UNIVERSITY OF CAPE COAST

Streptococcus agalactiae INFECTION AMONG PARTURIENTS AND THEIR NEONATES AT THE CAPE COAST TEACHING HOSPITAL: AN EVALUATION OF DIFFERENT DIAGNOSTIC METHODS, PREVALENCE AND RISK FACTORS

GERTRUDE ADU-AFARI

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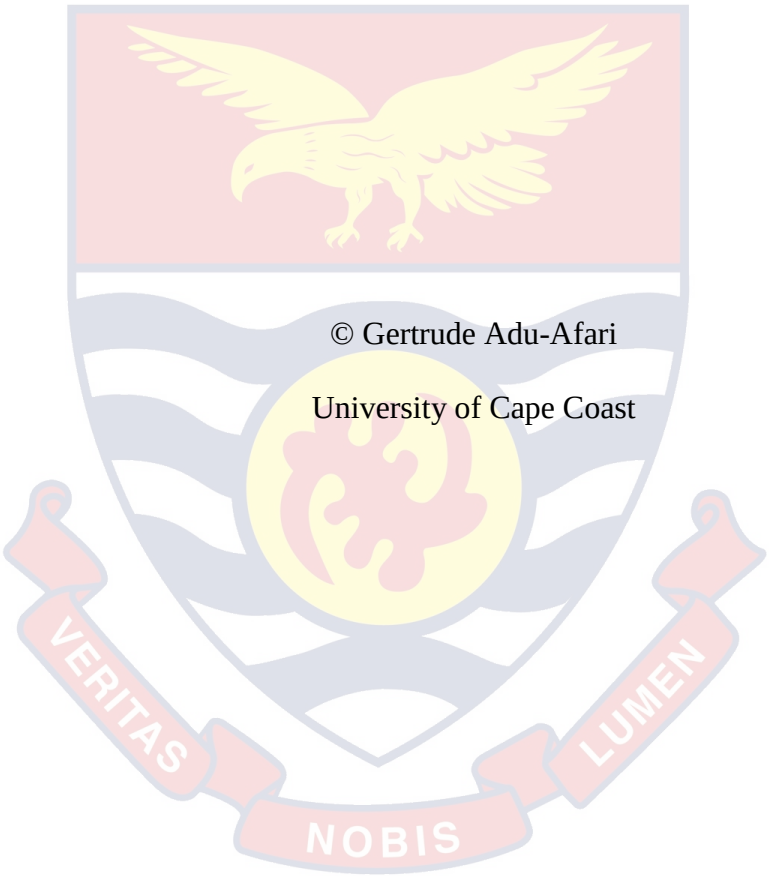

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Streptococcus agalactiae INFECTION AMONG PARTURIENTS AND THEIR NEONATES AT THE CAPE COAST TEACHING HOSPITAL: AN EVALUATION OF DIFFERENT DIAGNOSTIC METHODS, PREVALENCE AND RISK FACTORS

BY

GERTRUDE ADU-AFARI

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 fulfilment of the requirements for the award of Master of Philosophy degree in Infection and Immunity

OCTOBER 2021

DECLARATION

Candidate's Declaration

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

Candidate's Signature ……. Date ……

Name: ……

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Supervisor's Signature ……... Date ……

Name: ……

Co-Supervisor's Signature ……. Date ……

Name: ……

ABSTRACT

Streptococcus agalcatiae (GBS) is a relatively common; normal bacterium in a woman's gastrointestinal and genitourinary tract; however, implicated as a leading cause of neonatal invasive infections. Maternal GBS colonization during late pregnancy is a pre-requisite for neonatal GBS infections. This study aimed at determining the prevalence of *Streptococcus agalactiae* among parturients and their neonates in the Cape Coast Teaching Hospital. A total of 301;150 vaginal swabs(parturients) and 151 superficial swabs(neonates)were collected between June 2019 and August 2019 and investigated for the presence of *Streptococcus agalactiae* using the culture method,16S rRNA, *cfb*, *scpB*, and *atr* genes targeting assays. Prevalence of GBS infection observed among the mothers was 36(24.0%),34(22.7%),32(21.3%),20(13.3%), and 2(1.3%) for atr gene, ScpB gene, cfb gene,16SrRNA gene, and culture technique respectively. Using the atr as the standard, sensitivities 55.6% for scp, 47.2% for cfb,47.2% for 16s rRNA gene and 5.6%, for culture were reported. Among the neonates, prevalence was 57(37.5%), 43(28.3%), 42(27.6%) 40(26.3%) and 0(0.0%) for atr gene, cfb gene, scpB gene, 16s rRNA and culture technique respectively. Sensitivites of 64.9%,70.2%, 64.9%,0.0% were also reported among the neonates for 16s rRNA, ScpB, cfb, and culture respectively comparing with the standard, *atr* gene. Specificity for the culture method and assays used in this study were above 85.0% and 90% for parturients and neonates respectively. A significant relationship was observed between GBS infection and ≤37 weeks gestation among the parturients. Also, among the neonates, a statistically significant relationship was found between GBS infection and the risk of intrauterine fetal death (IUFD). A comparative analysis of the PCR assays and the culture method revealed a relatively high prevalence of GBS among parturients and their neonates. Therefore, the use of PCR assays in combination with the culture method is recommended to increase the diagnosis of Group B streptococcal infection. A systematic national guideline is therefore recommended for the prevention, diagnosis and treatment of GBS infection and its related complications among parturients and their neonates.

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DEDICATION

To my parents, Mr Johnson Adu-Afari and Miss Margaret Forson, my siblings and my son, Gideon Ansah-Boateng.

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LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic acid
GBS	Group B streptococcus
EOD	Early Onset Disease
LOD	Late Onset Disease
IAP	Intrapartum Antibiotic Prophylaxis
IUFD	Intrauterine foetal Death
CDC	Centre for Disease Control
CAMP	Christie Atkins Munch Peterson
NAAT	Nucleic acid amplification tests
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PID	Pelvic Inflammatory Diseases
PROM	Prolonged Rupture of Membranes
SBA	Sheep Blood Agar
SPSS	Statistical Package for Social Sciences
STGG	Skimmed milk, tryptone, glucose and glycerine
UTI	Urinary Tract Infections

CHAPTER ONE

INTRODUCTION

Chapter Introduction

Improving maternal and newborn health is key to the Global Sustainable Development Goal targets for 2030. Globally, a burden of 5.6 million deaths are reported as a result of vertical transmission from infected mothers around the time of birth (Lawn *et al.*, 2017). Therefore, accelerated efforts are required to prevent infections contributing to this burden, but data is limited.

Streptococcus agalactiae also known as Group B streptococcus (GBS) is identified as the main cause of pneumonia, neonatal sepsis, bacteremia and meningitis in neonates/infants and is connected with the increased rates of morbidity and mortality.

In Ghana, GBS epidemiology is poorly understood due to scarce data. This study therefore aims at estimating the prevalence and risk factors related with *streptococcus agalactiae* colonization among parturients and their neonates, and weigh the diagnostic value of culture and PCR assays. The outcome of this study will provide useful information on the GBS situation in Cape Coast and help formulate better interventions to improve maternal and child health.

Background to the study

Streptococcus agalactiae is a common colonizer among pregnant women which can also lead to invasive infections during perinatal period (Perez-Moreno *et al.*, 2017). Maternal genitourinary infection with GBS increases the risk for GBS related infections in new-borns. GBS is part of the

natural flora of the human gastrointestinal and genitourinary tract of 15–50% of women without disease (El Aila *et al.*, 2010; Huber *et al.*, 2011). GBS is spread from carrier mothers to newborns in the perinatal period particularly in cases of vaginal delivery (CDC, 2002). Pregnant women stand a higher chance of developing GBS related diseases like chorioamnionitis, urinary tract infections or sepsis before and after child birth. These maternal infections can lead to low birth weight, preterm birth and abortion (Vornhagen *et al.*, 2017). The skin of newborns at birth and mucosal surfaces like nasopharyngeal, oral, vaginal and anal mucosa have been reported to support the colonization of GBS (Chen *et al.*, 2018).

GBS colonization and infection represents major cause of morbidity and mortality especially in infants (Alfa *et al.*, 2010; Madzivhandila *et al.*, 2011). It is estimated that invasive disease develops in around 1–2% newborns infected with GBS (Madzivhandila *et al.*, 2011). In high-income settings, GBS is identified as the key cause of meningitis, pneumonia, neonatal sepsis and bacteraemia and is linked with increased rates of morbidity and mortality (Stoll *et al.*, 2011; Verani *et al.*, 2010). Reports from several studies have indicated the under appreciation of the weight of invasive GBS disease on under-developed settings especially in Africa (Gray *et al.*, 2007; Labi *et al.*, 2016; Vinnemeier *et al.*, 2015). In the prevention and management of GBS infections, risk factors associated with maternal and neonatal diseases are critically considered. Maternal risk factors including obstetric and demographic factors; maternal GBS colonization, history of neonatal or infant with streptococcal disease or GBS related bacteriuria during that pregnancy,

detection of maternal GBS colonization during labour or delivery, and low titres of anti-GBS antibodies are key for prevention and management.

Neonatal GBS disease is categorized into early-onset (EO) disease and late-onset (LO) disease manifesting 0-6 days and 7-89 days after birth respectively. EO-GBS septicaemia is often linked with pneumonia, which is thought to be as a result of the aspiration of GBS contaminated amniotic fluid in the course of birth, whereas late onset septicaemia is mostly linked with meningitis (Lee *et al.*, 2017; Takei *et al.*, 2013). The risk factors for neonatal GBS infection include peripartum fever, maternal GBS colonization, preterm labour, rupture of membranes (premature or prolonged), maternal GBS bacteriuria and a neonatal history of GBS disease (Berardi *et al.*, 2014; Slotved *et al.*, 2017a). LO-GBS may be by vertical transmission and nosocomial acquisition.

Timely and accurate detection of *Streptococcus agalactiae* colonization among pregnant women is key to prevent the infection in their neonates and its consequent sequelae. Reliable diagnostic methods depending on time of specimen collection is crucial due to the transient nature of GBS infection during pregnancy. Diagnosis is usually by bacterial culture and isolation, serological or molecular techniques. Although detection by culture and isolation is time consuming, it is recommended by the CDC for perinatal screening. However, several studies have reported the higher sensitivity and specificity of the molecular and serological techniques as compared to the culture method. Nevertheless, high cost of reagents and equipment hinder the availability of these techniques in most laboratories.

In Ghana, insufficiency of data on the epidemiology of GBS infection among parturients and their neonates with the burden of disease among neonates poorly understood. This project therefore forms part of an effort to contribute to the 'Ghana National Newborn Health Strategy and Action Plan 2014-2018' by understanding Group B Streptococcus epidemiology and diagnosis among parturients and their neonates to help in the formulation of better interventions to improve maternal and child health outcomes.

Problem Statement

In Ghana, the main causes of newborn mortality include prematurity complications (29%), septicaemia (31%) and negative obstetric events like birth asphyxia (27%), (MOH, 2014). Preterm babies most often die of complications due to respiratory problems, infections and adverse intrapartum events (MOH, 2014). A number of prevalence-based surveys on bacteraemia have emphasized worries on the increasing incidence of streptococcal disease among infants (Brimil *et al.*, 2006; Skoff *et al.*, 2009; Vornhagen *et al.*, 2017). Studies conducted in developed countries indicated high prevalence of GBS among neonates (Guo *et al.*, 2018; Madzivhandila *et al.*, 2011). In Ghana, documented surveys exist on the prevalence and serotype distribution of GBS in the Ashanti ((Vinnemeier *et al.*, 2015) and Greater Accra (Slotved *et al.*, 2017b) regions. Both studies observed high prevalence of GBS among pregnant women. However, there is inadequate data on GBS epidemiology which makes it difficult to draw conclusions that neonates who suffer from chorioamnionitis and sepsis are caused by GBS or other bacteria acquired congenitally. It is however estimated that at least 32% of neonatal mortalities in Ghana are caused by infections and a large number of these infections are

vertically transmitted (Enweronu-Laryea *et al.*, 2011). Generally, there is limited awareness of GBS infection among clinicians in Ghana, thus laboratory testing is rarely requested to address that. In light of this, the study looked at estimating the prevalence and risk factors of GBS colonization among parturients and their neonates. This is to sensitise the relevant stakeholders to implement effective national surveillance programmes on this medical burden.

Aim

The aim of this study is to determine the prevalence and risk factors associated with *Streptococcus agalactiae* colonization among parturients and their neonates, and compare the diagnostic value of culture and PCR assays.

Specific Objectives

The specific objectives of the study are to:

i. determine the clinical and socio-demographic characteristics of parturients and their neonates.
ii. determine the prevalence of group B streptococcus among parturients and their neonates.
iii. determine risk factors associated with GBS colonization among parturients and their neonates
iv. compare the diagnostic value of culture and PCR assays by determining the sensitivity, specificity, positive and negative predictive values in the detection of GBS among participants.

Significance of Study

The burden of GBS infection continues to pose as a public health concern even in high-income countries (Guo *et al.*, 2018; Madzivhandila *et*

al., 2011). Currently, the limited surveys conducted in Ghana targeted pregnant mothers only (Slotved *et al.*, 2017a; Vinnemeier *et al.*, 2015). The importance of this study cannot be overemphasized because there is limited data on GBS in Ghana. Also, epidemiology of maternal and neonatal GBS colonization is poorly understood in Ghana. It is therefore necessary to generate data on Streptococcus agalactiae colonization rate among parturients and their neonates since infection in neonates is mostly by vertical transmission. It will reveal the extent of GBS vaginal colonization among women in labour and consequently their neonates This is intended to be beneficial for the advancement of strategies to screen for GBS.

Also, data is needed regarding risk factors connected with GBS among parturients and their infants apart from colonization in pregnant women known to be the main risk factor for neonatal infections.

Organization of study

The study is systematized into five chapters. Chapter one introduces the background of the study. Chapter two explored literature on the microorganisms under study; its microbiology, epidemiology, serotype distribution, pathogenesis, risk factors for colonization 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 which include the study design, study sites, sampling, biochemical tests for phenotypic and genotypic identification of GBS isolates as well as the data analysis. Chapter four consists of the results from study and discussion of the key findings. Lastly, chapter five consists of the summary, conclusion and recommendations made from the study.

Chapter Summary

GBS, an asymptomatic colonizing organism of the gastrointestinal and genitourinary tracts of healthy women is implicated in invasive neonatal infections. Maternal *Streptococcus agalactiae* infection is a prerequisite for neonatal EO-GBS disease and other complications. Lack of data and availability of accurate clinical diagnosis of GBS continues to hamper surveillance of the infection in developing countries. Hence, the study looked at GBS infection among parturients and their neonates, risk factors and compared the diagnostic value of culture method and gene targeting PCR assays by determining the sensitivity, specificity, positive and negative predictive values in the detection of GBS among participants

CHAPTER TWO

LITERATURE REVIEW

Chapter Introduction

This chapter reviewed the microbiology, epidemiology, serotype distribution of Group B streptococcus, pathogenesis, risk factors for colonization of GBS in parturients and their neonates, signs, treatment and outcomes, laboratory detection and antimicrobial susceptibility pattern of GBS. Databases that were used in the conduction of the literature review included: PLOS, PubMed, Wiley online library, Medline, Google Scholar, Web of Science and WHO.

