10(6.7)
Parity				
≤2	34(100.0)	63(88.7)	24(53.3)	121(80.7)
3-4	0(0.0)	8(11.3)	12(26.7)	20(13.3)
≥5	0(0.0)	0(0.0)	9(20.0)	9(6.0)
Gestational Age				
≤37 weeks	26(76.5)	64(90.1)	38(84.4)	128(85.3)
38-42 weeks	8(23.5)	7(9.9)	7(15.6)	22(14.7)
>42 weeks	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Past Obstetrics History				
PROM	1(2.9)	5(7.0)	14(31.1)	20(13.3)
Preterm Birth	0(0.0)	4(5.6)	5(11.1)	9(6.0)
Puerperal Sepsis	1(2.9)	0(0.0)	2(4.4)	3(2.0)
IUFD	0(0.0)	3(4.2)	1(2.2)	4(2.7)
Chorioamnionitis	0(0.0)	0(0.0)	0(0.0)	0(0.0)
IUGR	1(2.9)	1(1.4)	0(0.0)	2(1.3)
None of the Above	32(94.1)	60(84.5)	26(57.8)	118(78.7)
Past Gynecological History				
Spontaneous Miscarriage	2(5.9)	17(23.9)	14(31.3)	33(22.0)
Induced Abortion	10(29.4)	10(14.1)	8(17.8)	28(18.7)
UTI	8(23.5)	26(36.6)	20(44.4)	54(36.0)
PID	1(2.9)	9(12.7)	5(11.1)	15(10.0)
Candidiasis	31(91.2)	50(70.4)	28(62.2)	109(72.7)
HIV	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Hepatitis B	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Syphilis	0(0.0)	0(0.0)	0(0.0)	0(0.0)
None of the Above	0(0.0)	9(12.7)	4(8.9)	13(8.7)

Total= total number of participants

Table 4: Characterization of the Clinical Information of the Participants Across Age

Variable	Age in Years			Total
	≤24yrs	25-32yrs	≥33yrs	
Past Neonatal History				
Neonatal Sepsis	1(2.9)	7(9.9)	5(11.1)	13(8.7)
Early Neonatal Death	0(0.0)	1(1.4)	2(4.4)	3(2.0)
Low Birth Weight	1(2.9)	4(5.6)	2(4.4)	7(4.7)
NICU Admission	2(5.9)	7(9.9)	10(22.2)	19(12.7)
None of the Above	32(94.1)	63(88.7)	34(75.6)	129(86.0)
Comorbidities				
Pregnancy with Chronic Hypertension	0(0.0)	1(1.4)	1(2.2)	2(1.3)
Gestational Hypertension	0(0.0)	1(1.4)	4(8.9)	5(3.3)
Diabetes in Pregnancy	0(0.0)	0(0.0)	2(4.4)	2(1.3)
Gestational Diabetes	0(0.0)	0(0.0)	1(2.2)	1(0.7)
Sickle Cell Anemia	1(2.9)	1(1.4)	0(0.0)	2(1.3)
Asthma	0(0.0)	3(4.2)	1(2.2)	4(2.7)
None of the Above	33(97.1)	64(90.1)	37(82.2)	134(89.3)

Total= total number of participants

Table 3 and 4 Characterization of the Clinical Information of the Participants According to Age. Participants, regardless of their age, had mainly not used any form of antibiotics, 130(86.7). None of the participants in the aged group ≤24yrs had taken antibiotics in ≤2 weeks. The least gravidity observed was ≥7, 10(6.7), and none of the participants between the ages ≤24yrs and 25-32 years were among this group. The most recorded gravidity was ≤3, 31(91.2), 51(71.8) and 8(17.8) among the age group ≤24yrs, 25-32yrs and ≥33yrs respectively. The pattern observed for gravidity is similar to that of parity and gestational age. With their past obstetric history, most of the participants had no

history, 118(78.7) whiles the most occurring obstetric history was PROM 20(13.3). PROM was high regardless of the age group. The next major observed obstetric history was preterm birth 9(6.0). This was observed among those aged 25-32yrs, 4(5.6) and ≥33yrs, 5(11.1). Whiles none of the participants ≤24yrs had a preterm birth. Candidiasis was the most prevalent gynaecological history irrespective of age group, followed by UTI, spontaneous miscarriage and induced abortion in descending order. None of the participants had HIV, Hepatitis B or syphilis. Most of the participants, age notwithstanding, had no past neonatal history or comorbidities. However, of the participants with neonatal history, NICU admissions were the most observed 19(12.7) and of the comorbidities, asthma was the most observed, 4(2.7).

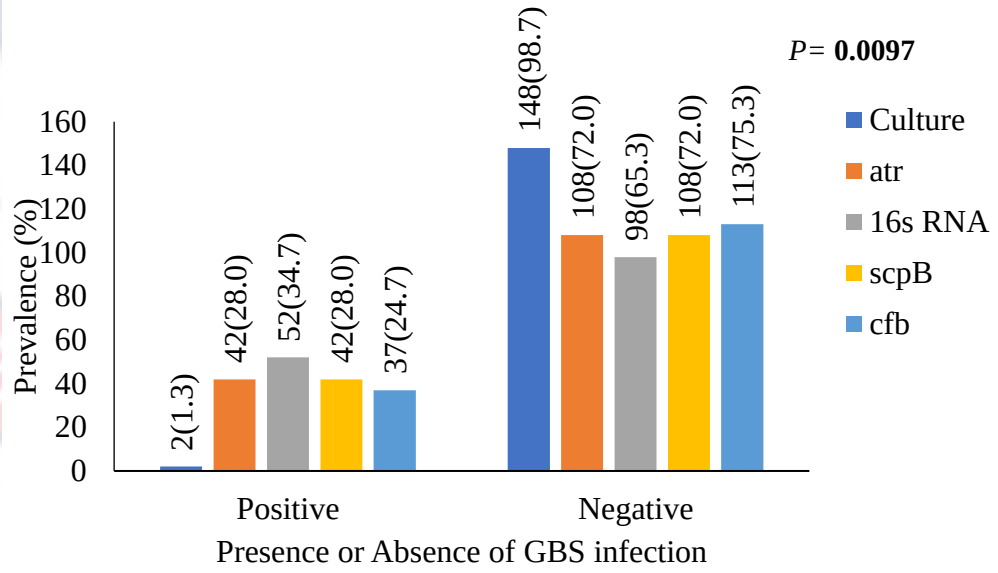

Figure 2: Prevalence of GBS Observed from Culture and the Various PCR Techniques

The prevalence of GBS infection observed was 52(34.7), 42(28.0), 42(28.0), 37(24.7) and 2(1.3) for 16s rRNA gene, atr gene, scpB gene, cfb gene

and culture technique respectively. A comparison of the infected and uninfected concerning the testing method used was significant, p<0.05. **(Figure 2)**

