CARRIAGE AND ANTIBIOTIC SUSCEPTIBILITY PROFILE OF GROUP B *STREPTOCOCCUS* DURING LATE PREGNANCY IN SELECTED HOSPITALS IN GREATER ACCRA

BY

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DECLARATION

I hereby Declare that this submission is my own work towards the MPhil and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text**.**

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DEDICATION

This thesis is dedicated to God Almighty, and to my wonderful family members Mr. D.K. Banini, Iswellam N, Shekinah A. and Agape E. Banini.

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ABSTRACT

Background: Group B *Streptococcus* (GBS) also known as *Streptococcus agalactiae* colonizes the genital and gastrointestinal tract found in 15–50% of healthy adults. Its infection is a major health problem among infants and adults. In pregnancy, vertical transmission of GBS to the newborn can cause neonatal sepsis, pneumonia and meningitis.

Aim: The aim of this study was to determine the prevalence of GBS in pregnant women in their third trimester, its antibiotic susceptibility profile and serotype distribution in two districts in Greater Accra.

Methods: Rectovaginal swabs were collected from a total number of 400 third trimester pregnant women between 35 - 37 weeks of gestation attending routine antenatal clinics at Mamprobi Polyclinic, Accra and Dangme West District Hospital between August 2012 and March 2013. The samples were cultured in Todd Hewitt Broth with 5% sheep blood, sub cultured on sheep blood agar and incubated at 37**º**c for 24 hours. Identification was based on the Gram staining, presence of β-haemolysis, absence of catalase production, and agglutination with group-specific and serotype antiserum. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk-diffusion method.

Results: GBS colonization on average was confirmed in 26.8% of pregnant women and proportion of GBS isolated from the vagina (77.4%) as compared to the rectum (17.9%) and both vagina and rectum (5.7%). The prevailing serotype were *Ia, II, III, IV, V, VII, VIII, IX (*0.9, 0.9, 6.6, 9.4, 6.6, 40.2, 6.6 and 29.8 %) respectively. Area of residence has not shown, significantly, to have any influence on GBS colonization (MPC = 28.0%, DWDH = 25.5%) $P < 0.05$. All isolates were found to be resistant to vancomycin, penicillin, ampicillin, erythromycin, ceftrixone and cefotaxime (15, 50, 26, 42, 33 and 38%) respectively.

Conclusion: A high prevalence of 26.8 % was detected among the pregnant women. There is a need for screening of pregnant women attending antenatal care for this bacterium, including knowing the antibiotic susceptibility so an appropriate intrapartum antimicrobial prophylaxis can be offered. Further studies are required to verify the presence of antibodies to the serotype and their immunobiological function in the pregnant women.

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LIST OF ABBREVIATIONS

AAP	American Academy of Pediatrics
ACOG	American College of Obstetricians and Gynecologists
ANC	Antenatal Care
CA	ChromID Strepto B Agar
CAMP	Christie Atkins Munch Peterson
CDC	Centre for Disease Control
CNA agar	Columbia Colistine Nalidixic Acid Agar
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic acid
DWDH	Dangme West District Hospital
ECM	Enriched Culture Medium
EOD	Early Onset of Disease
GBS	Group B *Streptococcus*
GBSDA	Group B *streptococcus* differential agar
IAP	Intrapartum Antibiotic Prophylaxis,
JHS	Junior High Secondary School
JUTH	Jos University Teaching Hospital
KVIP	Kumasi Improved Ventilation Project
LOD	Late Onset of Disease
MoH	Ministry of Health
MPC	Mamprobi Polyclinic
NAAT	Nucleic Acid Amplification Tests
NEL-GBS	Northeast Laboratory GBS Screening Medium
NMIMR	Noguchi Memorial Institute for Medical Research
PCR	Polymerase Chain Reaction
PNA-FISH	Peptide Nucleic Acid Fluorescence In Situ Hybridization
RNA	Ribonucleic acid
SBCB	StrepB Carrot Broth
SPSS	Statistical Package for Social Sciences
STI	Sexually Transmitted Infections
TTR	Time-To-Result
UTI	Urinary Tract Infection
WC	Water Closet

CHAPTER ONE

BACKGROUND OF THE STUDY

1.1 Introduction

Group B *Streptococcus* (GBS) also known as *Streptococcus agalactiae* is a Gram positive bacterium which causes invasive disease in infants, pregnant or postpartum women. It is also an important organism in elderly patients especially those with underlying medical condition such as diabetes mellitus, neurologic diseases, cirrhosis or other liver diseases, stroke, breast cancer, and renal failure, but with the highest incidence occurring among neonates (Huber *et al*., 2011; Wang *et al.,* 2010; Skoff *et al.,* 2009; Tyrrell *et al*., 2000).