Headings such as "Prevalence of neonatal colonization of Group B Streptococcus", "Microbiology and epidemiology of GBS in neonates", "Laboratory detection of GBS" among other relevant phrases were searched for in the databases.

Microbiology of Group B Streptococcus

General Characteristics of GBS

Streptococcus agalactiae is a spherical Gram-positive, encapsulated organism characteristically occuring in short chains but also in pairs and singles. GBS is fastidious requiring enriched media for growth, facultative anaerobes, catalase-negative, non-sporing and non-motile (Kim *et al.*, 2011). When cultured on SBA, a characteristic colourless zone is seen round the growing colonies as a result of complete lysis of red blood cells; phenomenon called beta haemolysis. Occasional pigmentation have been documented when deprived of oxygen on Columbia agar and MacConkey/bile agar with the

advantage of easy visual identification (Rosa-Fraile & Spellerberg, 2017; Tamayo *et al.*, 2004).

Capsular Polysaccharides (CPS)

GBS, the species representation of *Streptococci* and a member of the Lancefield group B. It is the only *species* harbouring the polysaccharide antigen ie Lancefield group B cell-wall-specific antigen which is shared by the entire *Streptococcus agalactiae* strains (Edwards *et al.*, 2016). Four different oligosaccharides including N-acetylglucosamine, rhamnose, glucitol and galactose form the basis of the complex structure of the group-B specific antigen. Recent studies have shown that, the capsular polysaccharide and the group B carbohydrate are bounded covalently to the mucopeptide at distinct points, i.e. to N-acetylmuramic acid and N-acetylglucosamine (Caliot *et al.*, 2012).

Based on the type-specific capsular polysaccharide, *Streptococcus agalactiae* can be subdivided into 10 different serotypes (1a, 1b, II, III, IV, V, VI, VII, VIII, and IX) (Edwards *et al.*, 2016). Among these, the most common serotypes that colonize pregnant women and cause neonatal infections include Ia, Ib, III, VI, and V (Furuichi *et al.*, 2017). The *cps* operon houses the entire genome accountable for the building of the cell wall and its attachment to the group B Streptococcus polysaccharide capsule (CPS). The differences in sequence of the 16–18 genes which form the operon is the basis for the different serotypes (Berti *et al.*, 2014).

Epidemiology of GBS

Group B Streptococcus is ubiquitous, a naturally occurring bacterium in the gastrointestinal tract which can colonize the genitourinary tract

recurrently or occasionally in 35 percent of women (Okon *et al.*, 2013; Shabayek & Spellerberg, 2018). The vagina and the rectum are the key reservoirs for streptococcus agalactiae and its presence in these areas is a risk factor for successive infection/sequelae in pregnant mothers and their new-borns (Martín *et al.*, 2019). Asymptomatic colonization of *Streptococcus agalactiae* occurs universally among pregnant women.

Globally, group B Streptococcus can be found in 4% to 30% of pregnant women (Mohammed *et al.*, 2012; Simoes *et al.*, 2007). Approximately 40–75% neonates born to these colonized mothers are infected with 1–2% of cases resulting invasive disease (Chan *et al.*, 2015; Patras & Nizet, 2018). The prevalence of Group b streptococcus colonization differs greatly worldwide depending on the medium used, number and type of sites cultured, culture methods, time of pregnancy, geographical area and race (Mohammed *et al.*, 2012; Quiroga *et al.*, 2008; Santhanam *et al.*, 2017).

In the Western world such as Europe, the reported prevalence of GBS ranges from 6.5% to 36% (Barcaite *et al.*, 2008). In the United States, GBS maternal colonization rates have stabilized around 20% to 25% (Gilbert, 2004; Musleh & Al Qahtani, 2018).

In low-middle-income settings, the prevalence observed are not different; In Ethiopia, an overall prevalence of GBS colonization of 14.6% was reported among 281 pregnant women in some chosen Public antenatal care centres (Assefa *et al.*, 2018). Another observational study in Ethiopia in 2014 among pregnant women at 35-37 weeks gestation reported a GBS carriage of 7.2% (22 out of the 300 pregnant women had positive rectovaginal isolates) (Woldu *et al.*, 2014). In Nigeria, a study at Obafemi

Awolowo,(a tertiary facility) among 150 pregnant women at 35-40 weeks observed a GBS carriage of 11.3% (Onipede *et al.*, 2012) while another observational cross-sectional study among 200 participants (24-35 week) in the Enugu state, observed carriage of 18% (Ezeonu & Agbo, 2014).In Tanzania (23%) and Malawi (16.5%) high GBS carriage rates have similarly been reported (Edmond *et al.*, 2012; Joachim *et al.*, 2009).

In Ghana, studies conducted in the Greater Accra region by Enweronu-Laryea *et al.* (2011) in a tertiary facility and Slotved *et al.* (2017b) in two health facilities revealed maternal GBS colonization rates of 19% and between 25.5% to 28.0% respectively. Another study by Vinnemeier *et al.* (2015) in the Ashanti region revealed an overall prevalence of 19.1% (rural Pramso 18.1% and in urban Kumasi (23.1%)) among study participants (519 pregnant women). In summary, these surveys demonstrated that *Streptococcus agalactiae* carriage in low and middle-income settings are comparable to that observed in developed countries.

GBS Serotype Distribution

The capsular polysaccharide of GBS has been studied to be a significant virulence factor. Differences in the polysaccharide capsule structure allow for the antigenic variation of ten distinct GBS serotypes that is Ia, Ib, II–IX (Guo *et al.*, 2018; Le Doare & Heath, 2013). Several studies have reported that serotype distribution differs with geographical region and ethnicity (Brochet *et al.*, 2009;Guo *et al.*, 2018; Le Doare & Heath, 2013; Mavenyengwa *et al.*, 2010).

Multitude of surveys in the US and Europe revealed that the serotypes Ia, II, III, and V are found in 80–90% of all clinical isolates, while serotypes

IV, VI, VII, VIII and IX are rarely observed (Diedrick *et al.*, 2010; Edwards & Baker, 2005). However, in the United Arab Emirates, type IV was the predominantly isolated serotype among colonized pregnant women and represented 26.3% of the GBS isolates (Diedrick *et al.*, 2010). Serotypes VI and VIII strains are more frequently isolated in Japan (Brimil *et al.*, 2006).

In East Africa, serotypes Ia, Ib, II, III, IV and V were common. The prevalence observed for these common serotypes were s 30.8 %, 30.2%, 17.2 %, 14.2%, 7.1 %, 0.6% for III, Ia, V, Ib, II and IV respectively (Huber *et al.*, 2011). Vaginal colonizing isolates in South Africa were distributed as follows: 37.3%, 30.1%, 11.3%, 10.2%, 6.7%, 3.7%, for III, Ia, II, V, Ib and IV respectively (Madzivhandila *et al.*, 2011).

Serotype III has been identified as the most invasive and these observations are consistent with those reported in other countries such as South Africa, Portugal, Sweden and Israel (Bisharat *et al.*, 2005; Madzivhandila *et al.*, 2011; Martins *et al.*, 2007). In Norway, both serotypes III and V were observed to be predominantly involved in invasive GBS infections with type III and V isolated frequently from infants and the elderly respectively (Bergseng *et al.*, 2008).

In a current systematic review, the serotypes Ia, Ib, II, III and V were estimated to make up 85% of all serotypes observed among geographical areas comprising Africa, the America, Western Pacific, Eastern Mediterranean and Europe. Specifically, serotype III is the most common serotype (49%) in all regions. Serotype I was the most predominant (40%) trailed by serotype III (37%) were found to be commonly associated with Early-Onset GBS cases.

Contrarily, serotype III was predominantly linked with Late-Onset GBS cases (53%) trailed by serotype I (30%) (Edmond *et al.*, 2012).

The prevalence of serotypes differ with time and geographical location; thus, information on serotype variation among regions is vital for the choice and advancement of serotype-based vaccine in a particular country (Fluegge *et al.*, 2005; Mukesi *et al.*, 2019)

Pathogenesis of GBS

Streptococcus agalactiae infection in pregnant women is a sign of the organism's attachment to the epithelial lining in the vagina and additionally its resistance to mucosal immune defences (Edwards *et al.*, 2016; Nizet *et al.*, 2000). Colonization may happen in the foetus if the GBS climbs into the amniotic cavity where it colonizes the foetus' mucous membranes or skin. The bacterium enters the foetus' lungs as a result of intake of the contaminated amniotic fluid. Infant can also be infected during passage through the birth canal. The main determinant of GBS disease in neonates is maternal genital tract GBS infection at the time of delivery (Edwards *et al.*, 2016).

For disease development after colonization in neonates, the bacterium must multiply in the alveoli, attach to the lining of the respiratory tract and employ neonate's resistance mechanisms such as pulmonary macrophages to avoid destruction. Infection caused by GBS occurs when pathogen invades the pulmonary epithelial and endothelial cells and enter the bloodstream. Organism becomes dispersed in the bloodstream results in meningitis and osteomyelitis, sepsis and death (Melin, 2011; Nizet *et al.*, 2000). The stages of neonatal infection as well as the cellular and molecular pathogenesis of GBS are summarized in (figure 1)

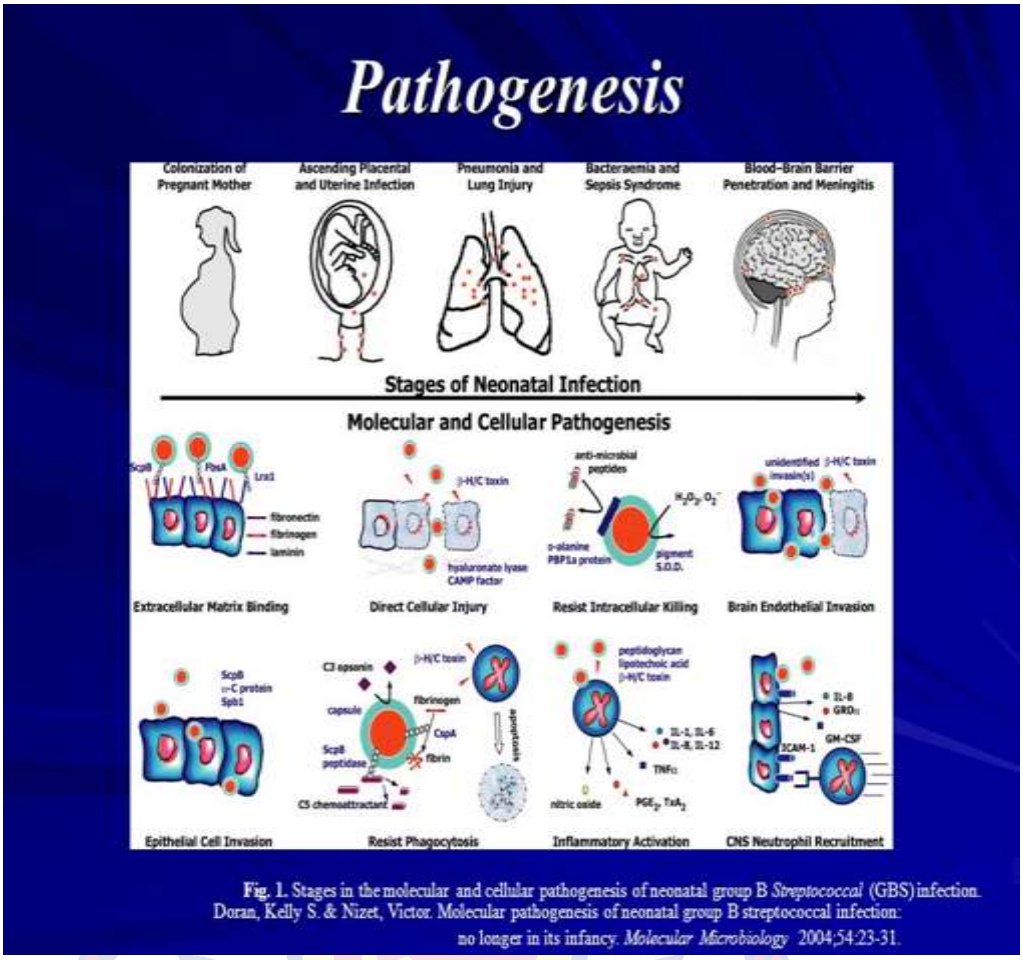


Fig. 1. Stages in the molecular and cellular pathogenesis of neonatal group B *Streptococcal* (GBS) infection.
Doran, Kelly S. & Nizet, Victor. Molecular pathogenesis of neonatal group B streptococcal infection: no longer in its infancy. *Molecular Microbiology* 2004;54:23-31.

Figure 1: Pathogenesis of GBS.
Source: (Doran & Nizet, 2004)

Maternal GBS Disease

The recognized maternal GBS disease sequelae includes:

Urinary tract infections (UTI): GBS causes symptomless bacteriuria and is found in 5-29 % of cases. GBS is similarly known to cause pyelonephritis and cystitis during pregnancy. *Streptococcus agalactiae* is also linked with preterm labour and premature birth. Treatment with penicillin G cannot be overemphasized especially in cases of high colony count in urine ie. ≥ 100 000 CFU/mL (Allen *et al.*, 2012).

Puerperal sepsis: *Streptococcus agalactiae* is responsible for around 17% of this condition normally happening within 12hrs after birth and this becomes

evident with tachycardia, endometritis, fever and abdominal distension (Mohamed, 2009).

Postpartum endometritis: GBS has been found to be associated with around twenty-eight percent of cases of endometritis, a multimicrobial infection caused by more than one naturally occurring organism due to ascending infections in the vagina. Postpartum endometritis is significantly linked with GBS Colonization during pregnancy. Apart from GBS, other organisms like *Ureaplasma urealyticum*, *Bacteroides bivius Peptostreptococcus, Gardnerella vaginalis*; are usually found in patients with endometritis thus management involves broad spectrum antibiotic. (Mohamed, 2009).

Neonatal GBS Disease

GBS disease is not solely known to be implicated in infections in newborns and expectant mothers. The bacterium can also cause invasive diseases in the aged; immunocompromised individuals (Melin, 2011) living with lasting conditions like cancer and diabetes (Edwards *et al.*, 2016). Preterm Babies are mostly prone to the infection than those born at term (Vornhagen *et al.*, 2017). Nevertheless, infants aged between 0-89 days have been reported to record the highest severity rate and incidence (Le Doare & Heath, 2013). Neonatal GBS disease is classified early or late based on the age of the infant as of the time of disease manifestation (Baker & Barrett, 1973; Franciosi *et al.*, 1973). Disease manifestation within the first 6 days of life is known as early-onset while that between 7-89 days is known as late-onset disease. An outline of the clinical symptoms linked with EO and LO -disease is defined below.