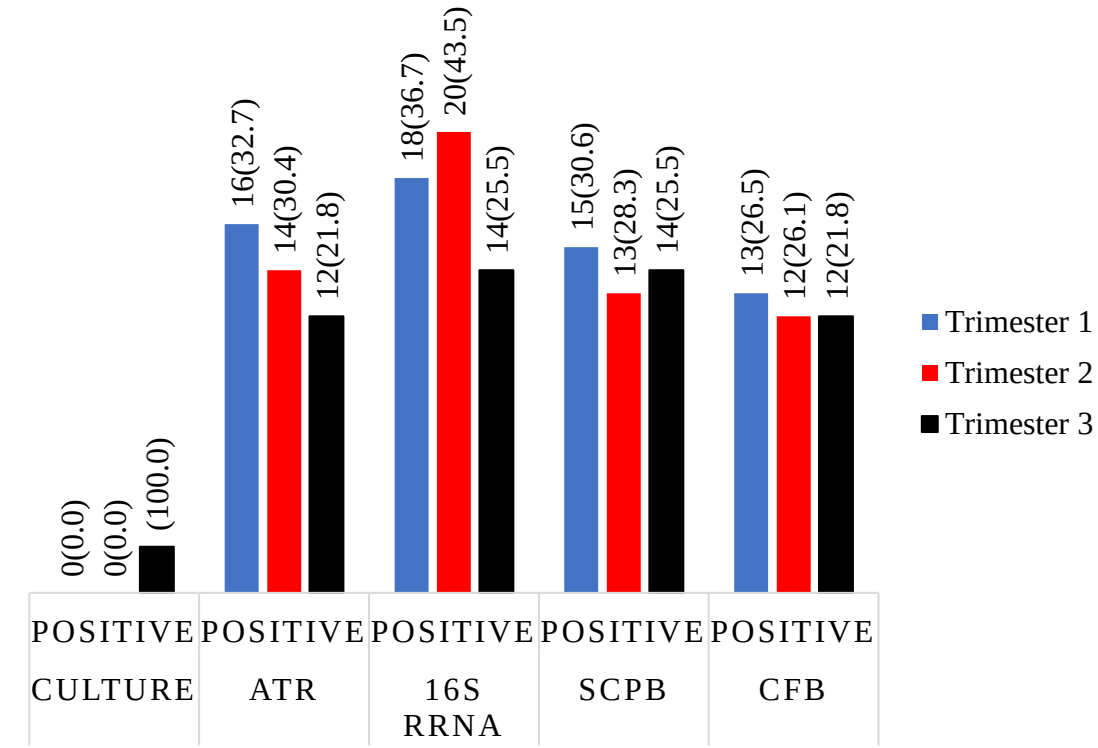

Figure 3: Occurrence of Group B *Streptococcus* at Different Trimesters Under Different Diagnostic Tests

POSITIVE= Positive for the infection under a test

Most of the infections in trimester 1 were detected by 16s rRNA, 18(36.7), followed by atr gene 16(32.7), whiles culture recorded no positives in this trimester. In trimester 2, 16s rRNA again recorded most of the infections 20(43.5), followed by atr gene 14(30.4), then ScpB recording 13(28.3), cfb gene 12(26.1) and culture recording no infections. Positive results from culture were all in the third trimester, 2(100.0). 16s rRNA and ScpB gene had 14(25.5) positives each, and atr gene and cfb gene also had 12(28.8) positives each. **(Figure 3).**

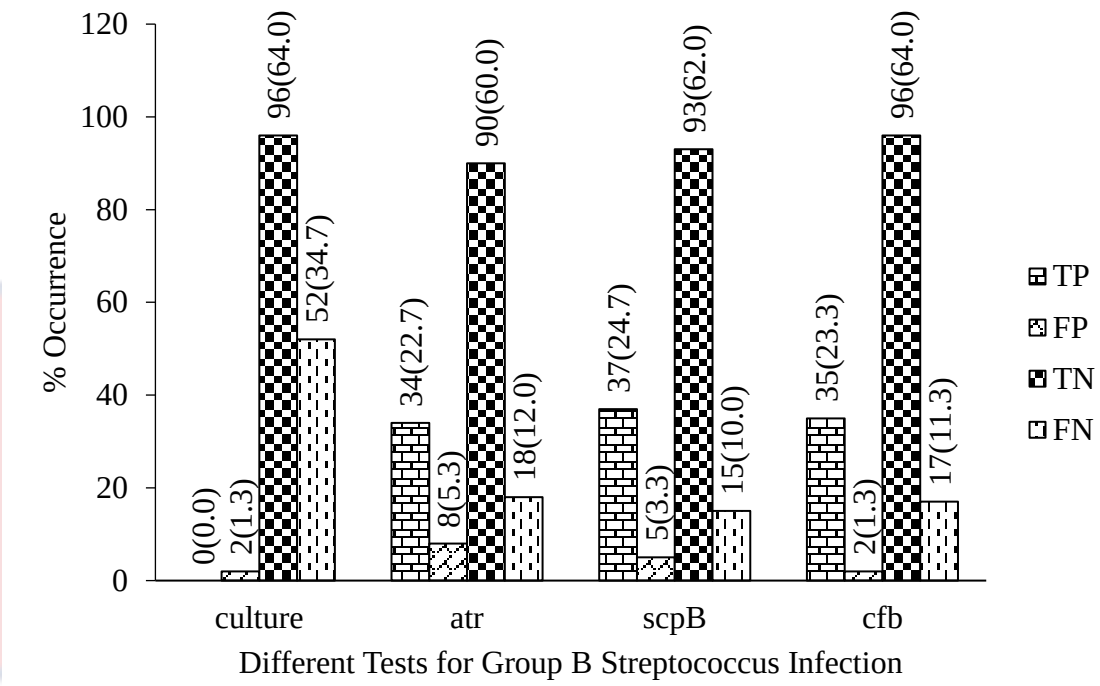

Figure 4: Comparing Diagnostic Performance of Culture and Other PCR Tests to the 16s rRNA PCR Test

TP: True Positive, FP: False Positive, TN: True Negative, FN: False Negative

Fifty-two samples were positive for the 16s rRNA gene PCR method. The results for the 16s rRNA test were compared to those obtained for other tests, i.e., culture, *atr*, *scpB*, and *cfb* PCR methods. The true positive result determined for culture, *atr*, *scpB* and *cfb* were 0(0.0), 34(22.7), 37(24.7) and 35(23.3), respectively. The true negatives for the culture, *atr*, *scpB* and *cfb* were also 96(64.0), 90(60.0),93(62.0) and 96(64.0), respectively. **(Figure 4).**

Table 5: Sensitivity, Specificity, PPV, NPV and Likelihood Ratio of the various test using 16s rRNA PCR Test as the Standard

Test	PPV		NPV		Sensitivity		Specificity		Likelihood Ratio	*P*
	%	95 % CI	%	95 % CI	%	95 % CI	%	95 % CI		
Culture	0.0	0.0-82.2	65.0	0.57-0.72	0.0	0.00-0.06	98.0	92.8-99.6	0.00	0.544
atr	80.9	0.67-0.90	83.3	0.75-0.89	65.4	0.51-0.80	91.8	0.84-0.95	8.01	<0.0001
scpB	88.1	0.75-0.94	86.1	0.78-0.91	71.1	0.57-0.81	95.0	0.88-0.97	13.95	<0.0001
cfb	95.0	0.82-0.99	85.0	0.77-0.90	67.3	0.54-0.79	98.0	0.92-0.99	32.98	<0.0001

PPV= Positive Predictive Value, NPV= Negative Predictive Value, 95%CI= 95% Confidence Interval, *P*= P-value (<0.05 implies statistically significant)

Table 5 above shows the sensitivity, specificity, negative predictive value, positive predictive value and the likelihood ratio of the various tests using 16s rRNA as the standard. All tests recorded specificity above 90%. Sensitivity, however, was 65.4% for atr gene, 71.1% for scpB and 67.3% for the cfb gene. There was no sensitivity in culture testing. All the tests above were statistically significant ($p<0.05$) except the culture method.