Group B *Streptococci* are bacteria found as a part of the normal flora of human gastrointestinal and the genitourinary tract of 15–50% of healthy women (Huber *et al.,* 2011; Aila *et al.,* 2010). GBS is known to cause diseases such as septicaemia, pneumonia, bacteremia and meningitis in neonates/infants and in elderly (Huber *et al.,* 2011; Wang *et al*., 2010; Skoff *et al.,* 2009). The bacterium also causes high risk of preterm deliveries in pregnant women (CDC, 2010; Brochet *et al.,* 2009; Borchard *et al.,* 2004).

GBS infection is one of the most common infections in the first week after birth (Van Dyke *et al.,* 2009), and it represents major causes of morbidity and mortality especially in neonates whose mothers are carriers (Madzivhandila *et al.,*2011; Alfa *et al.,* 2010; CDC, 2010; Brochet *et al.,*2009). It's estimated that about 1–2% of newborns colonized by GBS develop invasive disease (Madzivhandila *et al.,* 2011).

Despite substantial prevention of perinatal GBS diseases since the 1990s, GBS remains the leading cause of early-onset neonatal sepsis. It is recommended by CDC that GBS screening should be done in the third trimester (35- 37 week) since the state of collonisation is representative of intrapatum (CDC, 2010). And according to CDC, if a pregnant woman is tested positive and is not in the high risk category, then her chances of delivering a baby with GBS are 1 in 200 if antibiotics are not given and 1 in 4000 if antibiotics are given (CDC, 2010).

A comparative study on the occurrence of GBS and antimicrobial susceptibility among the people living in the Greater Accra will therefore provide useful information on the GBS situation in Greater Accra and the empirical treatment of the infection in pregnant women in Ghana.

1.2 Problem Statement

Population-based surveys of bacteremia have raised concerns about the growing incidence of GBS disease in neonates (Skoff *et al.,* 2009; Brimil *et al.,* 2006). Studies conducted outside Ghana indicated that high numbers of neonates are infected with GBS (Enweronu-Laryea *et al.,* 2011; Madzivhandila *et al.,* 2011). However, in Ghana, there is limited data on GBS epidemiology which makes it difficult to draw conclusion that the neonates who suffer from chorioamnionitis infections is caused by GBS or other bacteria. It is estimated that at least 32% of neonatal mortalities are coursed by infection in Ghana and a large number of these infections are acquired from the mother (Enweronu-Laryea *et al.,* 2011).

However, in Ghana there is less awareness of GBS disease among clinicians and laboratory detection of the possible GBS infection. Moreover, it appears there are no detailed national surveillance programs and routine laboratory detection technique in

place. Notwithstanding, the only known published study done in Ghana is over ten years old, which makes it difficult to get useful information on GBS disease in neonates (Enweronu-Laryea *et al.,* 2011). There are limited data on GBS serotype epidemiology associated with maternal recto-genital colonization or invasive disease in infants from industrialized countries as well as in Ghana. The study done by Enweronu-Laryea *et al.,* did not also look at the prevailing serotypes in Ghana (Enweronu-Laryea *et al.,* 2011).

1.3 Justification

The importance of this study cannot be overemphasized because there is limited data on GBS in Ghana. It is necessary to generate data on the GBS disease, especially on carriage of GBS in pregnant women since its infection in neonates is mostly by vertical transmission. It will reveal the extent of GBS recto-vaginal colonization, the serotypes and their antimicrobial resistance in Ghana, since serotype distribution varies, with geographical region, race and ethnic origin (Lachenauer *et al.,* 1999, Brimil *et al.,* 2006).

An antimicrobial susceptibility determination will show current resistance status for GBS isolates, though treatment is not a problem in Ghana (Enweronu-Laryea *et al.,* 2011) but a problem in other African countries such as Tanzania. It was reported that 17.6%, of GBS isolates were resistant to clindamycin, 13% to erythromycin and 9.4%, to penicillin G (Joachim *et al.,* 2009). Report from Taiwan indicated that, higher resistance to erythromycin (58.3%) and clindamycin (57.9%) was found in isolates with certain resistance phenotypes (Wang *et al.,* 2010).