Early-onset GBS Disease

EO-GBS disease is defined by the manifestation of a neonatal infection within the first week of birth (0-6 days) (Edwards *et al.*, 2016). EO-GBS disease is very common accounting for 60-70% of GBS infection among neonates (Le Doare & Heath, 2013). A prerequisite for neonatal EO disease is maternal GBS colonization of the gastrointestinal or genital tract; rupture of membranes preceeds vertical transmission usually occurring during labour. (Edwards *et al.*, 2016;Le Doare & Heath, 2013;Melin, 2011; Schrag & Verani, 2013). Maternal prophylaxis prior to labour will prevent fifty percent of infants born to infected mothers from becoming colonized and also stop the development of invasive disease among 1-2% of colonized infants (K. Le Doare & Heath, 2013). The most common serotypes recognized in Canada and US to cause Early- onset GBS disease are Ia, Ib, II, III and V (Alhhazmi *et al.*, 2016; Porta & Rizzolo, 2015).

Risk factors for early-onset GBS

Risk factors associated with EO-GBS disease are accurately defined and include maternal GBS colonization, prolonged rupture of membranes, regular vaginal examination in the course of labour, preterm delivery, GBS bacteriuria during pregnancy, history of an infant with invasive GBS disease, chorioamnionitis which manifest as intrapartum fever, young maternal age, African or Hispanic ethnicity and inadequate levels of maternal antibodies to the various serotypes that are found to be involved in invasive disease (Bergeron *et al.*, 2000; Lin *et al.*, 2004; Yancey *et al.*, 1996). Gestational diabetes, diabetes mellitus and frequent vaginal examination have been

documented to raise the possibilities of having a newborn with EOD-GBS infection (Raabe & Shane, 2019).

Signs, treatments and outcomes of neonatal EO-GBS disease

About ninety percent (90%) of infants show signs of illness of Early-onset GBS disease within 24 hours and occasionally at birth (Melin, 2011). Colonized infants usually develop pneumonia, osteomyelitis, sepsis, meningitis, septic arthritis and cellulitis (Schuchat, 1998). Signs of pneumonia include retractions, respiratory distress, hypoxemia and tachypnoea. Sepsis and meningitis are characterized by apnoea, irritability and poor feeding, (Ahmadzia & Heine, 2014; Porta & Rizzolo, 2015). Hypotension, usually occurring in 25% of all cases is also manifested during severe infections. New-borns infected in the uterus, may develop serious foetal asphyxia (Mullaney, 2001).

Late-onset (LO) GBS Disease

LO-GBS disease is presented by the start of signs of disease from a week after birth to the third month i.e. 7-89days (Edwards *et al.*, 2016). Serotype III has been reported to be the strain most frequently associated with LO GBS disease (Edmond *et al.*, 2012). The aetiology of Late-onset GBS disease is not well defined but presumed to be multifaceted. In the US, a prospective study revealed that forty-seven percent of infants with late-onset disease had the identical colonizing GBS strains as found in their mothers thus buttressing acquisition of infection through vertical transmission (Dillon Jr *et al.*, 1982). Direct person to person transmission has also been linked with late-onset disease. The faeco-oral route may be involved in this type of transmission. Horizontal transmission can be nosocomial and community

acquired (Ahmadzia & Heine, 2014; Melin, 2011; Pintye *et al.*, 2016). Hitherto, the hands of health care workers were observed to be a common source of GBS contamination (Berardi *et al.*, 2013). Conversely, improvement in infection prevention and control in neonatal care has made this source now uncommon. Breast milk and maternal infected placenta have also been suggested as possible transmission pathways in some studies (Buser *et al.*, 2017; Godambe *et al.*, 2005; Kotiw *et al.*, 2003).

Maternal Intrapartum prophylaxis which involves the administration of antibiotics during labour is the most effective means to reduce neonatal GBS infections and its accompanying complications. Antibiotics including penicillin G, cefazolin, clindamycin or vancomycin may be given (Money *et al.*, 2013). Treatment is also provided for neonates who show signs of being infected and those considered "at risk" as per the Canadian Paediatric Society (Jefferies, 2017). Infants without symptoms born to mothers who were adequately given prophylaxis are usually not treated.

Risk Factors for Late-onset GBS

Risk factors linked with LO-GBS infection are not well characterized. Hospital-associated acquisition and vertical transmission are the frequent possible sources (Ling *et al.*, 2010). African ethnicity, young maternal age (< 20 years of age), prematurity (less than 34-week gestation), positive GBS culture and neonatal male sex are recognized as risks factor for LO- GBS disease (Edwards *et al.*, 2016; Lin *et al.*, 2003; Pintye *et al.*, 2016). Although nosocomial acquisition has been reported, it does not seem to be the source for outbreaks in nurseries. Infants infected with GBS are not required to be

secluded. GBS colonization has been documented to persist for a number of months even after treatment in newborns (Verani *et al.*, 2010).

Signs, Treatment and Outcomes of Neonatal LO GBS Disease

The typical clinical presentation of late-onset GBS disease is meningitis. Poor feeding, induce fever, bacteraemia, irritability, lethargy, grunting and apnoea in neonates may also be as a result of late-onset disease with 50% of all Late-onset GBS cases presenting with meningitis comparable to 5% of Early-Onset cases (Schuchat *et al.*, 1994).Presentations include seizures, irritability, and lassitude (Porta & Rizzolo, 2015). The use of IAP at labour has no result on LO presentations as in the case of EO-GBS disease (Berardi *et al.*, 2013). Presently, there are no operative interventions to stop Late-onset GBS disease.

Table 1: Risk factors associated with early and late-onset GBS disease

Early or Late-Onset	Risk Factors
Early-Onset Only	High concentration maternal colonization, Maternal GBS bacteriuria Lengthy rupture of membranes (≥18 hours), Perinatal fever (≥38°C) Inadequate levels of maternal GBS-specific serotype antibodies Caesarean section deliveries Past spontaneous abortion or stillbirth, Poor prenatal care Past GBS disease, Low birth weight IUFD devices Multiple vaginal examinations
Early and Late-Onset	Prematurity (<37 weeks gestation), Young maternal age (<20), UTI Black race Male sex

Source:(Pintye *et al.*, 2016; Puopolo *et al.*, 2019).

Risk Factors for GBS Colonization

A number of maternal, obstetric and neonatal risk factors have been reported to be linked to both maternal and neonatal GBS colonization. These

factors subsequently affect transmission pathway (vertical) of the bacteria and disease development in newborns. Most of these risk factors are interconnected (Table 1).

GBS is a significant perinatal pathogen. An estimated 21.7 million pregnant women carry this bacterium according to the first worldwide study on GBS and most of them are currently unidentified and thus not treated. It is predictable that one in every five pregnant women round the world is colonized with group B streptococcus, a leading yet avoidable cause of maternal and infant ill health worldwide (Lawn *et al.*, 2017).

The gastrointestinal tract is the main GBS reservoir and the source of vaginal colonization in women. Maternal colonization is a key risk factor for perinatal transmission of GBS and consequently early-onset disease in their infants (Melin, 2011; Sherman *et al.*, 2012). Interestingly, an appreciable proportion of women will not experience the phenomenon even though there is reoccurrence of colonization in subsequent pregnancies (Turrentine & Ramirez, 2008). Personal hygiene and sexual practices have been reported to increase the risk of vaginal colonization (Manning *et al.*, 2004). Other risk factors include culture background, use of tampons, lack of lactobacilli in the gastrointestinal flora and obesity (Edwards *et al.*, 2016; Le Doare & Heath, 2013). Several prevalence-based surveys have reported remarkable variations in GBS carriage rates among black women as compared to Caucasian women (Schuchat, 1998; Turrentine & Ramirez, 2008). The concentration of maternal vaginal colonization is in direct relationship with the risk of perinatal transmission and the possibility of EO-GBS disease (Edwards *et al.*, 2016).

Management of Infants at Risk for GBS Disease

Guidelines for the prevention of perinatal GBS disease also recommends management of newborn whose mothers received IAP (Fultz-Butts *et al.*, 2002). Neonates born to mothers who received IAP during labour especially in cases of chorioamnionitis, are to be evaluated and treated empirically irrespective of signs and symptoms suggesting GBS infection; clinical condition at birth, period of IAP before delivery, or gestational age (Fultz-Butts *et al.*, 2002; Puopolo *et al.*, 2019). Empirical treatment should be given (ampicillin and gentamicin) awaiting culture results. A total diagnostic evaluation comprising FBC/(CBC), blood culture, chest x-ray with evident documented respiratory signs and a spinal tap for bacteriological assessment.

Symptomatic infants born preterm, late preterm, or term should also be given a total diagnostic assessment and empiric antibiotic treatment. In symptomatic infants whose spinal tap were postponed because of medical instability, medication should continue beyond 48 hours, CSF should be obtained for cell count, biochemical and bacteriological evaluation (Nandyal, 2008; Puopolo *et al.*, 2019).

Asymptomatic preterm babies below 35-week gestation are required to be closely monitored for any remarkable change in vital signs like temperature imbalance, under feeding, respiratory distress, aponea, abdominal distension, frequent vomiting, or lethargy. Immediate reassessment and repetition of laboratory tests becomes necessary. Asymptomatic late preterm and term neonates at 35 gestational age or later whose mothers received IAP may need adequate assessment which is not different from those who are closely observed (Nandyal, 2008).

Laboratory Diagnosis of GBS.

Streptococcus agalactiae, a major cause of invasive newborn infections and an important bacterium in immunocompromised individuals. Detection of GBS colonization in pregnant women is key for IAP administration and consequently the prevention of colonization in their neonates (Raabe & Shane, 2019). Effective detection of the GBS colonization status of pregnant women is critical. Techniques that increase the recovery of group B Streptococcus are required. Reliable detection of GBS colonization before delivery is key for efficient application of IAP (Rosa-Fraile & Spellerberg, 2017). Time of specimen collection is crucial due to the transient nature of GBS infection during pregnancy. Diagnosis is usually by bacterial culture and isolation, serological or molecular techniques.

Specimen Collection

Urine, blood, amniotic fluid, cerebrospinal fluid (CSF), breast milk, rectal, vaginal, ears, nose, umbilicus swabs and sometimes bone marrow samples are normally collected for GBS detection depending on the type of infection and research being carried out (Guo *et al.*, 2018; Skoff *et al.*, 2009).

The CDC recommends swabbing both the vagina and the rectum for high probability of Group b Streptococcus recovery as compared to swabbing the vagina only (El Aila *et al.*, 2010; Slotved *et al.*, 2017b). Employing appropriate transport media like Amies, Stuart medium or STGG is very important to maintain the viability of GBS (da Gloria Carvalho *et al.*, 2010;Verani *et al.*, 2010). Refrigeration at ambient temperature or 4°C is highly recommended within 24 hours of collection if swab processing will delay since GBS viability decreases over time (Trotman-Grant *et al.*, 2012;

Verani *et al.*, 2010). Currently, flocked swabs incorporated with liquid transport media have been developed for collecting microbiological samples. These swabs have been fashioned to reduce the sticking of specimens and hence reported to remarkably maximize the recovery of GBS (Rosa-Fraile & Spellerberg, 2017; Trotman-Grant *et al.*, 2012).

Sample Processing

The CDC recommends the isolation of GBS from rectovaginal swabs by initial cultivation on a selective enrichment broth, such as Lim broth or TransVag (Todd-Hewitt broth incorporated with selective antibiotics) and then sub-culturing onto SBA for detecting GBS colonization in pregnant women and hence enrichment culture method (ECM) as the "Gold Standard" and currently the best GBS testing technique (El Aila *et al.*, 2010; Verani *et al.*, 2010). Cultivation and isolation of GBS on an enriched medium which usually takes 24-48 hours is necessary to improve its viability due to its fastidious nature.

In this technique, swabs are initially inoculated into broth (selective /enrichment media) containing antibiotics for overnight incubation to suppress the overgrowth of any other microorganisms present in the sample and enhance the multiplication of GBS. The Todd-Hewitt broth containing colistin (10 μg/ml) and nalidixic acid (Lim broth) or Todd-Hewitt broth containing gentamicin (8 μg/ml) and nalidixic acid (15 μg/ml) (TransVag broth) are examples of selective enrichment media recommended by the CDC (Verani *et al.*, 2010). Five percent (5%) sheep blood may be added to some of the enrichment media to increase the recovery rate of GBS. Subsequently,

sample is sub-cultured on a solid media (sheep blood agar) for 18-24 hours under anaerobic conditions (El Aila *et al.*, 2010).

Phenotypic Methods for the Identification of GBS

Majority of the clinical GBS isolates readily grow on SBA after incubation for 24hours showing as bulky colonies (3 to 4 mm in diameter) with slender zone of β-haemolysis (Whiley & Hardie, 2009). GBS β-haemolysis are hard to observe in some strains, though can be detected when colonies are separated from SBA. In several laboratories, colonies exhibiting characteristic GBS pattern are subjected to latex agglutination tests to determine the existence of the Lancefield group B antigen and when test is positive, colonies are GBS (Brigtsen, 2018; Rosa-Fraile & Spellerberg, 2017). This decision is founded on the existence of the Lancefield group B antigen only in the streptococcal species and very specific thus only *S. agalactiae* harbours this antigen.

Nevertheless, *Streptococcus porcinus*, which could possibly be found in the vagina also grows readily on BA as β-haemolytic colonies and cross-reacts with latex agglutination kits (Rosa-Fraile & Spellerberg, 2017). Hence, β-haemolytic colonies confirmed as GBS using latex agglutination tests needs further testing (Stoner *et al.*, 2011; Suwantarat *et al.*, 2015). The region of beta-haemolysis around S. *porcinus* colonies on SBA are typically wider and more pronounced as compared to that observed with *S. agalactiae* (Duarte *et al.*, 2005). Detection of the presence of the reddish polyenic pigment known as granadaene in GBS is another easy and simple technique to distinguish GBS from other β-haemolytic streptococcal species (Melin & Efstratiou, 2013; Spellerberg *et al.*, 2000).

Isolation of GBS by Chrome Agar

Chromogenic media are differential media which indicate the presence of GBS by the development of specific colours (El Aila *et al.*, 2010). Chromogenic media contain enzymatic substrates connected to indoxyl chromogens systems that are metabolized by enzymes in the target microorganisms resulting in the release of chromogens. Consequently, upon oxidation, an indigoid dye is formed and dimerization of indoxyl molecules in the presence of oxygen precipitates in the colonies resulting in characteristic brightly different colors (Orenga *et al.*, 2009; Rosa-Fraile & Spellerberg, 2017).