Table 6: Prevalence and Multivariate Regression Analysis (OR) of GBS Infection Across the 16s RNA PCR Test of the Participants

Variable	Infection Status		Total	OR (95% CI)	*P*
	Positive	Negative			
Recent Antibiotic Usage					
≤2 Weeks	1(1.9)	2(2.0)	3(2.0)	0.91(0.08-10.34)	0.001
>2 Weeks	5(9.6)	12(12.2)	17(11.3)	0.76(0.25-2.29)	0.941
None of the Above	46(88.5)	84(85.7)	130(86.7)	1	-
Gravidity					
≤3	30(57.7)	60(61.2)	90(60.0)	0.75(0.20-2.86)	0.674
4-6	18(34.6)	32(32.7)	50(33.3)	0.84(0.21-3.39)	0.811
≥7	4(7.7)	6(6.1)	10(6.7)	1	-
Parity					
≤2	37(71.2)	84(85.7)	121(80.7)	0.22(0.05-0.93)	0.220
3-4	9(17.3)	11(11.2)	20(13.3)	0.41(0.08-2.11)	0.409
≥5	6(11.5)	3(3.1)	9(6.0)	1	-
Gestational Age					
≤37 weeks	47(90.4)	81(82.7)	128(85.3)	1.97(0.68-5.69)	0.209
38-42 weeks	5(9.6)	17(17.3)	22(14.7)	1	-
>42 weeks	0(0.0)	0(0.0)	0(0.0)	-	-
Past Obstetrics History					
PROM	7(13.5)	13(13.3)	20(13.3)	0.33(0.02-5.47)	0.438
Preterm Birth	1(1.9)	8(8.2)	9(6.0)	0.06(0.00-2.09)	0.120
Puerpersal Sepsis	1(1.9)	2(2.0)	3(2.0)	0.44(0.02-8.22)	0.584
IUFD	1(1.9)	3(3.1)	4(2.7)	0.24(0.01-5.37)	0.365
Chorioamnionitis	0(0.0)	0(0.0)	0(0.0)	-	-
IUGR	0(0.0)	2(2.0)	2(1.3)	1.749E-9(1.749E-9-1.749E-9)	-
None of the Above	43(82.7)	75(76.5)	118(78.7)	0.25(0.012-5.16)	0.371
Past Gynecological History					
Spontaneous Miscarriage	8(15.4)	25(25.5)	33(22.0)	0.38(0.13-1.08)	0.070
Induced Abortion	11(21.2)	17(17.3)	28(18.7)	1.01(0.41-2.53)	0.977
UTI	18(34.6)	36(36.7)	54(36.0)	0.82(0.39-1.73)	0.600
PID	9(17.3)	6(6.1)	15(10.0)	2.55(0.81-8.04)	0.110
Candidiasis	36(69.2)	73(74.5)	109(72.7)	0.40(0.15-1.08)	0.071
HIV	0(0.0)	0(0.0)	0(0.0)	-	-
Hepatitis B	0(0.0)	0(0.0)	0(0.0)	-	-
Syphilis	0(0.0)	0(0.0)	0(0.0)	-	-
None of the above	3(5.8)	10(10.2)	13(8.7)	0.22(0.04-1.18)	0.077

Negative= absence of the infection, Positive= Presence of the infection, Total= total number of participants, *P*= P-value (>0.05 implies statistically significant), OR (95%CI) = Odds Ratio (95% Confidence Interval)

Table 7: Prevalence and Multivariate Regression Analysis (OR) of GBS Infection Across the 16s RNA PCR Test of the Participants

Variable	Infection Status		Total	OR (95% CI)	*P*
	Positive	Negative			
Past Neonatal History					
Neonatal Sepsis	3(5.8)	10(10.2)	13(8.7)	0.79(0.09-7.39)	0.837
Early Neonatal Death	1(1.9)	2(2.0)	3(2.0)	1.30(0.06-26.86)	0.866
Low Birth Weight	1(1.9)	6(6.1)	7(4.7)	0.46(0.03-6.16)	0.555
NICU Admission	4(7.7)	15(15.3)	19(12.7)	0.39(0.01-11.39)	0.586
None of the above	47(90.4)	82(83.7)	129(86.0)	0.58(0.02-17.04)	0.753
Comorbidities					
Pregnancy with Chronic Hypertension	0(0.0)	2(2.0)	2(1.3)	3.418E-9(3.418E-9-3.418E-9)	-
Gestational Hypertension	3(5.9)	2(2.0)	5(3.4)	1.37(0.04-43.47)	0.859
Diabetes in Pregnancy	1(2.0)	1(1.0)	2(1.3)	0.73(0.02-23.25)	0.859
Gestational Diabetes	0(0.0)	1(1.0)	1(0.7)	3.418E-9(3.418E-9-3.418E-9)	-
Sickle Cell Anemia	0(0.0)	2(2.0)	2(1.3)	3.418E-9(3.418E-9-3.418E-9)	-
Asthma	2(3.9)	2(2.0)	4(2.7)	0.86(0.02-38.85)	0.936
None of the Above	46(90.2)	88(89.8)	134(89.9)	0.45(0.02-12.04)	0.632

Negative= absence of the infection, Positive= Presence of the infection, Total= total number of participants, *P*= P-value (>0.05 implies statistically significant), OR (95%CI) = Odds Ratio (95% Confidence Interval)

Tables 6 and 7 show the prevalence of GBS infection as determined by the 16s RNA PCR test of the participants and the multivariate regression analysis (OR) of this infection. No meaningful outcome was seen in regression analyses except for antibiotic usage ≤2. Participants with no antibiotic history were plentiful among both infected and uninfected individuals. When compared to individuals who had not used antibiotics, those who had taken them during the previous two weeks had a lower risk of infection (OR0.91, 95%CI (0.08-10.34), <0.05). Both infected and uninfected subjects were concentrated in groups with gravidities below three, parities ≤2, and gestational ages ≤37 weeks. No one in the study was ≥42 weeks along in their pregnancies.

Most infected (n=43; 82.7%) and the uninfected (n=75; 76.5%) had no previous obstetric history. Infected individuals with previous exposure to PROM outnumbered them by a factor of 7(13.5). Infected women also reported higher rates of preterm birth, puerperal sepsis, and intrauterine growth restriction (IUGR) in their medical records. Most infected and uninfected women (36/69.2%) and women (73/74.5%%) had experienced candidiasis at some point in their gynaecological history. Then, UTIs became commonplace among both infected and uninfected people. Although there was no statistical significance in the regression analysis, the probabilities of infected subjects having a history of induced abortion or PUD were 1.01(0.41-2.53) and 2.55(0.81-8.04), respectively. The vast majority of both infected and uninfected infants (47(90.4) and 82(83.7) had no history of infection at birth. Infected babies were more likely to have NICU hospitalisations than non-infected babies, with an odds ratio of 0.39(0.01-11.39) for such a diagnosis. Most study participants (134, or 89.9%) did not suffer from comorbidities.

Discussion

Perinatal infections caused by Group B Streptococcus (Streptococcus agalactiae) include chorioamnionitis, premature delivery, stillbirth, and meningitis, and are a prominent cause of both early-onset (7 days of life) and late-onset (7-89 days of life) neonatal sepsis. Clouse, Shehabi, Suleimat, Faouri, Khuri-Bulos, Al Jammal, et al. (2019) estimate a global burden of GBS illness of 0.49–0.53 per 1000 live births, with a case fatality rate of 8.4–9.6 percent. This research aimed to compare several diagnostic methods used to identify Group B streptococcus colonisation in pregnant women in the Central area of Ghana and to determine the prevalence of this condition and its associated characteristics.

Approximately 103 (68.7%), 48 (32.0%), and 120 (84.0%) of the participants were Akans, college educated and employed. Most participants were working adults with at least a bachelor's degree. Contrast this with research conducted in Zimbabwe and South Africa, where the vast majority of participants were either jobless or had dropped out of high school before graduating (Africa & Kaambo, 2018; Mavenyengwa, Afset, Schei, Berg, Caspersen, Bergseng, et al., 2010). Another research in Tanzania (Ernest, Ng'Walida, Ndaboine, Massinde, Kihunrwa, & Mshana, 2015) found the opposite, with participants likewise being jobless and only having completed elementary school. However, the findings of this investigation are consistent with those of a 2016 study conducted in Uganda (Namugongo, Bazira, Fajardot, & Joseph). Perhaps the large proportion of college-educated individuals contributed to the positive findings.