Since preliminary reports on this problem are needed to initiate nationwide surveillance and control measures the study will determine whether detailed national

surveillance programs and routine laboratory detection is needed in order to increase awareness by clinicians and encourage laboratory detection of a possible emerging problem.

1.4 Hypothesis

GBS colonization rate and serotype distribution can vary from one geographical area to another. In general serotype III has been observed as the most common cause of early onset disease in many countries.

1.5 Aim and Objectives

The aim of this study was to determine the prevalence of GBS in pregnant women in their third trimester of gestation, its antibiotic susceptibility profile and serotype distribution in two districts in Greater Accra.

1. To determine the prevalence of GBS in pregnant women attending antenatal clinic.
2. To determine the antibiotic susceptibility patterns of the GBS isolates.
3. To identify the various serotypes of the GBS isolates.
4. To determine the association between social demographic factors, obstetric factors and GBS colonization.

CHAPTER TWO

LITERATURE REVIEW

2.1 General Characteristics of Streptococci

Streptococci are Gram positive spherical and encapsulated organisms in chains, pairs and singles (Facklam, 2002; Hardie and Whilley, 1997), some are members of the normal human flora; others are associated with important human and animal diseases either by the organism itself or sensitization to them by convoluting variety of extracellular substances and enzymes (Jawetz, *et al*., 2010). *Streptococci* are characteristically found in the gastrointestinal tract, genitourinary tract, mucous membrane of the mouth, upper respiratory tract, and skin of human and animals (Aila *et al.*, 2010; Joachim *et al.*, 2009; Larsson, *et al.*, 1996).

In general, *Streptococci* are a large and complex group of bacteria, for clinical and epidemiological purposes, classification is based on (a) colonial morphology and hemolytic reactions on blood agar (b) serologic specificity of the cell wall group-specific substance and capsular antigens (c) biochemical reactions (d) resistance to physical and chemical factors (e) molecular genetics and (f) ecologic features (Jawetz, *et al*., 2010). *Streptococci* can be differentiated into three major groups based on the pattern of haemolysis on 5-10 % blood agar; (1) beta-haemolytic (β) strains producing a soluble haemolysin which causes a clear zone of haemolysis around colonies; eg. *Streptococcus agalactiae* and *Streptococcus pyogenes* (2) alpha-haemolytic (α) strains which do not produce soluble haemolysin and only cause partial haemolysis and a green colour on blood agar around the colonies eg. *Streptococcus pneumoniae* and *Streptococcus suis and* (3) non-haemolytic or gamma(γ) strains which do not produce soluble haemolysin and have no zone of

haemolysis around the colonies on blood agar plate as seen in Fig.: 2.1. But there are some haemolytic groups that have some variant well documented to be non hemolytic eg. *S. pyogenes, S .agalactiae,* and members of the *S. anginosus* (Jawetz, *et al*., 2010; Facklam, 2002).

Figure 2.1: Comparison of GBS & GAS Hemolysis, Non-hemolytic on blood agar

Source: Statens Serum Institut

The genera *Streptococci* possess Group-Specific Substance in the cell wall which is carbohydrate and forms the basis for serologic grouping into Lancefield groups A–H and K–U. These carbohydrates when extracted contain group–specific substance that helps in typing the groups. Among the Lancefield groups A–H and K–U, it is only groups A, B, C, F, and G which are well known to cause human diseases and for

which there are reagents that allow typing using simple serology (Jawetz, *et al*., 2010)

Many *Streptococci* are known to cause human infections and the most common ones include *S. pyogines, S. agalactiae*, *S. pneumoniae* but several others are involved in endocadieties abscesses and other diseases (Hardie and Whilley 1997).

2.2 Morphology and General Characteristics of *Streptococcus agalactiae*

Group B *Streptococci* are Gram-positive spherical organisms occurring characteristically in short chains but also in pairs and singles (Jawetz, *et al*., 2010; Nsagha *et al.,* 2000). They are non-motile, non-sporing, encapsulated and catalase-negative organisms. They are fastidious organisms, which need a rich medium such as blood agar to inoculate the specimen in order to obtain isolated colonies.

According to Lancefield grouping they belong to Group B. They are typically beta haemolytic and produce zones of hemolysis that are only slightly larger than the colonies (1–2 mm in diameter) when cultured on blood agar media with exception of around 4% which are non haemolytic (Aila *et al.,* 2010; Smith; *et al.,* 2008). The organism is covered by capsular polysaccharide which gives rise to antigenic characteristic during serotyping and produces positive reaction with capsular antisera (Madzivhandila *et al.,* 2011; Jawetz, *et al*., 2010).