Chrom Agar is a selective differential medium incorporated with three chromogenic substrates used for the detection of GBS usually appearing as red colonies that are rounded and pearly after incubation under aerobic environments. In the presence of chrom agar, most bacterial species are either supressed with the target organism in colonies identifiable with a specific colour (e.g. violet, blue, and colourless). It can detect all GBS strains as well as non ß- haemolytic strains (El Aila *et al.*, 2010). Orange-coloured haemotytic colonies on StrepB Carrot Broth (SBCB) are an indication of the presence of GBS. Chromogenic media offer a rapid method for GBS identification but are neither 100% specific nor 100% sensitive. ChromID Strepto B agar (CA), Group B streptococcus differential agar (GBSDA) Granada Medium, Columbia CNA agar (CNA) and Northeast Laboratory GBS Screening Medium (NEL-GBS are types of chrom agar (El Aila *et al.*, 2010; Rosa-Fraile & Spellerberg, 2017)). Currently, there are no GBS-specific chromogens and this limits their specificity leading to possible

misidentifications. Bacterial species like *Enterococcus spp., Streptococcus bovis, S. pseudoporcinus, S. porcinus, S. salivarius, S. thoraltensis, S. anginosus, S. pyogenes,* and *Staphylococcus* spp., mostly present in rectovaginal samples can develop GBS-like colonies (El Aila *et al.*, 2010; Morita *et al.*, 2014; Stoner *et al.*, 2011). Thus, the use of chromogenic media in GBS detection requires additional confirmatory tests for suspected GBS-like colonies like molecular techniques, the latex agglutination test, CAMP test and the use of Granada media (El Aila *et al.*, 2010; Rosa-Fraile & Spellerberg, 2017). Also, confirmation of GBS-like colonies can be done using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) on chromogenic media colonies. However, this technique is occasionally hard to perform as a result of the presence of substances which may interfere with mass peak signals (Joubrel *et al.*, 2014; Rosa-Fraile & Spellerberg, 2017).

Immunological Assay

GBS strains can be identified reliably by slide agglutination or a capillary precipitation using commercially produced antisera targeting the group B Lancefield antigen; polysaccharide antigens in the cell wall which remains a significant routine identification technique for beta-hemolytic streptococci and also the basis for vaccine development (Rosa-Fraile & Spellerberg, 2017).Consequently, multitude of latex agglutination kits and immunoassays capable of identifying the antigen found only in GBS have been manufactured. Antisera against polysaccharide antigens (antisera) have also been developed for both identification and serotyping. Clumping on a slide or precipitation in a capillary tube with suspension of broth culture is an

indication of the presence of GBS and subsequently the corresponding serotype (Brigtsen *et al.*, 2015). However, *S. pseudoporcinus* cross-reacts with GBS grouping antisera and can lead to a false positive result in the identification of GBS (Stoner *et al.*, 2011). Generally, the use of these commercially available assays is hindered by their low sensitivities. In comparison with the GBS colonization rates reported for selective broth cultures and rapid immunoassays among women from rectovaginal swabs ranged from 4% to 37% (Picard & Bergeron, 2004);rapid antigen tests are solely appropriate to identify GBS in overnight cultures and severely colonized patients in selective broth (Picard & Bergeron, 2004). Several antigen tests kits have been manufactured to identify GBS directly from rectovaginal samples. Nevertheless, results from these antigen testing are hindered by low sensitivity and specificity (Rosa-Fraile & Spellerberg, 2017).

Nucleic Acid Testing Assays

Currently, CDC recommends prenatal screening for GBS by collecting and cultivating rectovaginal swabs between 35-37 weeks of pregnancy on a selective media (enriched) and subculturing on a blood-base agar (Kim *et al.*, 2018; Rosa-Fraile & Spellerberg, 2017; Verani *et al.*, 2019). However, the period 24-72 hours required during culture is time consuming to be relevant during labour. Multitude of studies have reported that using the culture method only in the detection of GBS may lead to a higher percentage of missed false-negatives (Mashouf *et al.*, 2014; Mousavi *et al.*, 2016; Rosa-Fraile & Spellerberg, 2017). There is the need for a more sensitive, rapid and accurate testing for GBS since chromogenic media also suffered similar limitations as

the culture method (Control & Prevention, 2016; Mousavi *et al.*, 2016; Rosa-Fraile & Spellerberg, 2017).

A number of DNA probes and nucleic acid amplification tests have been fashioned to decrease the turnaround time for the detection of GBS. Currently GBS can be identified directly with (NAATs) from swabs incorporated into PCR like the Smart Cycler, BD MAX system (Becton, Dickinson), and Xpert technology (Cepheid). The Illumigene system (Meridian Bioscience) identifies GBS from enrichment broth (Rosa-Fraile & Spellerberg, 2017). Real-time based PCR such as BD GeneOhm assay, GeneXpert GBS assay and Strep B assay have also been developed to identify GBS from clinical samples, enrichment broths, or after subculturing (Verani *et al.*, 2010).

The (molecular method) PCR has a short turnaround time as compared to the gold standard making it relevant for determining GBS colonization status around time of childbirth. However, the high cost of PCR equipment and reagents hinders its availability in most Medical laboratories.

Several gene targeting PCR assays specific for GBS have been developed. Nevertheless, a number of them are too complicated to be relevant for clinical use. In recent times, the advancement in molecular methods necessary for definite diagnostic purposes has significantly transformed the face of clinical microbiology. Most often, simple species diagnosis of GBS doesn't necessitate the use of NAATs. In the case of atypical isolates (colonies), sequencing the 16SrRNA gene and the *sodA* gene can help attain reliable species designation. (Ke *et al.*, 2000).

Probe hybridization systems (PHS) are assays that targets explicitly the ribosomal RNA of the GBS.The assay is appropriate for detecting GBS from overnight cultures in selective broth. Sensitivity and specificity of 94.7–100% and 96.9–99.5% respectively were estimated in comparing PHS with the CDC recommended culture. (Picard & Bergeron, 2004; Williams-Bouyer *et al.*, 2000). However, PHS (DNA) cannot substitute antenatal culture, which is still most accurate for detection of GBS carriers. (Huang *et al.*, 2019; Verani *et al.*, 2010).

Biochemical Test

Several biochemical tests have been developed for the phenotypic detection and isolation of GBS. The CAMP, catalase, hippurate, pyrrolidonyl arylamidase (PYR) tests are important presumptive test employed in identifying GBS in the absence of the serological tests (Picard & Bergeron, 2004). The characteristic appearance of GBS colonies (colonial morphology) and beta-haemolysis on 5% sheep blood agar are also key for phenotypic identification (Rosa-Fraile & Spellerberg, 2017; Whiley & Hardie, 2009).

CAMP Test

The cytolytic toxin produced by almost all clinical GBS isolates is also known as the CAMP (Christie, Atkins, Munch-Petersen) factor. The toxin is not haemolytic *per se*, even though it breaks down sheep RBCs by acting synergistically with sphingomyelinase produced by *Staphylococcus aureus*. This cytolysin is different from the GBS β-haemolysin (Phillips *et al.*, 1980). The CAMP test is done by streaking the suspected GBS isolate perpendicular to a known haemolytic *Staphylococcus aureus* strain on SBA. A positive CAMP test shows as a distinctive arrowhead of haemolysis in line at where

the two streaks(line) come into closeness (Winn, 2006). Nevertheless, the test is not 100% specific. Some organism like *S. porcinus* strains occasionally isolated from the vagina (Facklam *et al.*, 1995) and other group A streptococci also yield positive CAMP reactions (Facklam, 2002; Guo *et al.*, 2019).The *cfb* gene encoding the CAMP factor is utilized for the molecular identification of GBS due to its presence in most GBS isolates (Rosa-Fraile & Spellerberg, 2017). The ability of most GBS strains to breakdown sodium hippurate to produce benzoic acid and glycine is the basis for the hippurate hydrolysis test (Winn, 2006).

Antimicrobial Susceptibility Pattern

Implementation of IAP was first studied in the 1980s (Verani *et al.*, 2010). Administration of antimicrobial prophylaxis to positive women (GBS) around labour decreases the possibility of EO-neonatal sepsis (Capanna *et al.*, 2013; Clifford *et al.*, 2011; Hong *et al.*, 2019).

Extensive usage of antibiotics in several medical settings as well as their recognized effectiveness in reducing early-onset neonatal GBS infections has also brought to bear concerns on the emergence of antibiotic resistance among GBS isolates (Quiroga *et al.*, 2008;Verani *et al.*, 2010). GBS infections still remain curable with penicillin especially for the prevention of early-onset GBS disease and also for the treatment GBS-related diseases. GBS has been studied to be susceptible to a number of β-lactams (Raabe & Shane, 2019). Conversely, few isolates with resistant to penicillin have been discovered in USA and Japan. This decrease in susceptibility is due to a point mutation discovered in the GBS pbp2x gene (Melin, 2011; Seki *et al.*, 2015). By reason of the increasing problem of antibiotic resistance among GBS isolates, it is

recommended that susceptibility testing be performed on the other antibiotics apart from those frequently administered for control measures so they can be used and monitored as alternatives (Piccinelli *et al.*, 2015;Quiroga *et al.*, 2008). Frequent susceptibility testing is also recommended for the routine drugs for GBS treatment to monitor their efficacy.

Prevention /Treatment of GBS Infection

Several preventive measures have been established to reduce the burden of invasive diseases on newborns (Collin *et al.*, 2019). Intrapartum antibiotic prophylaxis (IAP) is key to reducing infant GBS infection and the occurrence of EO-GBS disease. Intravenous penicillin administered every 4 hours during labor is the recommendation for prevention of vertical transmission (Raabe & Shane, 2019). IAP reduced EO disease among newborns born from 86 to 89% (Medugu *et al.*, 2018; Raabe & Shane, 2019). In developed countries where IAP is a policy, risk of EOD by vertical transmission is reduced to 0.03% whereas in under-developed settings rates as high as 1.1% are estimated among neonates born to colonized mothers (Raabe & Shane, 2019). Currently, administration of IAPs are dependent on risk and culture-based models worldwide and adherence protocols vary geographically (Guo *et al.*, 2018). The United Kingdom recommends the risk-based algorithms to monitor intrapartum antibiotics instead of routine GBS cultures for pregnant women while the United States, New Zealand, Australia and Canada recommend worldwide GBS screening cultures at 35-37 weeks (Chen *et al.*, 2018; Raabe & Shane, 2019).

Le Doare *et al.* (2017) published the variations in adherence to IAP guidelines which varied widely: they estimated that adherence among settings

with worldwide culture-based screening protocols ranged from 20 to 95% but in countries with risk-based screening programs ranged between 10 to 50%.

Currently, the IAP guidelines recommends culture of rectovaginal (swabs) of all 35- 37 weeks pregnant women for GBS colonization assessment and administer IAP to pregnant women with positive GBS cultures, GBS bacteriuria or with a past neonatal history with EO- GBS disease (Le Doare *et al.*, 2017; Verani *et al.*, 2010).

The inception of IAP in the US has reduced the incidence of invasive EO-GBS disease from 1.7 cases/1,000 live births in 1993 to 0.76 to 0.77 cases/1,000 live birth in 2005 to 2008 and a projected 0.21 cases/1,000 live births in 2015 (Ke *et al.*, 2000; Raabe & Shane, 2019).

Vaccination of pregnant women in the third trimester against GBS is also another strategy under development to curb the disease in mothers and their neonates. Currently, a number of Streptococcus agalactiae vaccine candidates like the trivalent protein-polysaccharide conjugate vaccine targeted at serotypes Ia, Ib, and III are under development (Fultz-Butts *et al.*, 2002; Raabe & Shane, 2019).

GBS remains susceptible to penicillin for intrapartum prophylaxis and treatment (Raabe & Shane, 2019) even though Seki *et al.* (2015) reported reduced penicillin susceptibility among some isolates in Japan. Beta-lactams like ampicillin, first, second and third generation cephalosporins and carbapenems with varied levels of activities have also been found to be effective against GBS infections. In pregnant women assessed to be allergic to penicillin with increased risk of anaphylactic shock, clindamycin, erythromycin, fluoroquinolones or vancomycin may be given although

clindamycin resistance have been reported among some isolates (Guo *et al.*, 2019; Piccinelli *et al.*, 2015). Several studies have reported increasing levels of GBS resistance with clindamycin, erythromycin, fluoroquinolones and vancomycin and should be given only if penicillin or cephalosporin is inappropriate and susceptibility pattern of isolate is known (Guo *et al.*, 2019; Piccinelli *et al.*, 2015; Raabe & Shane, 2019).

Newborns with EO-GBS disease are also treated with intravenous antibiotics as mothers. However, in the selection of appropriate antibiotics, the importance of antimicrobial susceptibility testing of GBS isolates cannot be overemphasized.

Chapter Summary

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

Group B *Streptococcus (Streptococcus agalactiae)* is a Gram-positive, encapsulated spherical organism occurring typically in short chains but also in pairs and singles. GBS is fastidious requiring enriched media for growth, facultative anaerobes, catalase-negative, non-sporing and non-motile. GBS is ubiquitous, and naturally occurring in the gastrointestinal tract and may inhabit the genitourinary tract persistently or occasionally in about thirty percent of women. Based on the type-specific polysaccharide capsule, *Streptococcus agalactiae* can be subdivided into 10 different serotypes (1a, 1b, II, III, IV, V, VI, VII, VIII, and IX). Among these, the most occurring serotypes that colonize pregnant women and result in neonatal infections

include Ia, Ib, III, VI, and V. GBS is not restricted to pregnant women. This pathogen can cause intense invasive disease in neonates. Disease manifesting within first week of life is known as early-onset. Late-onset disease presents within 7-89 days of life.

In Ghana, studies conducted in the Greater Accra region by Enweronu-Laryea *et al.* (2011) in a tertiary facility and Slotved *et al.* (2017b) in two health facilities revealed maternal GBS colonization rates of 19% and between 25.5% to 28.0% respectively. Another study by Vinnemeier *et al.* (2015) in the Ashanti region revealed 18.1% and 23.1% for rural Pramso and urban Kumasi respectively with an overall prevalence of 19.1% among study participants (519 pregnant women). Laboratory diagnosis including cultivation and isolation, phenotypic, biochemical and molecular techniques for GBS identification were reviewed.

CHAPTER THREE

METHODOLOGY

Chapter Introduction

The study was a hospital-based cross-sectional study aimed at determining the prevalence of Group B streptococcus among parturients and their neonates, with their socio-demographic and clinical features associated with GBS colonization and compare the diagnostic value of culture method and PCR assays. A total of 301 swabs; 150 vaginal swabs and 151 superficial swabs were collected from parturients and their neonates respectively for the study. A description of the study design, study sites, vaginal and superficial swab collection, as well as identification, biochemical, phenotypic and molecular tests used in the detection of the GBS have been explained. Inclusion and exclusion criteria were well defined.

Study Design

The study design employed was a facility-based descriptive cross-sectional study with the objective of determining the prevalence of Group B streptococcus among parturients and their neonates, socio-demographic and clinical factors of the parturients and their neonates, risk factors linked with GBS colonization and compare the diagnostic value of culture method and PCR assays by determining the sensitivity, specificity, positive and negative predictive values in the detection of GBS among participants.