This study determined GBS's prevalence using culture and different PCR tests. Among these tests, the 16s rRNA PCR method significantly recorded the most positives, 52(34.7), whiles the culture method recorded the least 2(1.3). Hence the 16s rRNA method was used as the standard for comparison to the other diagnostic approaches and the clinical data. The 34.7% prevalence observed from this study was high compared to the 19.7% (in two studies) and 23.7% observed 16s rRNA PCR and Xpert 16s rRNA GBS assay were employed, respectively (Mashouf, Mousavi, Rabiee, Alikhani, & Arabestani, 2014; Mousavi, Hosseini, Mashouf, & Arabestani, 2016; Said, Dangor, Mbelle, Sihlabela, Lekalakala, & Ismail, 2018). The prevalence recorded in this study was also higher than in previous studies conducted in Ghana in 2015 and 2017, where the culture technique was employed (Vinnemeier, Brust, Owusu-Dabo, Sarpong, Sarfo, Bio, et al., 2015; Völker, Cooper, Bader, Uy, Zimmermann, Lugert, et al., 2017). The differences in prevalence could result from the laboratory approach employed, geographical location, socio-economic status and healthcare availability among participants within this study compared to those in the other studies.

A sensitivity and specificity analysis of the various diagnostic tests to the 16s rRNA test revealed that while the *scpB* gene significantly recorded had the most true positive, the culture method had no true positives, thus making the *scpB* gene the most sensitive (71.1%) and culture the least sensitive (0.0%) when compared to the 16s rRNA. The most specific test concerning the 16s rRNA gene was culture and the *cfb* gene, each having a 98% specificity. The least specific test was the *atr* gene PCR test at 91.8%.

Furthermore, an assessment of the various test results across trimesters revealed that none of the infected participants fell within the first and the second trimester under culture testing, while in the PCR assessment, the 16s rRNA had the most positives in all trimesters with the *cfb* gene PCR test recording the least positives among the rest. Considering PCR, the prevalence was generally high in the first trimester and then decreased as the trimester increased, except for the 16s rRNA test (the standard test for the study), where the second trimester recorded the highest prevalence, followed by the first and third trimesters. This disagreed with a Michel *et al*. (2014) study where prevalence was mostly observed in the third trimester. The dissimilarities between this study and the previous study could be explained by the high sensitivity of the PCR tests used in this study compared to the culture method employed in the Michel *et al*. (2014) study. However, when the cultural results of this study and the previous study were compared, they agreed (Michel, Paul, Hortense, Koanga, & Sinata, 2014). Nonetheless, the findings of this study agreed with a previous study in which the researchers found that the prevalence of GBS decreased with an increase in gestational age (Mavenyengwa, Afset, Schei, Berg, Caspersen, Bergseng, et al., 2010). The conflicting findings observed from one study to the other could be resolved if a longitudinal case-control study monitors women from conception till birth.

Also, a risk analysis of the clinical information of the participants to the presence of infection revealed no significant associations between a participant being infected and their clinical data. However, the usage of antibiotics ≤2 weeks of infection was significantly associated with the infection level of a participant. An indication of taking antibiotics two weeks prior influences the

risk of infection by reducing the likelihood of infection. This could be because participants who had taken antibiotics perhaps had unknowingly treated the GBS infection. Yet in a Jordanian, Iranian and Chinese studies done recently, previous antibiotic usage had no significant effect on the infectivity of the participants (Africa & Kaambo, 2018; J. Chen, Fu, Du, Liu, Rongkavilit, Huang et al., 2018; Mousavi, Hosseini, Mashouf, & Arabestani, 2016).

Although insignificant, participants with ≤2 parity were the majority, and as parity increased, the number of infected participants decreased. This was dissimilar to results obtained in studies from Cameroon and Zimbabwe, where parity led to neither an increase nor decrease in the number of infections (Mavenyengwa, Afset, Schei, Berg, Caspersen, Bergseng, et al., 2010; Michel, Paul, Hortense, Koanga, & Sinata, 2014). It also disagreed with other recent studies where infection prevalence significantly increased with parity (Khan, Faiz, & Ashshi, 2015; Slotved, Dayie, Banini, & Frimodt-Møller, 2017), but it supported the findings of other studies in India and China (J. Chen, Fu, Du, Liu, Rongkavilit, Huang et al., 2018; Dechen, Sumit, & Ranabir, 2010). The results from this study add up to the inconsistent association between parity and GBS infections when previous studies are compared.

Most of the infected participants were in their ≤37 weeks of pregnancy, which agreed with a study by Dechen *et al*. (Dechen, Sumit, & Ranabir, 2010). As gestational age increased, the number of infected participants decreased. This was opposite to the findings of Khan and colleagues in 2015, which attributed an increase in gestational age to an increase in prevalence (Khan, Faiz, & Ashshi, 2015). It was also in disparity with another recent study that

reported a similar result to the Khan et al. study (Akadri, Osuolale, Shorunmu, & Odelola, 2019).

An additional assessment of their obstetric history revealed that most infected participants had no past complications. Yet among the participants with infection, a history of the premature rupture of membrane predominated. This agreed with previous studies conducted in India, where 28% and 43% prevalence of the infected had PROM (Dechen, Sumit, & Ranabir, 2010; Sharmila, Babu, & Chaturvedula, 2016). In studies conducted in 2014 and 2016, participants without a history of PROM were greatly infected, an assessment that disagreed with this study (Michel, Paul, Hortense, Koanga, & Sinata, 2014; Namugongo, Bazira, Fajardot, & Joseph, 2016). The overall lack of association between obstetric history and the prevalence of infection is similar to the report from a Brazilian study (Costa, Lamy Filho, Chein, Brito, Lamy, & Andrade, 2008).

On gynaecological history, it was observed that most of the infected with history had candidiasis followed by UTI and then induced abortion, although none was statistically significant. On UTI, the report from this study agreed with recent Jordanian and Ghanaian studies where no association was established between UTI and GBS infection (Clouse, Shehabi, Suleimat, Faouri, Khuri-Bulos, Al Jammal, et al., 2019; Slotved, Dayie, Banini, & Frimodt-Møller, 2017). However, these findings with regard to UTI disagreed with a study in northern India where the GBS-infected participants with UTI were substantially low (Khatoon, Nigam, Sharma, Srivastava, Sangal, & Malik, 2016).

In general, there was a lack of association between the clinical information obtained and the prevalence reported in this study, and this can be

explained by the fact that this current study was not a follow-up study and information from the participants was what this study mainly relied on.

Chapter Summary

Most of the participants were of Akan ethnicity (68.7%), had some form of formal education (92.0%) and were unemployed (84.0%). One hundred and thirty (86.7%) of the participants had not used any form of antibiotics. Gravidity and parity of ≤3(60.0) and ≤2(80.7), respectively, were found to be high among the participants. The *16s* rRNA gene 52(34.7) was identified to detect the highest prevalence and therefore was used as the gold standard. The culture technique was the least sensitive and specific test among the diagnostic tests performed. Infection detection to trimester revealed that generally, as the pregnancy trimester increased, the infection detection decreased when using the PCR technique. However, this was the opposite of the culture technique, which made all its detections in the third trimester.

Finally, there was no association between the clinical information obtained and the prevalence reported in this study. However, a significant relationship was established between the usage of antibiotics and the prevalence of the infection.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATION

Introduction

This chapter includes the summary, conclusion, and recommendation. The study reported a prevalence of 34.7% among the participants using the multiplex primer 16s rRNA PCR method as the standard and therefore recommends using PCR techniques together with the culture method to improve the detection of Group B streptococcus infection.