Group B *Streptococci* colonize human gastrointestinal and the genital tract of 15–50 % of healthy human (Huber *et al*., 2011; Aila *et al.,* 2010). An estimated 20 - 30 % of all pregnant women are carriers, its colonization duringz pregnancy can be transient, intermittent, or persistent (Huber *et al.,* 2011; CDC, 2010).

In Clinical laboratories, primary identification is often based on the typical colonial morphology, beta- haemolysis on blood agar plate, Gram‘s reaction (positive cocci) and catalase (negative) reaction. Other significant identification procedure for *S. agalactiae* is the CAMP test and hippurate hydrolysis test for presumptuous identification (Joachim *et al.,* 2009; Smith *et al.,* 2008). Aside serotyping which also can be referred to as phenotyping, GBS can also be identified using molecular techniques such as PCR (also referred to as genotyping) (Madzivhandila *et al.,* 2011; Aila *et al.,* 2010).

2.3 Epidemiology of Streptococci *agalactiae*

Since 1880s, Group B *Streptococcus* (GBS) has been a problem in veterinary medicine. It is an animal pathogen that causes infection in many animals including fishes and cattle which makes it a great economic importance in aqua culture and the milk and dairy products industries (Sørensen *et al.,* 2010; Correˆa *et al.,* 2009; Suanyuk *et al.,* 2008). In early 1970s, GBS was frequently associated with a large variety of serious human infections, such as sepsis and meningitis in neonates (Madzivhandila *et al.,* 2011; Corrêa *et al.,* 2009; Van Dyke *et al.,* 2009; Suanyuk *et al.,* 2008).

GBS is a bacteria found as a part of the normal flora of human gastrointestinal and the genital tract of 15–50% of healthy human (Huber *et al.,* 2011; Brochet *et al.,* 2009) and its colonization is mostly asymptomatic (Ippolito *et al.,* 2010; Florindo *et al.,* 2010). This maternal asymptomatic carriage of GBS in the genitourinary or gastrointestinal tract commonly leads to colonization in the neonate (Smith *et al.,* 2008). Because of the low immune status of neonate, this organism becomes a pathogen causing deadly neonatal diseases (Tazi *et al.,* 2010; Edwards *et al.,* 2008;

Edwards and Baker 2005). This infection is associated with two distinct clinical syndromes, referred to as early-onset disease (EOD) in neonates in their first week of life (age 0–6 day) and late-onset disease (LOD) at the age of 7–89 days (Mavenyengwa *et al.,* 2010; Brimil *et al.,* 2006; Edwards and Baker, 2005).

Although GBS is mostly acquired by vertical transmission (Diedrick *et al.,* 2010; Foxman *et al.,* 2007) there are some factors that contribute to its increased risk of invasiveness in infected mothers to their infants and these include; early rupturing of amniotic membrane, preterm delivery and GBS bacteriuria during pregnancy or with a previous infant with GBS infection, young maternal age and the black race (Amaya *et al.,* 2004).

It is estimated that in 1.8 cases per 1000 live births in the United States colonization leads to onset of invasive disease (Smith *et al.,* 2008; Zangwill *et al.,* 1992). After the implementation of CDC guidelines (CDC, 2010) which called for screening for GBS in pregnant women, it also encouraged initiation of systemic intrapartum antibiotic therapy for them in order to eradicate colonization and neonatal diseases. This strategy has been associated with about 33% reduction in early-onset invasive disease in the United States, with 1.2 cases per 1000 live births reported between 2003 and 2005 (Smith *et al.,* 2008). In 2006 the mean incidence of EOD in the USA was 0.37 per 1000 (Bergseng *et al.,* 2008).

Though more than a million pregnant women receive intravenous antibiotic during delivery to prevent perinatal GBS infection, intrapartum antibiotic prophylaxis (API) has shown less impact on the incidence of late onset disease (LOD) in infancy. Late onset disease incidence has been stable at 0.3-0.35 per 1000 live birth or an estimated 1,375 cases yearly in the US for decades (Edwards *et al.,* 2008).

Despite that the maternal invasive disease has been reduced by 21% with the implementation of the API in the US, the maternal pregnancy associated disease is still stable at 0.11-0.14 per 1000 live birth in 1999-2005. Among the nonpregnant adult the incidence is on the increase reaching the rate of 7.9 per 100,000 in 2005 (Edwards *et al.,* 2008).