Study Site

The study was conducted between June 2019 and August 2019 at the delivery suite of the Cape Coast teaching hospital (CCTH). Convenient sampling method was employed to collect vaginal and superficial swabs from

women in labour and their neonates respectively. Laboratory work was done in the CCTH. The Cape Coast Metropolis is the capital of the Central Region and one of the oldest districts in Ghana covering an area of 122 square kilometers and is the smallest metropolis in the country and it is located on longitude 1° 15'W and latitude 5°06'N. The Metropolis is bounded on the south by the Gulf of Guinea, west by Komenda Edina Eguafo /Abrem Municipal, east by the Abura Asebu Kwamankese District and north by the Twifo Hemang Lower Denkyira District.

The population of the Metropolis according to 2010 population and housing census stands at 169,894 with about 82,810 males and about 87,084 females. The Metropolis has two main hospitals; the Metropolitan hospital and the CCTH. The CCTH which was the collection site for this study is located in the northern part of Cape Coast. It is bounded on the north by Abura Township, on the south by Pedu Estate / 4th Ridge, Nkanfua on the East and Abura / Pedu Estate on the West. The facility, formerly the Regional Hospital was established in August, 1998 and served as a referral centre for the entire region. With the inception of School of Medical Sciences in the University of Cape Coast, the hospital was upgraded to a Teaching Hospital in 2013. The CCTH is a 400-bed capacity facility and the main referral centre for almost all health facilities in the Central Region. In 2019, the total number of pregnant women that attended antenatal clinic at the facility was estimated at 9,419 with 3021 deliveries recorded (CCTH, 2019).

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 consents were obtained from individuals who participated in the study. Participants were allowed to willingly agree to participate in the study without coercion and maximum precaution was taken not to harm participant or her new-borns.

Sample Size

The minimum sample size of parturient that the study intended to screen for GBS carriage was calculated with the formula;

N= Z2 (P) (1-P) / (ERROR) 2

With a prevalence from a previous study on GBS carriage in Ghana estimated at 25.5% (Slotved *et al.*).

A 5% allowable error, ERROR, will be used.

Where Z, 1.96 is the standard score for the confidence interval of 95%

Sample size, N = $\frac{(1.96)^2\ (0.255)\ (1-0.255)}{(5/100)^2}$ = 285

In all, 301 swabs were collected; 150 vaginal swabs from parturients and 151(set of twin) superficial swabs from neonates after considering critical factors like cost and time.

Inclusion and Exclusion Criteria

- Pregnant women in labour who understood and consented to be part of the study.
- Neonates whose mothers gave consent and were delivered per vagina were included in the study.
- Parturients who had planned caesarean section were excluded.

Sampling

In all, 150 parturients and 151 neonates delivered at the delivery suite of the CCTH were captured in this study. A total of 150 vaginal swabs and

151 superficial swabs (skin, ear, mouth and nose) were collected from informed and consenting parturients and their neonates respectively. One sterile swab was used to collect swabs from mother and one sterile swab for neonate; 1) swab from lower vagina of parturient and 2) swab from skin, ear, nose, and mouth of neonates immediately after delivery. Swabs from mothers (parturients) were taken by rotating the swab against the vaginal wall at the middle-portion of the arch and swab was carefully removed preventing contamination with flora from the introitus and vulva. Swabs from neonates were taken by midwives who delivered neonates at second-stage rooms after being taken through the appropriate instructions (Guo *et al.*, 2018; Verani *et al.*, 2010).

Demographic information such as age, educational level, employment status, and recent use of antibiotics were obtained using a questionnaire. Clinical data such as parity, gravidity, gestational age, past obstetric history, past gynaecological history, past neonatal history, present neonatal history and co-morbidities were also gathered from the parturients. Birth weight, APGAR scores, Status of new-borns at birth were also recorded.

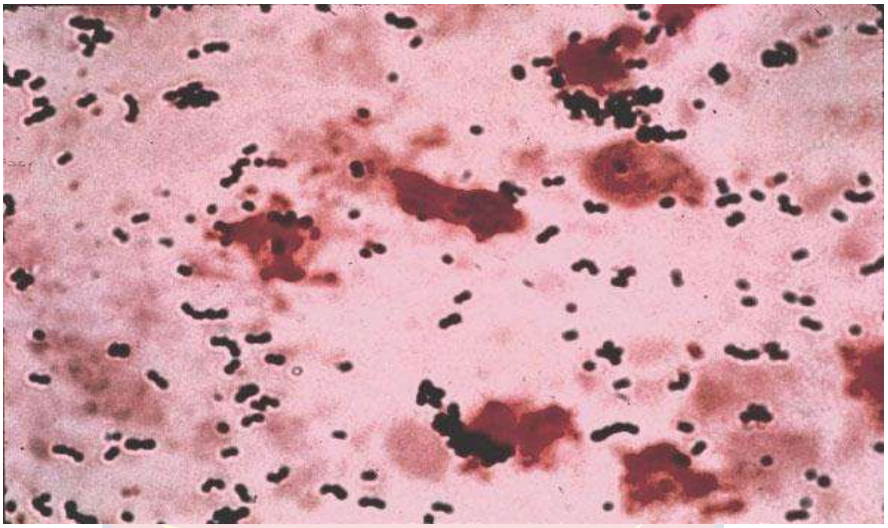
Specimen processing procedures and culture

Vaginal swabs from women in labour (parturients) and superficial swabs from the external ear, nose, skin, mouth and umbilical area of their neonates were collected and placed in STGG, well labelled and transported to the CCTH laboratory within an hour. Samples were vortexed to loosen bacteria from swab into STGG solution to improve viability (da Gloria Carvalho *et al.*, 2010).

The swabs inoculated into STGG broth were directly seeded onto 10 % sheep blood agar (SBA) plates prepared under aseptic conditions and tested for sterility (Organization, 2006). SBA was made selective with gentamicin (8 μg/ml), nalidixic acid (Sigma Aldrich) (15 μg/ml) and incubated overnight at 37°C in a carbon dioxide jar. Plates examined with no growth were re-incubated for 18-24 hours. Plates with significant growth were examined for characteristic GBS colonial morphology typical and haemolytic patterns on the SBA. GBS-like colonies were Gram stained (positive) and tested for *catalase* (negative) as described by Richard Facklam and Elliott (1995). All 301 swabs in STGG were tested for the presence of GBS with four gene assays using multiplex PCR (Imperi *et al.*, 2010).

Gram staining

Gram staining was performed on suspected colonies by making smears of the colonies on different slides. Smears were heat-fixed to prevent smears from washing off during subsequent staining steps. Smears were flooded with crystal violet stain for 30 seconds and was washed off with distilled water. A mordant, Gram's iodine was added to the smear. Decolourization of the smear was done rapidly after 30seconds with acetone-alcohol. Lastly smear was flooded with a secondary stain, safranin for 30 seconds to 1 minute and stain was washed off with distilled water. Smears were examined microscopically under oil immersion objective for Gram-positive bacteria that stained blue to purple and Gram-negative bacteria that stained red to pink as shown in figure below in (figure 2):



Gram Stain Reaction of GBS
Source: ASM Microbe Library CDC, 2010

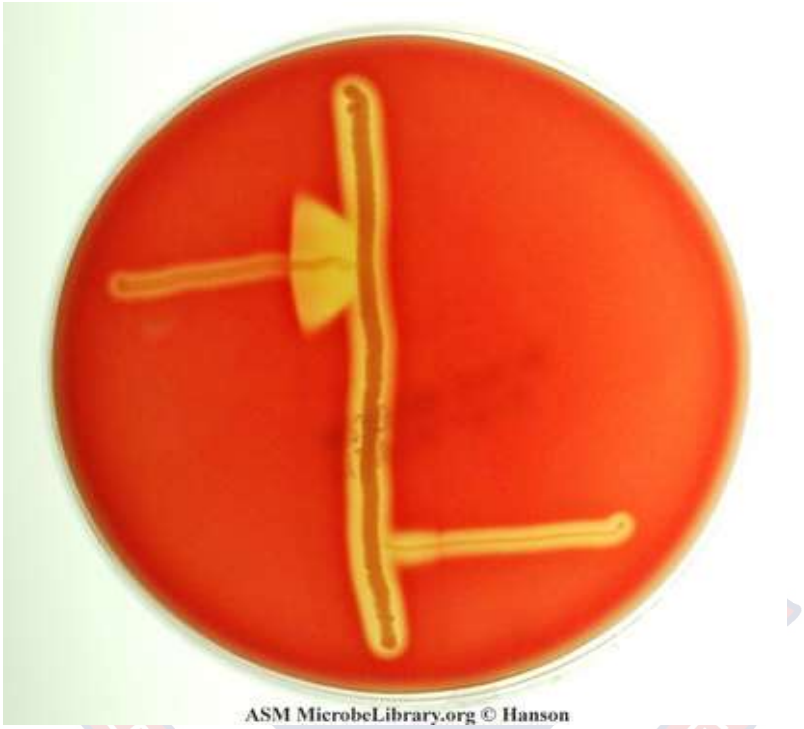
Catalase test

Catalase test was necessary to differentiate *Streptococcus spp.* from *Staphylococcus spp.* since both were Gram-positive cocci and stains blue to purple. Identification of the enzyme, catalase found in some organisms and its ability to mediate the breakdown hydrogen peroxide, into oxygen and water is the main principle behind this test. Bacterial colonies found to be gram-positive were picked with inoculating loop and immersed in a drop of 3% hydrogen peroxide on a glass slide. The evolution of bubbles (oxygen gas) was an indication of the existence of the catalase enzyme and the non-appearance of bubbles showed catalase negative. The positive control for the test was a known staphylococcus aureus and the negative control was *Streptococcus agalactiae* strain (ATCC 12386).

CAMP test

The principle of this test is based on an extracellular protein produced by GBS called the CAMP factor that works in synergy with *Staphylococcus aureus* β-toxin to cause enhanced lysis of red cells. The suspected GBS was

streaked perpendicular to a known *Staphylococcus aureus* on SBA without both streaks directly touching each other. Plates were incubated overnight at 37°C. An "arrowhead" between the junction of growth of *S. aureus* and GBS is an indication of a positive test for CAMP reaction appearing as erythrolysis (figure3). The absence of an arrowhead haemolysis indicated that the isolate tested was not GBS.



Source: ASM Microbe Library CDC, 2010

DNA Extraction

Swabs were incubated for 15-18hrs in Todd Hewitt selective medium at 37°C. Incubated swabs were vortexed and 1 ml of sample then pipetted into 2 ml Eppendorf tubes. The sample was centrifuged at 9000 rpm for 3 minutes. Additionally, 700 µl of the supernatant was discarded. Five hundred microlitres of PBS was added to the sample, vortexed and centrifuged at 9000 rpm for 3 minutes. All the supernatant was discarded (about 800 µl of supernatant). Approximately 200 µl of 1×TE buffer was added to the sample

and vortexed till pellets dissolved in the buffer. Using a heat block, the buffer was boiled for 10 minutes at 100°C and frozen at -20°C for 10 minutes. The buffer was centrifuged at 14000 rpm for 10 minutes and 100 µl of the supernatant pipetted and discarded. The deposit will then serve as the DNA sample for PCR.

PCR Testing

A primer mix was prepared consisting of the 16srRNA, cfb, atr and ScpB primers. The Multiplex PCR reaction volume (25 µl) was prepared as follows: 12.5 µl of 2x Gotaq master mix (Promega Corporation, USA), 6.3 µl Nuclease free water,0.6 µl of the forward primer mix, 0.6 µl of the reverse primer mix and 5 µl of the DNA sample. The DNA samples as well as a positive control (a sample including DNA from *Streptococcus agalactiae*) and a negative control (a sample without template) were amplified by an initial denaturation step for five minutes at 94° C, followed by 35 cycles of 94° C for 45 seconds, 60° C for 60 seconds, and 72° C for one minute, and a final cycle of 72° C for seven minutes in a thermal cycler. After amplification, 5 µl of each amplification product was analysed by electrophoresis on a 2% (w/v) agarose gel, stained with Gel red (Huang *et al.*, 2010).

Table 2: Primers selected for *Streptococcus agalactiae* identification

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

Source: (Mashouf *et al.*, 2014; Mousavi *et al.*, 2016)

Storage

Isolates obtained were stored at -80 ºC in STGG broth at the Biomedical Science Department, University of Cape Coast.

Quality Control

Laboratory work done by strict adherence to protocols and use of quality assured consumables. Control strain (ATCC 12386) was used to monitor the strength of all media and reagents used during sample collection and laboratory work. Stored isolates were sub-cultured before use.

Data Analysis

Data was entered into Microsoft Office Excel, and results analysed by means of IBM SPSS Version 25. Proportions of variables were expressed in percentages and Multivariate regression analysis reporting odds ratio was used for risk analysis. Using GraphPad Prism Version 8, sensitivity analysis was done. Data was presented in tables and graphs. Level of significance was associated with P value less than 0.05 ($p < 0.05$).

Chapter summary

A facility-based cross-sectional study was conducted in CCTH between June and August, 2019. 150 Vaginal and 151superficial swabs were collected from parturients and their neonates respectively. 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 GBS was conducted on all 301 samples with *atr, scpB, cfb* and 16SrRNA gene assays at the Bacteriology laboratory of Noguchi Memorial Institute of Medical Research, University of Ghana.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

Chapter Introduction

The purpose of this study is to determine the prevalence and risk factors associated with GBS colonization among parturients and their neonates and compare the diagnostic value of culture and PCR assays. The result section is furnished with presentation of the 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.

Description of Study Population

In all total of 301 participants were involved in the study; 150 of them were pregnant women in labour (parturients); 151 of them were their newborns (a set of twins). A total of 301 swabs; 150 vaginal swabs were sampled from the lower vagina and 151 superficial swabs from parturients and their newborns respectively. The ages of the parturients in the study ranged between 15 and 44 years with the mean age of 27.08 SD± 6.40 years.

Table 3: Characterization of the Demographic Information of the Participants across their Age Groups

Variable	Age in Years			Total
	≤24yrs n(%)	25-32yrs n(%)	≥33yrs n(%)	n(%)
Ethnicity				
Akan	38(69.1)	40(61.5)	25(83.3)	103(68.7)
Ga	5(9.1)	6(9.2)	1(3.3)	12(8.0)
Ewe	10(18.2)	10(15.4)	1(3.3)	21(14.0)
Nzema	1(1.8)	3(4.6)	0(0.0)	4(2.7)
Others	1(1.8)	6(9.2)	3(10.0)	10(6.7)
Education Level				
Non-Formal	4(7.3)	5(7.7)	5(16.7)	14(9.3)
Primary	10(18.2)	9(13.8)	2(6.7)	21(14.0)
JHS	29(52.7)	24(36.9)	10(33.3)	63(42.0)
SHS	11(20.0)	18(27.7)	9(30.0)	38(25.3)
Tertiary	1(1.8)	9(13.8)	4(13.3)	14(9.3)
Occupation				
Employed	27(49.1)	7(10.8)	5(16.7)	39(26.0)
Unemployed	28(50.9)	58(89.2)	25(83.3)	111(74.0)

The Akans 103(68.7%) were the major ethnic group that participated, followed by the Ga's 16 (10.7%) and the least ethnic group being the Nzema's 6 (4.0%). Also, irrespective of the age group the Akans dominated. Among the Ewe's, participants were mainly between 25-32yrs, i.e. 10/14 of the parturients. Most of the parturients had Junior High School 63(42.0%) followed by senior high school education, 38(25.3%) level of education. About 14(9.3%) had no formal education. Participants were mostly educated up to JHS level and unemployed, irrespective of the age group (Table 3).