Summary

The hospital-based cross-sectional study aimed to determine the prevalence and risk factors associated with group B Streptococcus colonization among pregnant women and compare the diagnostic value of culture and PCR assays. One hundred and fifty (150) vaginal swabs were collected from pregnant women attending CCTH. Swabs were directly seeded unto SBA supplemented with gentamicin (8 μg/ml), nalidixic acid (Sigma Aldrich) (15 μg/ml) and incubated at 37°C for 24 hours in a carbon dioxide-rich condition. All 150 samples were tested to confirm the presence of GBS with four gene assays (*atr, ScpB, cfb* and *16SrRNA*), each using multiplex PCR.

Demographically, most of the participants were Akans, tertiary level educated and working, 103(68.7), 48(32.0) and 120(84.0), respectively. Seventy-four (49.3%) of the participants had not used any form of antibiotics. Gravidity and parity of ≤3(60.0) and ≤2(80.7), respectively, were found to be high among the participants. Most participants were at ≤37 weeks (85.3%) week gestational age.

The prevalence of GBS infection observed was 52(34.7), 42(28.0), 42(28.0), 37(24.7) and 2(1.3) for 16s rRNA gene, atr gene, scpB gene, cfb gene and culture technique respectively. Most of the infections in trimester 1 were detected by 16s rRNA, 18(36.7), followed by atr gene 16(32.7), whiles culture recorded no positives in this trimester. In trimester 2, 16s rRNA again recorded most of the infections 20(43.5), followed by atr gene 14(30.4), then ScpB recording 13(28.3), cfb gene 12(26.1) and culture recording no infections. Positive results from culture were all in the third trimester, 2(100.0). 16s rRNA and ScpB gene had 14(25.5) positives each, and atr gene and cfb gene also had 12(28.8) positives; every 52 samples were positive for the 16s rRNA gene PCR method. All sensitivity and specificity tests were obtained by comparing the 16s rRNA test with the other methods, i.e., culture, *atr*, *scpB,* and *cfb* PCR method and a recorded specificity above 90% was observed among all. However, Sensitivity was highest for the scpB gene, 71.1%, while culture testing was the least sensitive (0.0%).

Multivariate regression analysis of the clinical demographics among participants against 16s rRNA gene assay revealed a statistical significance between the prevalence of infection and participants who had used antibiotics for ≤ 2 weeks. No other significant association was established as a risk factor for GBS.

Conclusion

The study reported a prevalence of 34.7% among the participants using the multiplex primer 16s rRNA PCR method as the standard. A comparison of the other PCR techniques and the culture method reported that the culture method was insignificantly insensitive but specific. It, however, confirmed that

the other PCR techniques were both significantly sensitive and specific to use. Trimester analysis still indicated that the 16s rRNA was the most sensitive test comparatively, and the culture method was best employed when participants were in their third trimester. No meaningful association was established between the prevalence of the infection and the clinical information provided by the participants.

Recommendation

From the findings of this study, we recommend using PCR techniques together with the culture method to improve the detection of Group B streptococcus infection. A comprehensive national policy for preventing, diagnosing, and treating GBS infection in pregnant women and newborns is also required to further reduce the high frequency of GBS and its associated complications.

REFERENCES

Acosta, C. D., Kurinczuk, J. J., Lucas, D. N., Tuffnell, D. J., Sellers, S., & Knight, M. (2014). Severe maternal sepsis in the UK, 2011–2012: a national case-control study. *PLoS Med*, *11*(7), e1001672.

Africa, C. W. J., & Kaambo, E. (2018). Group B Streptococcus serotypes in pregnant women from the Western Cape region of South Africa. *Frontiers in Public Health*, *6*(DEC). https://doi.org/10.3389/fpubh.2018.00356

Afshar, B., Broughton, K., Creti, R., Decheva, A., Hufnagel, M., Kriz, P., Lambertsen, L., Lovgren, M., Melin, P., & Orefici, G. (2011). International external quality assurance for laboratory identification and typing of Streptococcus agalactiae (Group B streptococci). *Journal of Clinical Microbiology*, *49*(4), 1475–1482.

Ahmadzia, H. K., & Heine, R. P. (2014). Diagnosis and management of group B streptococcus in pregnancy. *Obstetrics and Gynecology Clinics of North America*, *41*(4), 629–647. https://doi.org/10.1016/j.ogc.2014.08.009

Akadri, A. A., Osuolale, B. D., Shorunmu, T. O., & Odelola, O. I. (2019). Group B Streptococcus Colonization in Pregnancy: Prevalence, Determinants and Antibacterial Susceptibility Pattern in Sagamu, Nigeria. *Journal of Advances in Medicine and Medical Research*, *29*(December 2017), 1–8. https://doi.org/10.9734/jammr/2019/v29i1 030131

Assefa, S., Desta, K., & Lema, T. (2018). Group B streptococci vaginal colonization and drug susceptibility pattern among pregnant women attending in selected public antenatal care centers in Addis Ababa,

Ethiopia. *BMC Pregnancy and Childbirth*, *18*(1), 135.

Baker, C. J., Barrett, F. F., Gordon, R. C., & Yow, M. D. (1973). Suppurative meningitis due to streptococci of Lancefield group B: a study of 33 infants. *The Journal of Pediatrics*, *82*(4), 724—729. https://doi.org/10.1016/s0022-3476(73)80606-7

Banini, J. A. N. (2015). *Carriage and antibiotic susceptibility profile of Group B Streptococcus during late pregnancy in selected hospitals in greater Accra* (Doctoral dissertation).

Belard, S., Toepfner, N., Capan-Melser, M., Mombo-Ngoma, G., Zoleko-Manego, R., Groger, M., Matsiegui, P.-B., Agnandji, S. T., Adegnika, A. A., González, R., Kremsner, P. G., Menendez, C., Ramharter, M., & Berner, R. (2015). Streptococcus agalactiae Serotype Distribution and Antimicrobial Susceptibility in Pregnant Women in Gabon, Central Africa. *Scientific Reports*, *5*(1), 17281. https://doi.org/10.1038/srep17281

Berardi, A., Rossi, C., Lugli, L., Creti, R., Reggiani, M. L. B., Lanari, M., Memo, L., Pedna, M. F., Venturelli, C., & Perrone, E. (2013). Group B streptococcus late-onset disease: 2003–2010. *Pediatrics*, *131*(2), e361–e368.

Blumberg, H. M., Stephens, D. S., Modansky, M., Erwin, M., Elliot, J., Facklam, R. R., Schuchat, A., Baughman, W., & Farley, M. M. (1996). Invasive Group B Streptococcal Disease: The Emergence of Serotype V. *The Journal of Infectious Diseases*, *173*(2), 365–373. https://doi.org/10.1093/infdis/173.2.365

Borchardt, S. M., Foxman, B., Chaffin, D. O., Rubens, C. E., Tallman, P. A.,

Manning, S. D., Baker, C. J., & Marrs, C. F. (2004). Comparison of DNA dot blot hybridization and Lancefield capillary precipitin methods for group B streptococcal capsular typing. *Journal of Clinical Microbiology*, *42*(1), 146–150.

Brigtsen, A. K. (2018). Maternal Colonization with Group B Streptococcus (GBS). Epidemiology, Microbiology and Clinical Outcomes in the Oslo GBS Study.

Buser, G. L., Mató, S., Zhang, A. Y., Metcalf, B. J., Beall, B., & Thomas, A. R. (2017). Notes from the field: late-onset infant Group B streptococcus infection associated with maternal consumption of capsules containing dehydrated placenta—Oregon, 2016. *MMWR. Morbidity and Mortality Weekly Report*, *66*(25), 677.