A recent population-based surveillance report revealed that, GBS infection is also very common in old age. This is supported by the fact that, 22 - 26 cases per 100,000 persons are adults aged 65 and older mostly suffers from GBS infection (Phares *et al.,* 2008; Skoff *et al.,* 2009), and roughly two-thirds of the deaths in the United States are attributed to GBS infection (Amaya *et al.,* 2004). While the case fatality rate in neonate has declined in developed countries in recent years, the lethality of GBS disease in developing countries is still high (Mavenyengwa *et al.,* 2010). Vaginal colonization rates in Mexico is approximately 10% in pregnant women and a neonatal infection rate of 1/1,500 live births with a case fatality rate of 38.5% has also been recorded (Palacios *et al.,* 1997).

The overall GBS colonization rate reported in the Sub-Saharan Africa was 19% (Mavenyengwa *et al.,* 2010). In a study from Dares Salaam, Tanzania by Joachim and his group found that GBS colonization was confirmed in 23% of pregnant women and 8.9% of neonates. A prevalence rate of 31.6% was reported in Zimbabwe and 32.9% in Trinidad (Joachim *et al.,* 2009).

In Nigeria a study performed by Nsagha *et al.,* (2000) in Jos University Teaching Hospital (JUTH) and the Vom Christian Hospital revealed that 7% of Nigeria population is colonized with GBS. In Zimbabwe 38.0% cases were reported among the people living in an urban area and 62.0% in a rural area giving an overall

cumulative colonization rate in Zimbabwe as 60.3% (Mavenyengwa *et al.,* 2010). The overall GBS colonization rate in Belgium was 22% (Aila *et al.,* 2010) and in 2005-2007, Portugal recorded colonization rate of 6.2% (Florindo *et al.,* 2010). Finland reported (0.62 per 1000) whereas Germany reported (0.28 per 1000) Bergseng *et al.,* 2008). Whilst in Ghana, Enweronu-Laryea *et al.,* recorded a prevalence rate of 23% in the year 2000 (Enweronu-Laryea *et al.,* 2011).

In Norway, the incidence of invasive GBS disease increased significantly in the elderly (The mean incidence in the elderly (>70 years) increased from 3.9 per 100,000 in 1996–1998 to 9.15 in 1999–2006). While the incidence of early-onset disease was stable with 0.46 cases per 1,000 live births the incidence of late-onset disease increased in 2005 and 2006 (increased from 3.9 per 100,000 in 1996–1998 to 9.15 in 1999–2006 in infants). Additionally, the lethality of GBS in infants increased from an average of 6.5% in 1996–2005 to 20% in Norway 2006 (Bergseng *et al.,* 2009).

2.4 Serotype Distribution

The polysaccharide capsule of *S. agalactiae* has been recognized as an important virulence factors. Variations of the capsular polysaccharide structure allow the antigenic distinction of ten different *S. agalactiae* serotypes that is Ia, Ib, II–IX (Doare & Heath 2013; Martins *et al.,* 2010).

Studies have shown that serotype distribution varies with geographical region and ethnic origin (Doare & Heath 2013; Mavenyengwa *et al.,* 2010; Brochet *et al.,* 2009). Data from US and Europe have shown that in general 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 and Baker 2005; Hickman *et al.,*

1999). However, in the United Arab Emirates, type IV was the predominant serotype among colonized pregnant women, representing 26.3% of the GBS isolates (Diedrick *et al.,* 2010). In colonization studies from Japan, serotypes VI and VIII strains are detected most frequently (Brimil *et al.*, 2006; Lachenauer *et al.,* 1999).

In East Africa, the capsular serotypes detected included Ia, Ib, II, III, IV and V. The most common serotype was serotype III (30.8 %), followed by serotype Ia (30.2 %), serotype V (17.2 %), serotype Ib (14.2 %), serotype II (7.1 %), and serotype IV (0.6%) (Huber *et al.,* 2011). In South Africa, the serotype distribution among vaginal colonizing isolates was as follows: III (37.3%), Ia (30.1%), and II (11.3%), V (10.2%), Ib (6.7%) and IV (3.7%) (Madzivhandila *et al.*, 2011).

It has been described that serotype III is the most invasive and these findings are consistent with those found in many countries such as South Africa, Portugal, Sweden and Israel (Madzivhandila *et al.*, 2011; Brueggemann *et al.,* 2003; Martins *et al.,* 2007; Bisharat, *et al.*, 2005; Brueggemann *et al.,* 2003; Berg *et al.,* 2000; Palacios *et al.,* 1997). In Norway, both serotypes III and V were predominant in invasive GBS strains characterized type III in infants and type V in the elderly (Bergseng *et al.,* 2009).