Table 4: Characterization of the Clinical Information of the Parturients by Age.

Variable	Age in Years			Total n(%)
	≤24yrs n(%)	25-32yrs n(%)	≥33yrs n(%)	
Recent Antibiotic Usage				
≤2 Weeks	8(14.5)	14(21.5)	3(10.0)	25(16.7)
>2 Weeks	18(32.7)	20(30.8)	13(43.3)	51(34.0)
None of the Above	29(52.7)	31(47.7)	14(46.7)	74(49.3)
Gravidity				
≤3	52(94.5)	44(67.7)	10(33.3)	106(70.7)
4-6	3(5.5)	19(29.2)	18(60.0)	40(26.7)
≥7	0(0.0)	2(3.1)	2(6.7)	4(2.7)
Parity				
≤2	52(94.5)	49(75.4)	15(50.0)	116(77.3)
3-4	3(5.5)	15(23.1)	12(40.0)	30(20.0)
≥5	0(0.0)	1(1.5)	3(10.0)	4(2.7)
Gestational Age				
Preterm (≤37 weeks)	12(21.8)	4(6.2)	2(6.7)	18(12.0)
Term (38-42 weeks)	43(78.2)	59(90.8)	27(90.0)	129(86.0)
Post-term (>42 weeks)	0(0.0)	2(3.1)	1(3.3)	3(2.0)
Past Obstetrics History				
PROM	3(5.5)	3(4.6)	5(16.7)	11(7.3)
Preterm Birth	5(9.1)	6(9.2)	7(23.3)	18(12.0)
Puerperal Sepsis	1(1.8)	1(1.5)	1(3.3)	3(2.0)
IUFD	1(1.8)	2(3.1)	2(6.7)	5(3.3)
Chorioamnionitis	2(3.6)	3(4.6)	2(6.7)	7(4.7)
IUGR	0(0.0)	1(1.5)	0(0.0)	1(0.7)
None of the Above	44(80.0)	51(78.5)	16(53.3)	111(74.0)
Past Gynecological History				
Spontaneous Miscarriage	5(9.1)	13(20.3)	7(23.3)	25(16.8)
Induced Abortion	0(0.0)	3(4.7)	1(3.3)	4(2.7)
UTI	23(41.8)	28(43.8)	17(56.7)	68(45.6)
PID	6(10.9)	5(7.8)	4(13.3)	15(10.1)
Candidiasis	16(29.1)	11(17.2)	5(16.7)	32(21.5)
HIV	1(1.8)	3(4.7)	2(6.7)	6(4.0)
Hepatitis B	5(9.1)	7(10.9)	2(6.7)	14(9.4)
Syphilis	7(12.7)	9(14.1)	5(16.7)	21(14.1)
None of the Above	6(10.9)	8(12.5)	1(3.3)	15(10.1)
Past Neonatal History				
Neonatal Sepsis	0(0.0)	2(3.1)	0(0.0)	2(1.3)
Early Neonatal Death	0(0.0)	6(9.2)	4(13.3)	10(6.7)
Low Birth Weight	2(3.6)	1(1.5)	2(6.7)	5(3.3)
NICU Admission	6(10.9)	7(10.8)	8(26.7)	21(14.0)
None of the Above	48(87.3)	50(76.9)	17(56.7)	115(76.7)
Comorbidities				
Pregnancy with Chronic Hypertension	1(1.8)	0(0.0)	0(0.0)	1(0.7)
Gestational Hypertension	0(0.0)	4(6.2)	6(20.0)	10(6.7)
Diabetes in Pregnancy	0(0.0)	1(1.5)	0(0.0)	1(0.7)

Gestational Diabetes	1(1.8)	0(0.0)	1(3.3)	2(1.3)
Sickle Cell Anemia	2(3.6)	1(1.5)	0(0.0)	3(2.0)
Asthma	2(3.6)	5(7.7)	0(0.0)	7(4.7)
None of the Above	49(89.1)	54(83.1)	23(76.7)	126(84.0)

PID: Pelvic Inflammatory Disease, UTI: Urinary Tract Infection, IUFD: Intrauterine Foetal Death, PROM: Prolonged Rupture of Membrane, NICU: Neonatal Intensive Care Unit.

In Table 4, 74(49.3%) participants regardless of their age had not used any form of antibiotics. Participants in the age groups ≤24yrs and 25-32yrs who had taken antibiotics in >2 weeks were 18(32.7%) and 20(30.8%), respectively. The least gravidity observed was ≥7, 4(2.7%) and none of the participants within the age ≤24yrs had gravidity ≥7. The most recorded gravidity was ≤3, 52(94.5%), 44(67.7%) and 10(33.3%) among the age group ≤24yrs, 25-32yrs and ≥33yrs respectively. The pattern observed for gravidity is similar to that of parity. By their past obstetric history, most of the participants had no history 111(74.0%) whiles the most occurring obstetric history was preterm birth, 18(12.0%). Preterm birth was high among those with history regardless of the age group. The next major observed obstetric history was PROM 11(7.3%). On UTI, 68(45.6%) was the most prevalent gynaecological history irrespective of their age group followed by candidiasis, 32(21.5%), spontaneous miscarriage 25(16.8%) and syphilis, 21(14.1%) in descending order. Most of the participants had no past neonatal history or comorbidities. However, of the participants with neonatal history, NICU admissions were the most observed 21(14.0%) and of the comorbidities, gestational hypertension was the most observed 10(6.7%).

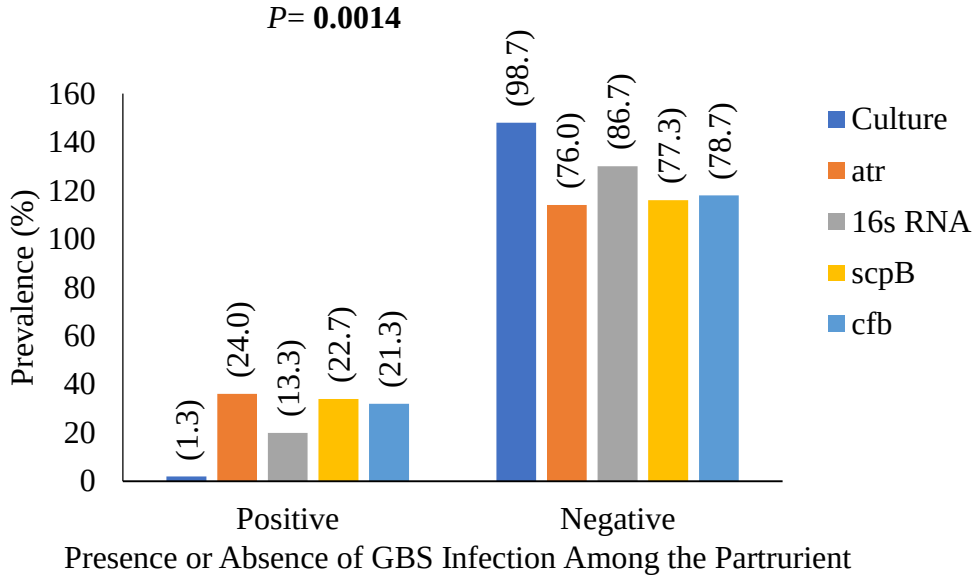


Figure 4: Prevalence of GBS Observed from Culture and the Various PCR Techniques Among the Parturients

The prevalence of GBS infection observed among parturients was 36(24.0%), 34(22.7%), 32(21.3%), 20(13.3%), and 2(1.3%) for *atr* gene, *ScpB* gene, *cfb* gene,16s rRNA gene, and culture technique respectively. A comparison of the infected and uninfected concerning the testing methods used was significant $p<0.05$. (Figure 4)

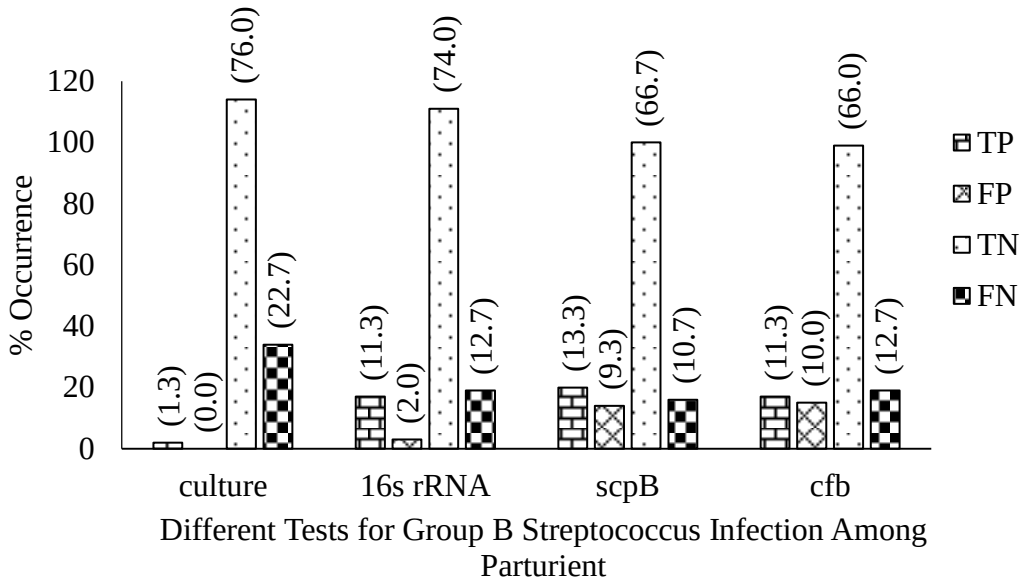


Figure 5: Comparing Diagnostic Performance of Culture and Other PCR assays to the atr PCR test among the Parturients.

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

Thirty-six (36) samples were positive for the atr gene PCR assay. The results obtained for atr gene PCR assay were compared to the results obtained for other tests, i.e. culture, 16s rRNA, scpB, and cfb PCR method. The true positive result determined for culture, 16s rRNA, scpB and cfb were 2(1.3%), 17(11.3%), 20(13.3%) and 17(11.3%) respectively. The true negatives for the culture, 16s rRNA, scpB and cfb were also 114(76.0%), 111(74.0%), 100(66.7%) and 99(66.0%) respectively. (Figure 5).

Table 5: Sensitivity, Specificity, PPV, NPV and Likelihood ratio of the Various Tests using atr PCR Test as the Standard (Parturient)

PCR	Sensitivity		Specificity		PPV		NPV		Likelihood	*P*
Test	%	95% CI	%	95% CI	%	95% CI	%	95% CI	Ratio	
Culture	5.6	0.01-0.18	100	0.96-1.00	100	0.78-1.00	0.77	0.69-0.83	-	0.056
16s rRNA	47.2	0.32-0.62	97.4	0.93-0.99	85.0	0.64-0.94	85.4	0.78-90	17.9	**<0.0001**
scpB	55.6	0.39-0.71	87.7	0.80-0.92	58.8	0.42-0.73	86.2	0.78-0.91	4.5	**<0.0001**
Cfb	47.2	0.32-0.62	86.8	0.79-0.91	53.1	0.36-0.69	84.0	0.76-0.89	3.6	**<0.0001**

PPV= Positive Predictive Value, NPV= Negative Predictive Value, 95%CI= 95% Confidence Interval, *P*= P-value (<0.05 implies statistically significant). Sensitivity and specificity analysis were done through contingency tables and the p-value determined by Fisher's exact test.

Table 5 above shows the sensitivity, specificity, negative predictive value, positive predictive value and the likelihood ratio of the various tests using the atr gene as the standard. All the tests recorded specificity above 85.0%. Sensitivity however was 5.6% for culture, 47.2% for 16s rRNA gene, 55.6% for scpB and 47.2% for cfb gene. 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 According to atr PCR Test of the Parturients

Variable	Infection Status		Total	OR	P
	Positive	Negative		(95% CI)	
Recent Antibiotic Usage					
≤2 Weeks	5(13.9)	20(17.5)	25(16.7)	0.68(0.22-2.04)	0.486
>2 Weeks	11(30.6)	40(35.1)	51(34.0)	0.74(0.32-1.72)	0.488
None of the Above	20(55.6)	54(47.4)	74(49.3)	1	-
Gravidity					
≤3	24(66.7)	82(71.9)	106(70.7)	0.88(0.09-8.83)	0.912
4-6	11(30.6)	29(25.4)	40(26.7)	1.14(0.11-12.14)	0.915
≥7	1(2.8)	3(2.6)	4(2.7)	1	-
Parity					
≤2	28(77.8)	88(77.2)	116(77.3)	0.96(0.10-9.95)	0.968
3-4	7(19.4)	23(20.2)	30(20.0)	0.94(0.91-0.08)	0.941
≥5	1(2.8)	3(2.6)	4(2.7)	1	-
Gestational Age					
Preterm (≤37 weeks)	2(5.6)	16(14.0)	18(12.0)	2019779.11(441192.13-9246555.84)	**<0.0001**
Term (38-42 weeks)	34(94.4)	95(83.3)	129(86.0)	5782946.50(5782946.50-5782946.50)	-
Post term (>42 weeks)	0(0.0)	3(2.6)	3(2.0)	1	
Past Obstetrics History					
Prom	5(13.9)	6(5.3)	11(7.3)	0.11(0.05-1.95)	0.132
Preterm Birth	5(13.9)	13(11.4)	18(12.0)	0.29(0.02-4.23)	0.362
Puerpersal Sepsis	0(0.0)	3(2.6)	3(2.0)	509825.05(0.00-0.00)	0.993
IUFD	0(0.0)	5(4.4)	5(3.3)	1556481(0.00-0.00)	0.989
Chorioamnionitis	2(5.6)	5(4.4)	7(4.7)	0.265(0.01-5.62)	0.394
IUGR	0(0.0)	1(0.9)	1(0.7)	509825.06(509825.06-509825.06)	
None of the Above	26(72.2)	85(74.6)	111(74.0)	0.28(0.01-5.74)	0.409