Chan, G. J., Lee, A. C. C., Baqui, A. H., Tan, J., & Black, R. E. (2015). Prevalence of early-onset neonatal infection among newborns of mothers with bacterial infection or colonization: a systematic review and meta-analysis. *BMC Infectious Diseases*, *15*(1), 118.

Chen, J., Fu, J., Du, W., Liu, X., Rongkavilit, C., Huang, X., Wu, Y., Wang, Y., & McGrath, E. (2018). Group B streptococcal colonization in mothers and infants in western China: Prevalences and risk factors. *BMC Infectious Diseases*, *18*(1), 1–8. https://doi.org/10.1186/s12879-018-3216-4

Chen, V. L., Avci, F. Y., & Kasper, D. L. (2013). A maternal vaccine against group B Streptococcus: past, present, and future. *Vaccine*, *31 Suppl 4*(0 4), D13–D19. https://doi.org/10.1016/j.vaccine.2012.12.080

Clouse, K., Shehabi, A., Suleimat, A. M., Faouri, S., Khuri-Bulos, N., Al

Jammal, A., Chappell, J., Fortner, K. B., Chamby, A. B., Randis, T. M., Ratner, A. J., Aronoff, D. M., & Halasa, N. (2019). High prevalence of Group B Streptococcus colonization among pregnant women in Amman, Jordan. *BMC Pregnancy and Childbirth*, *19*(1), 1–8. https://doi.org/10.1186/s12884-019-2317-4

Costa, A. L. dos R., Lamy Filho, F., Chein, M. B. da 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 obstetricia : revista da Federacao Brasileira das Sociedades de Ginecologia e Obstetricia*, *30*(6), 274–280. https://doi.org/10.1590/ s0100-72032008000600002

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

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.

Dechen, T. C., Sumit, K., & Ranabir, P. (2010). Correlates of Vaginal Colonization with Group B Streptococci among Pregnant Women. *Journal of Global Infectious Diseases*, *2*(3), 236–241. https://doi.org/10.4103/0974-777X.68536

Denny, F. J., Stevens, D. L., & Kaplan, E. L. (2000). History of hemolytic streptococci and associated diseases.

Edmond, K. M., Kortsalioudaki, C., Scott, S., Schrag, S. J., Zaidi, A. K. M., Cousens, S., & Heath, P. T. (2012). Group B streptococcal disease in infants aged younger than 3 months: systematic review and meta-analysis. *Lancet (London, England)*, *379*(9815), 547–556. https://doi.org/10.1016/S0140-6736(11)61651-6

El Helali, N., Giovangrandi, Y., Guyot, K., Chevet, K., Gutmann, L., & Durand-Zaleski, I. (2012). Cost and effectiveness of intrapartum group B streptococcus polymerase chain reaction screening for term deliveries. *Obstetrics & Gynecology*, *119*(4), 822–829.

El Helali, N., Nguyen, J.-C., Ly, A., Giovangrandi, Y., & Trinquart, L. (2009). Diagnostic accuracy of a rapid real-time polymerase chain reaction assay for universal intrapartum group B streptococcus screening. *Clinical Infectious Diseases*, *49*(3), 417–423.

Enweronu-Laryea, C. C., Damale, N. R., & Newman, M. J. (2011). Prevalence of group B streptococcus in pregnant women attending a tertiary hospital in Ghana in 2001. *Archives of Clinical Microbiology*, *2*(2).

Ernest, A. I., Ng'Walida, N., Ndaboine, E., Massinde, A., Kihunrwa, A., & Mshana, S. (2015). Maternal vaginorectal colonization by Group B Streptococcus and Listeria monocytogenes and its risk factors among pregnant women attending tertiary hospital in Mwanza, Tanzania. *Tanzania Journal of Health Research*, *17*(2), 1–9. https://doi.org/10.4314/thrb.v17i2.4

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.

Farley, M. M., Harvey, R. C., Stull, T., Smith, J. D., Schuchat, A., Wenger, J. D., & Stephens, D. S. (1993). A population-based assessment of invasive disease due to group B Streptococcus in nonpregnant adults. *The New England Journal of Medicine*, *328*(25), 1807–1811. https://doi.org/10.1056/NEJM199306243282503

Fenton, L. J., & Harper, M. H. (1979). Evaluation of colistin and nalidixic acid in Todd-Hewitt broth for selective isolation of group B streptocci. *Journal of Clinical Microbiology*, *9*(2), 167–169.

Florindo, C., Viegas, S., Paulino, A., Rodrigues, E., Gomes, J. P., & Borrego, M. J. (2010). Molecular characterization and antimicrobial susceptibility profiles in Streptococcus agalactiae colonizing strains: association of erythromycin resistance with subtype III-1 genetic clone family. *Clinical Microbiology and Infection*, *16*(9), 1458–1463.

Fultz-Butts, K., Gorwitz, R. J., Schuchat, A., & Schrag, S. (2002). *Prevention of perinatal group B streptococcal disease; revised guidelines from CDC*.

Håkansson, S., Axemo, P., Bremme, K., Bryngelsson, A.-L., Carlsson Wallin, M., Ekström, C.-M., Granlund, M., Jacobsson, B., Källén, K., & Spetz, E. (2008). Group B streptococcal carriage in Sweden: a national study on risk factors for mother and infant colonisation. *Acta Obstetricia et Gynecologica Scandinavica*, *87*(1), 50–58.

Heath, P. T. (2011). An update on vaccination against group B streptococcus. *Expert Review of Vaccines*, *10*(5), 685–694.

Imperi, M., Pataracchia, M., Alfarone, G., Baldassarri, L., Orefici, G., & Creti, R. (2010). A multiplex PCR assay for the direct identification of the capsular type (Ia to IX) of Streptococcus agalactiae. *Journal of Microbiological Methods*, *80*(2), 212–214.

Jefferies, A. L. (2017). Management of term infants at increased risk for early-onset bacterial sepsis. *Paediatrics & Child Health*, *22*(4), 223–228.

Joachim, A., Matee, M. I., Massawe, F. A., & Lyamuya, E. F. (2009). Maternal and neonatal colonisation of group B streptococcus at Muhimbili National Hospital in Dar es Salaam, Tanzania: prevalence, risk factors and antimicrobial resistance. *BMC Public Health*, *9*, 437. https://doi.org/10.1186/1471-2458-9-437

Joubrel, C., Tazi, A., Six, A., Dmytruk, N., Touak, G., Bidet, P., Raymond, J., Trieu Cuot, P., Fouet, A., Kernéis, S., & Poyart, C. (2015). Group B streptococcus neonatal invasive infections, France 2007-2012. *Clinical Microbiology and Infection : The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, *21*(10), 910–916. https://doi.org/10.1016/j.cmi.2015.05.039

Kalin, A., Acosta, C., Kurinczuk, J. J., Brocklehurst, P., & Knight, M. (2015). Severe sepsis in women with group B Streptococcus in pregnancy: An exploratory UK national case–control study. *BMJ Open*, *5*(10).

Karnabi, P. (2017). *Neonatal group B streptococcal disease : burden of illness and assessment of preventability in British Columbia*.

Khan, M. A., Faiz, A., & Ashshi, A. M. (2015). Maternal colonization of group

B streptococcus: Prevalence, associated factors and antimicrobial resistance. *Annals of Saudi Medicine*, *35*(6), 423–427. https://doi.org/10.5144/0256-4947.2015.423

Khatoon, F., Nigam, A., Sharma, N., Srivastava, R., Sangal, R., & Malik, N. (2016). Prevalence and risk factors for group B streptococcal colonization in pregnant women in northern India. *International Journal of Reproduction, Contraception, Obstetrics and Gynecology*, *5*(12), 4361–4364. https://doi.org/10.18203/2320-1770.ijrcog20164343

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

Kong, F., Gowan, S., Martin, D., James, G., & Gilbert, G. L. (2002). Serotype identification of group B streptococci by PCR and sequencing. *Journal of Clinical Microbiology*, *40*(1), 216–226.