The prevalence of serotypes varies with time and geographical location; thus, knowledge of serotype distribution is necessary for the selection and development of serotype-based vaccine in a given country (Fluegge *et al.*, 2005; Berg *et al.,* 2000).

2.5 Mode of Infection

Group B *Streptococcus* (GBS) is a common inhabitant of the gastrointestinal tracts and because of anatomical proximity it colonizes vagina as well (Martins *et al.,*

2010). Approximately 10 to 30 percent of pregnant women carry GBS in the vagina or rectum or surrounding area. The known transmission routes of GBS include vertical transmission from mother to infant and sexual contact (Doare & Heath, 2013). Food-borne transmission is also possible, as GBS is a known fish and bovine pathogen (Sørensen *et al.*, 2010; Correˆa *et al.*, 2009; Foxman *et al.,* 2007).

In neonates, GBS infection can be acquired during labour or in uterus via intact or ruptured amniotic membrane or during passage through a colonized birth canal of the mother or anorectal-colonized mucosa. (Madzivhandila *et al.,* 2011; Diedrick *et al.,* 2010; Foxman *et al.,* 2007; Aila *et al.,* 2010).

Vertical acquisition of GBS, involving colonization of the skin or mucous membranes, occurs in 15% to 50% of newborns born from GBS colonized mother (Madzivhandila *et al.,* 2011; Beal and Dancer, 2006; Eren *et al.,* 2005).

Although sexual transmission is possible, GBS is not considered to be a sexually transmitted infection since the genital area may become colonized from bacteria that the individual is caring in his or her own gastrointestinal tract (Foxman *et al.,* 2007).

2.6 Group B *Streptococcus* Diseases

GBS was first identified as cause of puperal sepsis in 1935 when Lancefied and Hare observed differences in haemolytic culture. Fry subsequently reported several cases of fatal puerperal sepsis related to GBS disease (Koenig and Keenan, 2009; Edwards *et al.,* 2008). Until 1970s, GBS was only of an economically important in the fish and dairy cattle industry as it causes bovine mastitis throughout the world hence the name “agalactiae” meaning “lack of milk” (Suanyuk *et al.,* 2008; Rato *et al.,* 2008;

Zhao *et al.,* 2006b**)**. For unknown reasons, this organism emerged as the leading cause of neonatal septicaemia and meningitis (Al Safadi *et al.,* 2010) in North America and many countries in Western Europe (Dahl *et al.,* 2003).

GBS, in peripartum women, causes preterm labour, miscarriages, chorioamnionitis, endometritis, and sepsis stillbirths, puerperal or gynaecological sepsis, bladder infections, endocarditis, cellulitis, arthritis, and occasionally urinary tract infection. GBS is also a common cause of osteoarticular infections, bacteremia, pneumonia, mastitis, urosepsis, streptococcal toxic shock syndrome, skin and soft tissue infections, which may be invasive (Huber *et al.,* 2011; Wang *et al.,* 2010; Radtke *et al.,*2010; Skoff *et al.,* 2009, Tyrrell *et al.,* 2000; Zaleznik *et al.,* 2000).

Early onset GBS presents as: Breathing problems, heart and blood pressure instability gastrointestinal and kidney problems, sepsis, pneumonia and meningitis are the most common complications whereas late-onset GBS presents mostly as Meningitis (Gray *et al.,* 2007). Late-onset GBS infection is not as common as early-onset Late-onset of GBS could be a result of delivery, or contact after birth GBS.

2.6.1 Risk Factors Associated with Developing Invasive GBS Disease in Pregnant Women

According to the Center of Disease Control and Prevention and other collaborating groups such as American College of Obstetricians and Gynecologists (ACOG) and American Academy of Pediatrics (AAP), there are various risk factors associated with the GBS disease in pregnant women and neonate which must be conceded in the control and management of disease. These clinical and microbiologic characteristics

have been targeted in prevention strategies (CDC, 2010). These factors include; 1) Obstetric risk factors: Preterm delivery or membrane rupture before 37 weeks, Prolonged rupture of membranes for at least 18 hours, Infection of the placental tissues or amniotic fluid (amnionitis) or intrapartum fever of temperature greater than 38ºC. 2) Maternal GBS colonization late in pregnancy i.e. during labor and delivery. 3) Previous delivery of an infant with GBS disease, maternal GBS bacteriuria during the pregnancy (marker for heavy colonization). 4) Low maternal levels of anti-GBS antibodies, 5) Demographic risk factors: African American (black race), Young maternal age (Doare & Heath, 2013; Berardi *et al.*, 2013; Joachim et al., 2009).