Past Gynecological History					
Spontaneous Miscarriage	9(25.7)	16(14.0)	25(16.8)	0.35(0.12-1.02)	0.054
Induced Abortion	2(5.7)	2(1.8)	4(2.7)	0.25(0.03-2.17)	0.208
UTI	18(51.4)	50(43.9)	68(45.6)	0.80(0.29-2.20)	0.668
PID	1(2.9)	14(12.3)	15(10.1)	3.61(0.38-34.67)	0.266
Candidiasis	8(22.9)	24(21.1)	32(21.5)	0.79(0.25-2.46)	0.680
HIV	1(2.9)	5(4.4)	6(4.0)	2.29(0.20-25.83)	0.504
Hepatitis B	5(14.3)	9(7.9)	14(9.4)	0.48(0.13-1.79)	0.271
Syphilis	7(20.0)	14(12.3)	21(14.1)	0.46(0.14-1.46)	0.185
None of the above	2(5.7)	13(11.4)	15(10.1)	1.27(0.19-8.35)	0.805
Past Neonatal History					
Neonatal Sepsis	0(0.0)	2(1.8)	2(1.3)	1	-
Early Neonatal Death	1(2.8)	9(7.9)	10(6.7)	2.63(0.12-55.77)	0.536
Low Birth Weight	2(2.8)	3(2.6)	5(3.3)	0.41(0.016-10.29)	0.585
NICU Admission	8(22.2)	13(11.4)	21(14.0)	0.34(0.02-7.86)	0.501
None of the above	26(72.2)	89(59.3)	115(76.7)	0.74(0.03-20.26)	0.860
Comsorbidities					
Pregnancy with Chronic Hypertension	0(0.0)	1(0.9)	1(0.7)	2.680E-8(2.680E-8-2.680E-8)	-
Gestational Hypertension	3(8.3)	7(6.1)	10(6.7)	1.37(0.33-5.64)	0.661
Diabetes in Pregnancy	0(0.0)	1(0.9)	1(0.7)	2.680E-8(2.680E-8-2.680E-8)	-
Gestational Diabetes	0(0.0)	2(1.8)	2(1.3)	2.680E-8(0.00-0.00)	0.998
Sickle Cell Anemia	1(2.8)	2(1.8)	2(1.3)	1.60(0.14-18.27)	0.705
Asthma	2(5.6)	5(4.4)	7(4.7)	1.28(0.24-6.94)	0.775
None of the Above	30(83.3)	96(84.2)	126(84.0)	1	

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)

From Table 6, according to atr gene PCR Test of the Participants. Except for the gestational age ≤37 weeks, none of the regression analysis yielded a significant result. Considering the use of antibiotics, participants who had no history of antibiotic usage were high in both the infected and uninfected participants. The risk of those who had taken antibiotics ≤2 weeks being infected using the no antibiotic usage as a reference was 0.68, 95%CI (0.22-2.04), P>0.05. Concerning gravidity, parity and gestational age, both the infected and uninfected participants were mostly in ≤3 gravidity group, ≤2 parity group and 38-42week gestational age group. None of the participants at >42 weeks had the infection. Majority of both the infected and the uninfected were participants with no obstetric history, 26(72.2) and 85(74.6) respectively. Considering the infected with obstetric history, participants with PROM and preterm birth in their history predominated 5(13.9) respectively. While none of the infected participants recorded in their obstetric history puerperal sepsis, IUFD and IUGR. With their gynaecological history, majority of both the infected and the uninfected had UTI in their history, 18(51.4) and 50(43.9) respectively. This is followed by candidiasis predominating in both the infected and the uninfected. While none of the regression analysis was significant, the odds of infected participants having induced abortion and PID in their history were 0.25, 95%CI (0.03-2.17) and 3.61, 95%CI (0.38-34.67) respectively. Majority of both the infected and the uninfected had no past neonatal history 26(72.2%) and 89(59.3%) respectively. With the neonatal history, NICU admissions were the most recorded among the infected 8(22.2%) at an OR 0.34, 95%CI (0.02-7.86). Most of the infected participants had no comorbidities 30(83.3%).

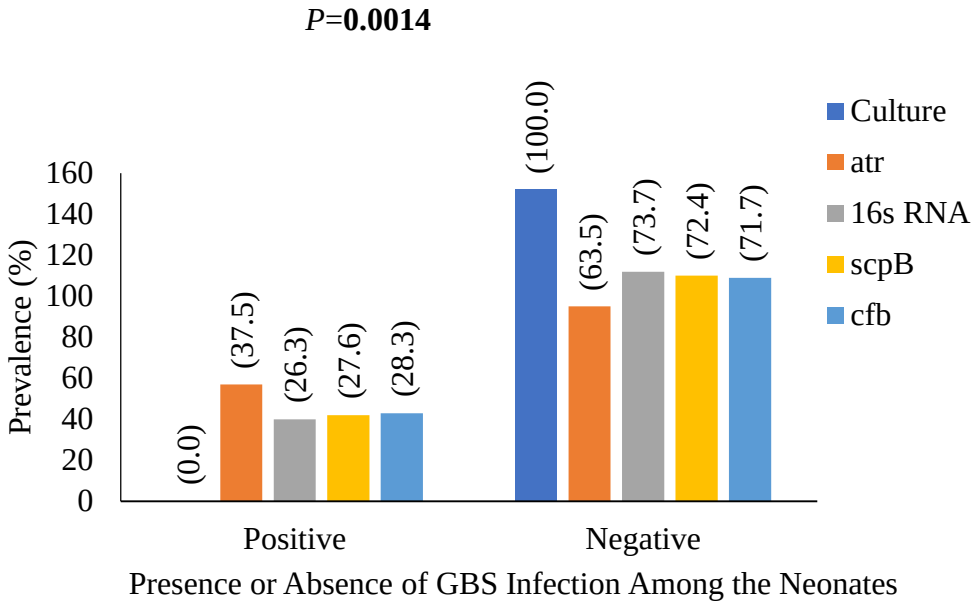


Figure 6: Prevalence of GBS Observed from Culture and the Various PCR Techniques in the Neonates.

The prevalence of GBS infection observed among the neonates was 57(37.5%), 43(28.3%), 42(27.6%) 40(26.3%) and 0(0.0%) for atr gene, cfb gene, scpB gene, 16s rRNA and culture technique respectively. A comparison of the infected and uninfected to the testing method used was significant $p<0.05$. (Figure 6)

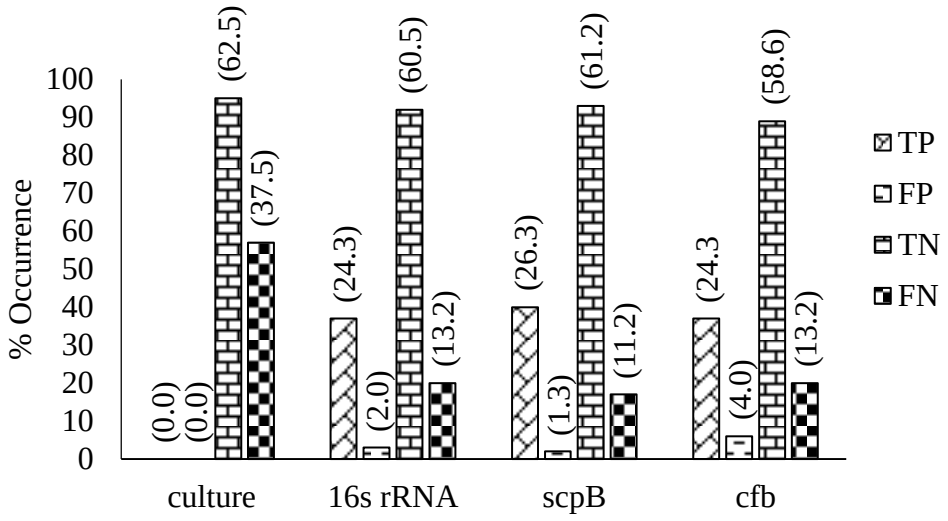


Figure 7: Comparing Diagnostic Performance of Culture and Other PCR Tests to the *atr* PCR assay Among the Neonates.

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

Fifty-seven (57) samples were positive for the *atr* PCR assay. The results obtained for *atr* assay were compared to the results obtained for other tests, i.e. culture, 16s rRNA, *scpB*, and cfb PCR assay. True positive result determined for culture, 16s rRNA, *scpB* and *cfb* were 0(0.0%), 37(24.3%), 40(26.3%) and 37(24.3%) respectively. The true negatives for the culture, 16s rRNA, *scpB* and *cfb* were also 95(62.5%), 92(60.5), 93(61.2%) and 86(58.6%) respectively. (Figure 7).

Table 7: Sensitivity, Specificity, PPV, NPV and Likelihood ratio of the Various Tests using atr PCR Test as the Standard. (Neonates).

PCR	Sensitivity		Specificity		PPV		NPV		Likelihood	*P*
Test	%	95% CI	%	95% CI	%	95% CI	%	95% CI	Ratio	
Culture	0.0	0.00-0.06	100	0.96-1.00	-	-	62.5	0.55-0.70	-	>0.999
16s rRNA	64.9	0.51-0.76	96.8	0.91-0.99	92.5	0.80-0.97	82.1	0.74-0.88	20.56	**<0.0001**
scpB	70.2	0.57-0.81	98.0	0.93-0.99	95.2	0.84-0.99	84.6	0.76-0.90	33.33	**<0.0001**
cfb	64.9	0.51-0.76	93.6	0.87-0.97	86.1	0.72-0.93	81.6	0.73-0.88	10.25	**<0.0001**

PPV= Positive Predictive Value, NPV= Negative Predictive Value, 95%CI= 95% Confidence Interval, *P*= P-value (<0.05 implies statistically significant) Sensitivity and specificity analysis was done through contingency tables and the p-value determined by Fisher's exact test.

Table 7 above shows the sensitivity, specificity, negative predictive value, positive predictive value and the likelihood ratio of the various tests using the *atr* gene as the standard. All tests recorded specificity above 90.0%. Sensitivity for 64.9% for 16s rRNA gene, 70.2% for scpB and 64.9% for cfb gene and none for culture. All of the tests above were statistically significant ($P<0.05$) except the culture method.

Table 8: Prevalence and Multivariate Regression Analysis (OR) of GBS Infection According to atr PCR Test of the Neonates.

Variable	Infection Status		Total	OR (95%CI)	*P*
	Positive	Negative			
Birthweight (n ± SD)	2.80±0.57	2.88±0.58		-	-
Birthweight Grouped					
Low (>2.5kg)	11(19.3)	17(17.9)	28(18.4)	1.08(0.47-2.51)	0.853
Normal (2.5-4.5kg)	46(80.7)	77(81.1)	123(80.9)	1	
High (>4.5kg)	0(0.0)	1(1.1)	1(0.7)	6.99E(6.99E-6.99E)	-
PROM					
No	52(91.2)	91(95.8)	143(94.1)	1	
Yes	5(8.8)	4(4.2)	9(5.9)	0.46(0.19-1.78)	0.259
APGAR Scores 1 Minute					
Low (7)	31(54.4)	41(43.2)	72(47.4)	1.57(0.81-3.04)	0.181
Normal (≥7)	26(45.6)	54(56.8)	80(52.6)	1	
APGAR Scores 5 Minute					
Low (7)	14(24.6)	16(16.8)	30(19.7)	1.61(0.72-3.61)	0.249
Normal (≥7)	43(75.4)	79(83.2)	122(80.3)	1	
Gestational Age at Birth					
Preterm (≤37 weeks)	8(14.0)	11(11.6)	19(12.5)	0.36(0.03-4.74)	0.440
Term (38-42 weeks)	47(82.5)	83(87.4)	130(85.5)	0.28(0.03-3.21)	0.308
Post term (>42 weeks)	2(3.5)	1(1.1)	3(2.0)	1	
Still Birth					
No	53(93.0)	93(97.9)	146(96.1)	1	
Yes	4(7.0)	2(2.1)	6(3.9)	0.29(0.05-1.61)	0.155
IUFD					
No	52(91.2)	94(98.9)	146(96.1)	1	
Yes	5(8.8)	1(1.1)	6(3.9)	0.11(0.01-0.97)	**0.047**

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 8, Prevalence and Multivariate Regression Analysis (OR) of GBS Infection According to *atr* assay among the Neonates. Except for IUFD,

none of the regression tests was significant statistically. Both the infected and uninfected neonates had normal (2.5-4.5kg) weighted and without PROM, 46(80.7) and 77(81.1) and 52(91.2) and 91(95.8) respectively. Most of the infected had APGAR scores low under 1 minute 31(54.4) but normal 43(75.4) under 5 minutes. This pattern applied to the uninfected participants too. Majority of both the infected and uninfected were at a gestational age of 38-42 weeks, 47(82.5) and 83(87.4) respectively. The odd of an infected participant being at 38-42 weeks of pregnancy was OR 0.28, 95%CI (0.03-3.21), P>0.05. Five (8.8%) of the participating neonates had IUFD and the odds of neonates having IUFD was OR 0.11, 95%CI (0.01-0.97), P<0.05.

Discussion

Group B *Streptococcus* causes perinatal infections of the mother, fetus, and neonate, like chorioamnionitis, preterm delivery, stillbirth and meningitis. It is also a major cause of both EO and LO-GBS infection which manifests within first week of life and between seven and eighty-nine days respectively in pediatric sepsis. Worldwide, the incidence of GBS disease is projected at 0.49–0.53 per 1000 live births, with a case-fatality rate of 8.4–9.6% (Clouse *et al.*, 2019). This research looked at the prevalence and risk factors linked with *Streptococcus agalactiae* colonization among parturients and their neonates in Central Region of Ghana while comparing the various diagnostic approaches employed.

Demographically, the bulk of participants were Akans, JHS graduates and unemployed, 103(68.7%), 63(42.0%) and 111(74.0%) respectively. This result was similar to reports in Zimbabwe, Tanzania and South Africa in which most participants enrolled were unemployed as well as either primary

or senior high school graduates (Africa & Kaambo, 2018; Ernest *et al.*, 2015; Mavenyengwa *et al.*, 2010). However, the result of this study conflicts with that reported in a Ugandan study in 2016, where many of the participants were working and senior high school graduates (Namugongo *et al.*, 2016). This could be due to the difference in educational development in the different geographical settings.

In this research, the prevalence of GBS was estimated using culture method and four PCR gene targeting assays (*atr, ScpB, cfb* and*16SrRNA*). Of these tests used, the *atr* assay substantially reported the most positive for both mothers and neonates, 36(24.0%) and 57(37.5%), whereas the culture method reported the least 2(1.3%) and 0(0.0%) comparatively. The *atr* gene was then used as a standard of reference for the other diagnostic methods and clinical data. The prevalence of the *atr* assay (24.0%) observed from this study was close to the 26.0% reported in an earlier study (Wollheim *et al.*, 2017). It was however higher than previous reports among pregnant women and their neonates respectively, where the culture method was employed (Ali *et al.*, 2019; Chen *et al.*, 2018; Santhanam *et al.*, 2017). The prevalence reported in this research was also higher than those earlier reported in 2015(19.1%) and 2017(10.6%) in Ghana (Vinnemeier *et al.*, 2015; Völker *et al.*, 2017). The high prevalence reported in this study may be due to the laboratory approach employed (*atr* gene PCR as used as standard instead of culture), the geographical location of the study as well as the socio-economic properties of the participants within this research compared to those in the other studies.