Kwatra, G., Cunnington, M. C., Merrall, E., Adrian, P. V, Ip, M., Klugman, K. P., Tam, W. H., & Madhi, S. A. (2016). Prevalence of maternal colonisation with group B streptococcus: a systematic review and meta-analysis. *The Lancet Infectious Diseases*, *16*(9), 1076–1084.

Lancefield, R. C. (1933). A serological differentiation of human and other groups of hemolytic streptococci. *The Journal of Experimental Medicine*, *57*(4), 571–595.

Lawn, J. E., Bianchi-Jassir, F., Russell, N. J., Kohli-Lynch, M., Tann, C. J., Hall, J., Madrid, L., Baker, C. J., Bartlett, L., Cutland, C., Gravett, M. G., Heath, P. T., Ip, M., Le Doare, K., Madhi, S. A., Rubens, C. E., Saha,

S. K., Schrag, S., Sobanjo-Ter Meulen, A., … Seale, A. C. (2017). Group B Streptococcal Disease Worldwide for Pregnant Women, Stillbirths, and Children: Why, What, and How to Undertake Estimates? *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *65*(suppl_2), S89–S99. https://doi.org/10.1093/cid/cix653

Le Doare, K., & Heath, P. T. (2013). An overview of global GBS epidemiology. *Vaccine*, *31*, D7–D12.

Longtin, J., Vermeiren, C., Shahinas, D., Tamber, G. S., McGeer, A., Low, D. E., Katz, K., & 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.

Lu, B., Chen, X., Wang, J., Wang, D., Zeng, J., Li, Y., Li, D., Zhu, F., Cui, Y., & Huang, L. (2016). Molecular characteristics and antimicrobial resistance in invasive and noninvasive Group B Streptococcus between 2008 and 2015 in China. *Diagnostic Microbiology and Infectious Disease*, *86*(4), 351–357.

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.

Martín, V., Cárdenas, N., Ocaña, S., Marín, M., Arroyo, R., Beltrán, D., Badiola, C., Fernández, L., & Rodríguez, J. M. (2019). Rectal and vaginal eradication of Streptococcus agalactiae (GBS) in pregnant

women by using Lactobacillus salivarius CECT 9145, a target-specific probiotic strain. *Nutrients*, *11*(4), 810.

Martins, E. R., Melo-Cristino, J., & Ramirez, M. (2010). Evidence for rare capsular switching in Streptococcus agalactiae. *Journal of Bacteriology*, *192*(5), 1361–1369.

Mashouf, R. Y., Mousavi, S. M., Rabiee, S., Alikhani, M. Y., & Arabestani, M. R. (2014). Direct identification of Streptococcus agalactiae in vaginal colonization in pregnant women using polymerase chain reaction. *Journal of Comprehensive Pediatrics*, *5*(4), 0–5. https://doi.org/10.17795/compreped-23339

Matuschek, E., Brown, D. F. J., & Kahlmeter, G. (2014). Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. *Clinical Microbiology and Infection*, *20*(4), O255–O266.

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. https://doi.org/10.3109/00016340903398029

McCracken, G. H. J. (1973). Group B streptococci: the new challenge in neonatal infections. *The Journal of Pediatrics*, *82*(4), 703–706. https://doi.org/10.1016/s0022-3476(73)80603-1

McGee, L., Schrag, S., & Verani, J. R. (2010). *Prevention of perinatal Group B streptococcal disease; revised guidelines from CDC, 2010*.

Medugu, N., Iregbu, K., Iroh Tam, P.-Y., & Obaro, S. (2018). Aetiology of

neonatal sepsis in Nigeria, and relevance of Group b streptococcus: A systematic review. *PloS One*, *13*(7), e0200350.

Melin, P. (2011). Neonatal group B streptococcal disease: from pathogenesis to preventive strategies. *Clinical Microbiology and Infection : The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, *17*(9), 1294–1303. https://doi.org/10.1111 /j.1469-0691.2011.03576.x

Melo, S. C. C. S. de, Costa, A. B., Silva, F. T. R. da, Silva, N. M. M. G., Tashima, C. M., Cardoso, R. F., Pádua, R. A. F. de, Previdelli, I., Carvalho, M. D. de B., & Pelloso, S. M. (2018). Prevalence of Streptococcus agalactiae colonization in pregnant women from the 18th Health Region of Paraná State. *Revista Do Instituto de Medicina Tropical de São Paulo*, *60*.

Michel, T., Paul*, A. J., Hortense, G., Koanga, M., & Sinata, K. S. (2014). Vaginal colonization and resistance profile of group B Streptococcus among pregnant women in Yaound Gynecology, Obstetric and Pediatric Hospital in Cameroon. *Journal of Clinical Medicine and Research*, *6*(6), 16–21. https://doi.org/10.5897/JCMR2014.0249

Mitima, K. T., Ntamako, S., Birindwa, A. M., Mukanire, N., Kivukuto, J. M., Tsongo, K., & Mubagwa, K. (2014). Prevalence of colonization by Streptococcus agalactiae among pregnant women in Bukavu, Democratic Republic of the Congo. *Journal of Infection in Developing Countries*, *8*(9), 1195—1200. https://doi.org/10.3855/jidc.5030

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.

Money, D., Allen, V. M., Yudin, M. H., Bouchard, C., Boucher, M., Caddy, S., Castillo, E., Murphy, K. E., Ogilvie, G., & Paquet, C. (2013). The prevention of early-onset neonatal group B streptococcal disease. *Journal of Obstetrics and Gynaecology Canada*, *35*(10), 939–948.

Mousavi, S. M., Hosseini, S. M., Mashouf, R. Y., & Arabestani, M. R. (2016). Identification of group B streptococci using 16S rRNA, cfb, scpB, and atr genes in pregnant women by PCR. *Acta Medica Iranica*, *54*(12), 765–770.

Namugongo, A., Bazira, J., Fajardot, Y., & Joseph, N. (2016). Group B Streptococcus Colonization among Pregnant Women Attending Antenatal Care at Tertiary Hospital in Rural Southwestern Uganda. *International Journal of Microbiology, 2016*. https://doi.org/10.1155/2016/3816184

Nan, C., Dangor, Z., Cutland, C. L., Edwards, M. S., Madhi, S. A., & Cunnington, M. C. (2015). Maternal group B Streptococcus-related stillbirth: a systematic review. *BJOG: An International Journal of Obstetrics & Gynaecology*, *122*(11), 1437–1445.

NIZET, V., FERRIERI, P., & RUBENS, C. E. (2000). Molecular Pathogenesis of Group B Streptococcal. *Streptococcal Infections: Clinical Aspects, Microbiology, and Molecular Pathogenesis*, 180.

Nsagha, D. S., Bello, C. S., & Kandakai-Olukemi, Y. T. (2000). Hippurate hydrolysis and Christie, Atkins, Munch-Peterson tests as epidemiological diagnostic tools for Streptococcus agalactiae carriage

in pregnancy. *East African Medical Journal*, *77*(1), 34–36. https://doi.org/10.4314/eamj.v77i1.46373

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), 135–143.

Paoletti, L. C., & Kasper, D. L. (2003). Glycoconjugate vaccines to prevent group B streptococcal infections. *Expert Opinion on Biological Therapy*, *3*(6), 975–984. https://doi.org/10.1517/14712598.3.6.975

Patras, K. A., & Nizet, V. (2018). Group B streptococcal maternal colonization and neonatal disease: molecular mechanisms and preventative approaches. *Frontiers in Pediatrics*, *6*, 27.