2.6.2 Risk Factors Associated with Developing Invasive GBS Disease in Elderly

According to Amaya *et al.*, the risk factors associated with invasive GBS disease in elderly subjects depend on medical underlying conditions (Amaya *et al.*, 2004). These include patients with immuno compromised conditions; diabetes mellitus, neurological disease, advanced age, cirrhosis, chronic renal failure, cardiac disease and certain malignancies e.g. breast cancer (Huber *et al.*, 2011; Bergseng *et al.*, 2008; Edwards and Baker, 2005).

2.6.3 GBS Disease in Babies

While GBS is generally harmless in healthy adults, it may cause serious infections eg. Meningitis, sepsis, pneumonia or result in stillbirth of the newborn. For early-onset disease, GBS most commonly cause sepsis, pneumonia, and sometimes meningitis. Similar illnesses are associated with late-onset Group B *Streptococcus* disease but meningitis is more common with late-onset GBS disease than with EOD group B strep disease (Doare & Heath, 2013).

For both early and late-onset GBS disease and particularly for infants about 25% of the GBS diseased infants develop meningitis, and its survival goes with high risk of long-term consequences of the GBS infection. These include neurological sequelae, hearing or visual loss, cerebral palsy hydrocephalus, mild to severe mental retardation and other developmental disabilities (Edward *et al.,* 2008; Jordan *et al.,* 2008). On average, about 1,200 babies in the U.S. suffers early-onset Group B *Streptococci* disease each year with rates of GBS disease higher among blacks (Jordan *et al.,* 2008).

Today, in the developed countries, because of effective early-onset disease prevention, early and late-onset diseases occur at similar low rates**.** But in sub-Saharan Africa neonatal morbidity due to GBS remains a public-health problem. GBS neonatal disease occurs in 3.06 per 1,000 live births in South Africa and 1.81 per 1,000 live births in Malawi (Madzivhandila *et al.,* 2011).

Although the GBS infection is more common among adults than in neonates, the overall mortality is higher in neonates than in adults. Moreover risk of disease is greater in pregnant women than in men and non-pregnant women (Joachim *et al.,* 2009). Approximately 1 out of every 200 babies who are born to GBS carry mothers manifests the disease. However, there are certain symptoms that put a mother at a higher risk than others.

Though GBS is mostly acquired by vertical transmission (Foxman *et al.,* 2007, Diedrick *et al.,* 2010); there are some risk factors associated with early-onset disease and these include GBS colonization; prenatal cultures late in pregnancy can predict delivery status. There are some factors that contribute to its increased risk of invasiveness in infected mothers to their infants and this include; early rupturing of

amniotic membrane, preterm delivery and GBS bacteriuria during pregnancy or with a previous infant with GBS infection, young maternal age, black race. Heavy colonization with GBS has been identified more frequently among African American women, a finding which may explain the higher risk of both early- and late-onset GBS disease among black race, and low concentration of capsular polysaccharide (CPS) - specific immunoglobulin-G (IgG) in sera of pregnant women at delivery (Aila *et al.,* 2010; Amaya *et al.,* 2004). And also when infants born to mothers with adequate antibody levels are born before 34 weeks gestation, since transplacental transport of immunoglobulin G is reduced early in gestation. The inoculums of GBS to which the baby is exposed can be large either because maternal colonization is particularly dense or because obstetric complications develop which permit multiplication of the bacteria at the time of labour.

2.7 Prevention of GBS Infection: Vaccination and Prophylaxis.

The incidence of early-onset neonatal infections has reduced significantly by 62% in the developed countries since the introduction of GBS Prevention guidelines for perinatal GBS disease by CDC in 1996, and revised in 2002 (Wang *et al.,* 2010; Joachim *et al.,* 2009). These substantial reductions in early-onset GBS disease have been realised through improved use of intrapartum antimicrobial prophylaxis. However, the consensus prophylaxis strategies and its association with frequent use antibiotic may lead to the emergence of resistant strains of GBS or other organisms during the perinatal period (Edwards *et al.,* 2008). Furthermore, antimicrobial prophylaxis is not likely to prevent most late-onset infections, GBS-related stillbirths, or prematurity and does not address GBS disease in non pregnant adults (Edwards *et al.,* 2008).