Sensitivity and specificity assessment of the different diagnostic approaches by comparing them to the *atr* gene PCR test for both mothers and

neonates revealed that while the *scpB* gene considerably reported more true positives 13.3% in mothers and 26.3% in neonates), the culture method reported the least true positives 1.3% in mothers and 0.0% in neonates). Thus, a sensitivity of 55.6% in mothers and 70.2% neonates for the *scpB* gene whiles the culture technique reported a sensitivity of 5.6% in mothers and 0.0% in their neonates. The culture method was also the most specific test among the various diagnostic tests. This finding was in agreement with that of Rallu *et al.* (2006).

Also, a risk analysis on the clinical information of the mothers with regards to the presence of infection revealed that in all instances there were no significant associations between a participant being infected and their clinical data. However, the participants with gestation ≤37 weeks were significantly at risk of getting infected, and also an increase in gestational age is likely to result in a decline in the risk of GBS infection among participants in this study. The finding is in agreement with a prior study where the researchers associated the phenomenon to the prevalence of GBS infection (Mavenyengwa *et al.*, 2010) but disagreed with earlier studies where a link was established between increased prevalence and increase in gestational age (Akadri *et al.*, 2019; Khan *et al.*, 2015; Michel *et al.*, 2014). Perhaps the inconsistency in reports can be resolved if a follow-up cross-sectional study is conducted targeting the pregnant women from the beginning of pregnancy to the end.

Though it was insignificant, mothers with parity of ≤2 were predominantly infected in this research but as parity rose, the number of infected participants reduced. This differed from a prior finding in studies

from Cameroon and Zimbabwe, where parity contributed neither to an increase nor decrease in the number of participants infected (Mavenyengwa *et al.*, 2010; Michel *et al.*, 2014). However, this finding was consistent with other recent studies where infection prevalence either significantly or insignificantly increased with parity (Chen *et al.*, 2018; Dechen *et al.*, 2010; Khan *et al.*, 2015; Slotved *et al.*, 2017a). The findings from this study complements the inconsistent linkage between parity and GBS infections when previous studies are compared. Perhaps a more controlled approach should be considered when studying the prevalence of GBS among women before birth and throughout subsequent births to help bridge this gab.

An additional assessment of their obstetric history revealed that most infected participants were without any past complications. Yet among the participants with infection, history of premature rupture of membrane 5(13.9%) and preterm birth 5(13.9%) predominated. The incidence of PROM in this study agreed with findings from past studies conducted in India where 28% and 43% prevalence of the infected had PROM (Dechen *et al.*, 2010; Sharmila *et al.*, 2016). In other studies, conducted in 2010 and 2014, participants without history of PROM were greatly infected, an assessment that disagreed with this study (Dechen *et al.*, 2010; Michel *et al.*, 2014). The high nature of the preterm birth as an obstetric complication among the participants is inconsonance with the results of (Dechen *et al.*, 2010) where about 64% of the infected had a history of preterm birth but disagrees with another study from India where none of the infected had a history of preterm delivery (Sharmila *et al.*, 2016). However, the overall lack of association between obstetric history and the prevalence of GBS infection is comparable

to that reported by some Brazilian studies (Costa *et al.*, 2008; Vieira *et al.*, 2019). Therefore, a more specific approach would be necessary to examine the relationship between GBS colonization and obstetric factors.

On gynaecological history, it was observed that most of the infected parturients had history of UTI, even though there was no statistically significant association observed. The UTI report from this study agreed with a recent Jordan and Ghanaian studies where no link was established between UTI and GBS infection (Clouse *et al.*, 2019; Slotved *et al.*, 2017a). These findings with regards to UTI disagreed with a study in northern India where the infected participants with history of UTI were comparably low (Khatoon *et al.*, 2016). However, history of UTI among the infected was in agreement with a recent Iranian study where a significant number of the infected individuals had UTI (Ghanbarzadeh *et al.*, 2017). Subsequently, the high number of urinary tract infections among participants calls for concern, although there is no significant link to GBS infection. Perhaps a more controlled study that will look at the colonization of GBS and UTI in-vitro could answer these questions.

A risk assessment conducted between the neonates and possible risk factors also revealed a general lack of association between the colonization of GBS within neonates and possible clinical factors that could pose as a risk. Except in the case of intrauterine fetal death (IUFD) where GBS infection resulted in a significant risk. This current report disagrees with prior findings from (Michel *et al.*, 2014) where no association was established between neonatal death and GBS infection. On the other hand, this report agrees with a

recent study where a significant relationship was established between IUFD and GBS infection (Ghanbarzadeh *et al.*, 2017).

In general, the lack of correlation between the clinical information gathered and the prevalence identified among parturients and neonates in this research can be clarified by the fact that the study was neither a follow-up nor a control case study and also much of the information gathered came from the participants.

Chapter Summary

Majority of the parturients were between the ages of 25-32 years (60.8%), of Akan ethnicity (68.7%), had formal education up to JHS (42.0%) and were unemployed (74.0%). Seventy-four (49.3%) of the parturients had not used any form of antibiotics. Gravidity and parity of ≤3(70.7%) and ≤2(77.3%) respectively were found to be high among the parturients. Majority of the parturients were at 38-42 weeks (86.0%) gestational age. The *atr* gene assay was identified to detect the highest prevalence among both parturients and their neonates. Also, a significant relationship was found between the prevalence of GBS infection and ≤ 37 weeks gestational age and that against IUFD.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATION

Summary

The hospital-based cross-sectional study was aimed at determining the prevalence and risk factors associated with GBS colonization among parturients and their neonates and compare the diagnostic value of culture and PCR assays. One hundred and fifty (150) vaginal swabs and one hundred and fifty-one (151) superficial swabs were collected from parturients and their neonates respectively at the delivery suite of the CCTH. Swabs were directly seeded unto SBA incorporated 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 301 samples were also tested to confirm the presence of GBS with four gene assays (*atr*,*ScpB*,*cfb* and *16SrRNA*) each using multiplex PCR.

Most of the parturients were within the ages of 25-32 years (60.8%), of Akan ethnicity (68.7%), had formal education up to JHS (42.0%) and were unemployed (74.0%). Seventy-four (49.3%) of the parturients had not used any form of antibiotics. Gravidity and parity of ≤3(70.7%) and ≤2(77.3%) respectively were found to be high among the parturients. Majority of the parturients were at 38-42(86.0%) week gestational age.

Prevalence observed among parturients was 36(24.0%), 34(22.7%), 32(21.3%), 20(13.3%), and 2(1.3%) for atr gene, *ScpB* gene, *cfb* gene, 16srRNA gene and culture technique respectively. All sensitivity and specificity analysis were done using atr as the standard. Sensitivities reported for parturients were 55.6% for *ScpB*, 47.2% for *cfb*, 47.2% for 16s rRNA gene

and 5.6% for culture. Specificity of culture and PCR assays among parturients were all observed to be above 85.0%. Among the neonates, rates reported were 57 (37.5%), 43 (28.3%), 42 (27.6%), 40 (26.3%) and 0 (0.0%) for atr gene, cfb gene, scpB gene, 16srRNA gene and culture technique respectively. Sensitivities of 64.9%,70.2%, 64.9%, 0.0% were also reported among the neonates for 16s rRNA, *ScpB*, *cfb*, and culture respectively with *atr* gene as the standard. Specificities were all above 90.0%.

Multivariate regression analysis of the clinical demographics among parturients against *atr* gene assay revealed a statistical significance between prevalence of GBS infection and ≤ 37-weeks gestational age. Also, a significant association was found between GBS infection in neonates and IUFD.

Conclusion

The study reported a prevalence of 36/150 (24.0%) and 57/151 (37.5%) among the mothers and neonates respectively using the multiplex PCR primer *atr* gene assay as the standard. A comparative analysis of the various PCR assays and the culture method revealed that the culture method was the most insensitive technique. A significant relationship was identified among mothers between the prevalence of GBS infection and ≤37 weeks gestation. Among the neonates, an association was established between the prevalence of GBS infection and the risk of intrauterine fetal death (IUFD).

Recommendation

Based on the results of this research, there is relatively high prevalence of GBS among parturients and their neonates in the Cape Coast Metropolis. Therefore, we propose the use of PCR techniques in combination with the

culture method to increase the diagnosis of GBS infection among pregnant women and their neonates. Also, a systematic national guideline for the prevention, diagnosis and treatment of GBS infection among parturients and their neonates is required to help minimize the high prevalence of GBS and its related complications.

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APPENDICES

APPENDIX I

Ethical Clearance

In case of reply the reference number and the date of this Letter should be quoted

Our Ref.: CCTH

Your Ref.:

P. O. Box CT 1363
Cape Coast
CC-170-9967
Tel: 03321-34010-14
Fax: 03321-34016
Website: www.ccthghana.org
email: info@ccthghana.com

1st April 2019

Samuel Essien-Baidoo
School of Allied Health Sciences
University of Cape Coast
Cape Coast

Dear Sir/Madam,

ETHICAL CLEARANCE – REF: CCTHERC/EC/2019/050

The Cape Coast Teaching Hospital Ethical Review Committee (CCTHERC) have reviewed your research protocol titled, "Molecular Characterization and corresponding antibodies to group B streptococcus among pregnant women and their neonates: A Hospital-Based Cross Sectional Study in the Cape Coast Metropolis" ***which was submitted for Ethical Clearance.***

The ERC have made some recommendations (see in table below) for modification of your protocol. You are required to work on the recommendations and incorporate it in the proposal to warrant a provisional approval for implementation of your research protocol.

Research Title	**Molecular Characterization and corresponding antibodies to group B streptococcus among pregnant women and their neonates: A Hospital-Based Cross Sectional Study in the Cape Coast Metropolis**
Principal Investigator	Samuel Essien-Baidoo
Recommendations	To divide the project and work on various aspects.
Conclusion	**Contingent approval given subject to modifications on the issues raised.**

Always quote the protocol identification number in all future correspondence with us in relation to this protocol.

Yours sincerely

Dr. Stephen Laryea
Medical Director
For: Prof. Ganiyu Rahman, Chairman ERC

APPENDIX II

QUESTIONNAIRE

Streptococcus agalactiae INFECTION AMONG PARTURIENTS AND THEIR NEONATES AT CAPE COAST TEACHING HOSPITAL: AN EVALUATION OF DIFFERENT DIAGNOSTIC METHODS, PREVALENCE AND RISK FACTORS

STUDY CODE Date:

	SECTION A: DEMOGRAPHICS		
	VARIABLE	**DESCRIPTION**	**CODE**
1	Age	Year(s)	
2	Ethnicity	1.Akan 2.Ga 3.Ewe 4.Nzema 5.others	
3	Gravidity		
4	Parity		
5	Gestational age at labour		
6	Educational Background	1. No formal education 2. Primary 3. JHS 4. SHS 5. Tertiary	
7	Occupation	1.Employed 2.Unemployed	
8	Recent use of Antibiotics	1. Less than two weeks 2. More than two weeks 3. None of the above (those who have not taken antibiotics at all and do not fall in any of the categories above)	
9	Past obstetric history	1. PROM 2. Preterm birth 3. Puerperal sepsis 4. IUFD 5. Chorioamnionitis 6. IUGR 7. none	

10	Past gynaecology history	1. Spontaneous miscarriage 2. Induced abortion 3. UTI 4. PID 5. candidiasis 6. HIV 7. Hep b 8. Syphilis 9. none	
11	Past neonatal history	1. Neonatal sepsis 2. Early neonatal death 3. Low birth weight 4. NICU admission 5. none	
12	Present neonatal history	1. Gestational age at birth 2. Birth weight 3. PROM 4. APGAR score 5. Still birth 6. IUFD 7. none	
13	Co-morbidites	1.pregnancy with chronic hypertension 2.Gestational hypertension 3.Diabetes in pregnancy 4.Gestational diabetes 5.sickle cell disease 6.Asthma 7. none	

APPENDIX III

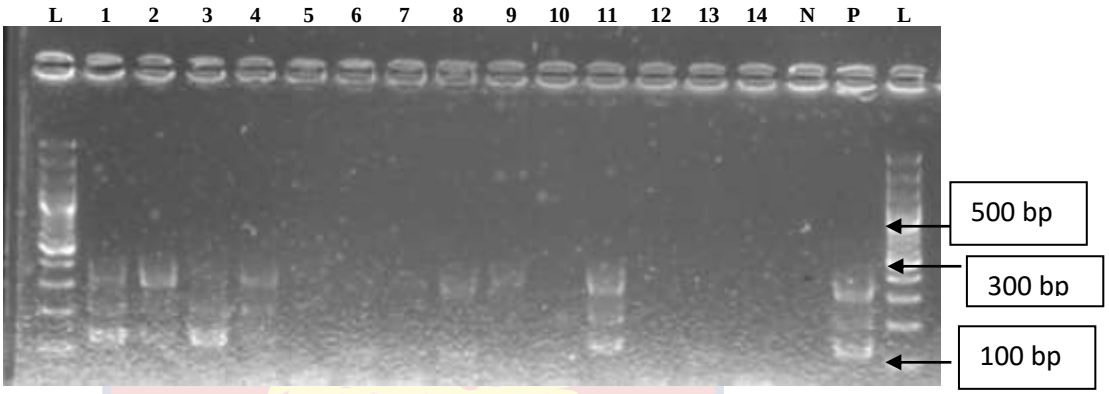
Streptococcus agalactiae **strain (ATCC 12386) for positive control.**

APPENDIX IV

Gram stained slides of clinical isolates

APPENDIX V

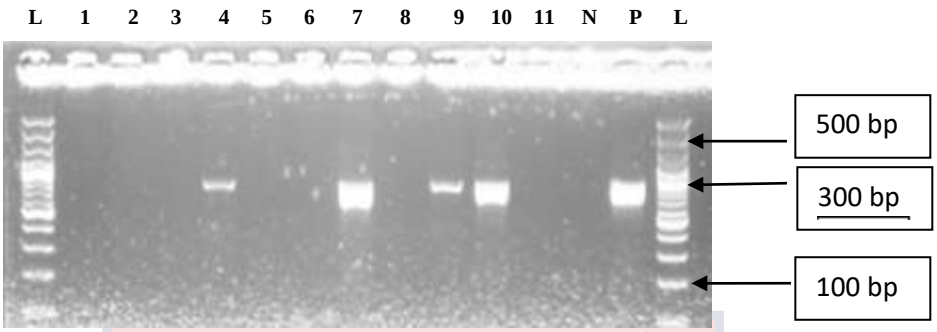
Gel photograph of the multiplex PCR Products of 16S rRNA (405bp), *scpB* (255bp) and *cfb* (153bp) fragments.



L – Ladder N – Negative control P – Positive control

APPENDIX VI

Gel photograph of the multiplex PCR Products of the *atr* (780bp) fragment.



L – Ladder N – Negative control P – Positive control