Phares, C. R., Lynfield, R., Farley, M. M., Mohle-Boetani, J., Harrison, L. H., Petit, S., Craig, A. S., Schaffner, W., Zansky, S. M., Gershman, K., Stefonek, K. R., Albanese, B. A., Zell, E. R., Schuchat, A., & Schrag, S. J. (2008). Epidemiology of invasive group B streptococcal disease in the United States, 1999-2005. *JAMA*, *299*(17), 2056–2065. https://doi.org/10.1001/jama.299.17.2056

Pintye, J., Saltzman, B., Wolf, E., & Crowell, C. S. (2016). Risk factors for late-onset group B streptococcal disease before and after implementation of universal screening and intrapartum antibiotic prophylaxis. *Journal of*

the Pediatric Infectious Diseases Society, *5*(4), 431–438.

Porta, K., & Rizzolo, D. (2015). Preventing group B streptococcal infections in newborns. *Journal of the American Academy of PAs*, *28*(3), 24–29.

Poyart, C., Tazi, A., Réglier-Poupet, H., Billoët, A., Tavares, N., Raymond, J., & Trieu-Cuot, P. (2007). Multiplex PCR assay for rapid and accurate capsular typing of group B streptococci. *Journal of Clinical Microbiology*, *45*(6), 1985–1988.

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*.

Rosa-Fraile, M., & Spellerberg, B. (2017). Reliable detection of group B Streptococcus in the clinical laboratory. *Journal of Clinical Microbiology*, *55*(9), 2590–2598.

Said, M., Dangor, Y., Mbelle, N., Sihlabela, A., Lekalakala, M. R., & Ismail, F. (2018). Comparison of xpert GBS v. Culture for rapid detection of group b streptococcus in pregnant women: Sensitivity, specificity and predictive values. *South African Medical Journal*, *108*(12), 1032–1035. https://doi.org/10.7196/SAMJ.2018.v108i12.13079

Santhanam, S., Jose, R., Sahni, R. D., Thomas, N., & Beck, M. M. (2017). Prevalence of group B streptococcal colonization among pregnant women and neonates in a tertiary hospital in India. *Journal of the Turkish German Gynecology Association*, *18*(4), 181–184.

https://doi.org/10.4274/jtgga.2017.0032

Schrag, S. J., Zywicki, S., Farley, M. M., Reingold, A. L., Harrison, L. H., Lefkowitz, L. B., Hadler, J. L., Danila, R., Cieslak, P. R., & Schuchat, A. (2000). Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis. *The New England Journal of Medicine*, *342*(1), 15–20. https://doi.org/10.1056/NEJM200001063420103

Seale, A. C., Koech, A. C., Sheppard, A. E., Barsosio, H. C., Langat, J., Anyango, E., Mwakio, S., Mwarumba, S., Morpeth, S. C., & Anampiu, K. (2016). Maternal colonization with Streptococcus agalactiae and associated stillbirth and neonatal disease in coastal Kenya. *Nature Microbiology*, *1*(7), 1–10.

Seki, T., Kimura, K., Reid, M. E., Miyazaki, A., Banno, H., Jin, W., Wachino, J., Yamada, K., & Arakawa, Y. (2015). High isolation rate of MDR group B streptococci with reduced penicillin susceptibility in Japan. *Journal of Antimicrobial Chemotherapy*, *70*(10), 2725–2728.

Shabayek, S., & Spellerberg, B. (2018). Group B streptococcal colonization, molecular characteristics, and epidemiology. *Frontiers in Microbiology*, *9*, 437.

Sharmila, V., Babu, T., & Chaturvedula, L. (2016). Neonatal outcome in maternal genital tract group B streptococcal colonization. *International Journal of Reproduction, Contraception, Obstetrics and Gynecology*, *5*(10), 3444–3447. https://doi.org/10.18203/2320-1770.ijrcog20163420

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.

Simoes, J. A., Alves, V. M. N., Fracalanzza, S. E. L., Camargo, R. P. S. de, 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.

Slotved, H. C., Dayie, N. T. K. D., Banini, J. A. N., & Frimodt-Møller, N. (2017). Carriage and serotype distribution of Streptococcus agalactiae in third trimester pregnancy in southern Ghana. *BMC Pregnancy and Childbirth*, *17*(1), 10–16. https://doi.org/10.1186/s12884-017-1419-0

Stoll, B. J., Hansen, N., Fanaroff, A. A., Wright, L. L., Carlo, W. A., Ehrenkranz, R. A., Lemons, J. A., Donovan, E. F., Stark, A. R., Tyson, J. E., Oh, W., Bauer, C. R., Korones, S. B., Shankaran, S., Laptook, A. R., Stevenson, D. K., Papile, L.-A., & Poole, W. K. (2002). Changes in pathogens causing early-onset sepsis in very-low-birth-weight infants. *The New England Journal of Medicine*, *347*(4), 240–247. https://doi.org/10.1056/NEJMoa012657

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.

Turrentine, M. A., & Ramirez, M. M. (2008). Recurrence of group B streptococci colonization in subsequent pregnancy. *Obstetrics & Gynecology*, *112*(2), 259–264.

Verani, J. R., McGee, L., & Schrag, S. J. (2010). Prevention of perinatal group

B streptococcal disease. *Morbidity and Mortality Weekly Report (MMWR), Revised Guidelines from CDC, Recommendations and Reports*, *59*(RR10), 1–32.

Vinnemeier, C. D., Brust, P., Owusu-Dabo, E., Sarpong, N., Sarfo, E. Y., Bio, Y., Rolling, T., Dekker, D., Adu-Sarkodie, Y., Eberhardt, K. A., May, J., & Cramer, J. P. (2015). Group B Streptococci serotype distribution in pregnant women in Ghana: assessment of potential coverage through future vaccines. *Tropical Medicine and International Health*, *20*(11), 1516–1524. https://doi.org/10.1111/tmi.12589

Völker, F., Cooper, P., Bader, O., Uy, A., Zimmermann, O., Lugert, R., & Groß, U. (2017). Prevalence of pregnancy-relevant infections in a rural setting of Ghana. *BMC Pregnancy and Childbirth*, *17*(1), 1–7. https://doi.org/10.1186/s12884-017-1351-3

Weisner, A. M., Johnson, A. P., Lamagni, T. L., Arnold, E., Warner, M., Heath, P. T., & Efstratiou, A. (2004). Characterization of group B streptococci recovered from infants with invasive disease in England and Wales. *Clinical Infectious Diseases*, *38*(9), 1203–1208.

Wessels, M. R. (1997). Biology of streptococcal capsular polysaccharides. *Journal of Applied Microbiology*, *83*(S1), 20S-31S.

Woldu, Z. L., Teklehaimanot, T. G., Waji, S. T., & Gebremariam, M. Y. (2014). The prevalence of Group B Streptococus recto-vaginal colonization and antimicrobial susceptibility pattern in pregnant mothers at two hospitals of Addis Ababa, Ethiopia. *Reproductive Health*, *11*, 80. https://doi.org/10.1186/1742-4755-11-80

Yao, K., Poulsen, K., Maione, D., Rinaudo, C. D., Baldassarri, L., Telford, J.

L., Sørensen, U. B. S., Kilian, M., & Group, D. S. (2013). Capsular gene typing of Streptococcus agalactiae compared to serotyping by latex agglutination. *Journal of Clinical Microbiology*, *51*(2), 503–507.

Zaleznik, D. F., Rench, M. A., Hillier, S., Krohn, M. A., Platt, R., Lee, M.-L. T., Flores, A. E., Ferrieri, P., & Baker, C. J. (2000). Invasive Disease Due to Group B Streptococcus in Pregnant Women and Neonates from Diverse Population Groups. *Clinical Infectious Diseases*, *30*(2), 276–281. https://doi.org/10.1086/313665