It has long been acknowledged that maternal API is an interim strategy for the prevention of GBS infection in neonate but not a permanent solution (Edwards *et al.*, 2008). The development of GBS vaccine is a better option in combating all forms of GBS diseases. The success of maternal immunization in preventing young infant morbidity and mortality is best characterized by the success of tetanus immunization program and inactivated influenza vaccine studies during pregnancy (Madzivhandila *et al.*, 2011; Zaman *et al.*, 2008). The GBS serotypes are defined by differences in capsular polysaccharide antigens (Kong *et al.*, 2003) which are major antigenic and virulence factor also the main target of antibody-mediated killing (Martins *et al.*, 2010; Imperi *et al.*, 2009). The production of specific antibodies against polysaccharides plays a significant role in the defense against bacterial infection (Johnson *et al.*, 2003).

Aside this, GBS have either the Rib (R proteins R1–R4) or a protein (alpha-C protein, Rib, Alp2, Alp3, Alp4, the IgA-binding protein and the epsilon protein) which also contribute to virulence (alpha-protein-like proteins) (Diedrick *et al.*, 2010; Zhao *et al.*, 2006; Brimil *et al.*, 2006). It is immunogenic and immunization with highly purified form can prevent fatal GBS infections. Maternal vaccinations with capsular polysaccharide type-specific serum IgG will possibly reduce maternal colonization, vertical transmission and enhance transplacental transfer of anti-GBS antibody to the foetus hence protect against invasive disease in their infants (Madzivhandila *et al.*, 2011 CDC, 2010). The protein and Rib conferred protection on individuals. Thus immunization with these two proteins may protect against the large majority of GBS strains causing invasive infections (Larsson *et al.*, 1996).

The Phase I and II clinical trials of the monovalent polysaccharide-protein conjugate vaccines of GBS disease-associated types have been done among healthy non

pregnant adults, have shown these vaccines to be immunogenic and can be well tolerated (CDC, 2010; Guttormsen *et al.,* 2008). A recent, double-blind randomized trial of a conjugate vaccine against GBS serotype III among non pregnant women of reproductive age found a significant delay in acquisition of colonization with the vaccine-serotype among vaccine recipients. Although an effective GBS vaccine would be a powerful tool against GBS disease, no licensed vaccine has yet been available (CDC, 2010).

For intrapartum antibiotic prophylaxis to be given, the pregnant woman should have one or more of the following conditions: GBS positive screening test, GBS colonization status unknown with, Delivery <37 weeks, temperature during labor ≥100.4˚ F (≥38.0˚ C), rupture of membranes ≥18 hours, previous infant with GBS disease, GBS in the mother's urine during current pregnancy.

2.8 Treatment of GBS Infection

Antimicrobial therapy of invasive GBS infection is with penicillin Ampicillin, vancomycin, clindamycin, erythromycin, cotrimoxazole, and ceftriaxone of GBS isolated from different sites. A study done by Joachim *et al*., revealed that the GBS isolates were all (100%) sensitive and Ampicillin, (90% - 100%) sensitive to penicillin G and ciprofloxacin and (80-90%) sensitive to clindamycin, erythromycin, cotrimoxazole and ceftriaxone (Joachim *et al.,* 2009). Penicillin is a drug of choice for antimicrobial intrapatum prophylaxis (API) and treatment, however, women who are allergic to penicillin and have high risk for anaphylaxis erythromycin or clindamycin may be used (CDC, 2010; Florindo *et al.*,2010). Moreover, it is important to perform antimicrobial susceptibility testing of GBS isolates in order to select appropriate antibiotic prophylaxis.

Since GBS is now developing resistance to clindamycin, D-zone testing using the double-disk diffusion method has been used to identify isolates that are erythromycin-resistant and clindamycin-susceptible, yet have inducible resistance to clindamycin (CDC, 2010). Isolates that are D-zone positive are considered to have inducible clindamycin resistance. Newborns with early-onset are treated the same as the mothers, which is through intravenous antibiotics.

2.9 Laboratory Diagnosis of GBS

In human being, GBS disease is diagnosed by isolation of the organism from a sterile site, primarily blood or cerebrospinal fluid (Jordan *et al.*, 2008; Edwards and Baker, 2005). Diagnosis is usually done by using the following techniques; bacterial culture and isolation, serological or molecular techniques. In the laboratory, the isolation of GBS depends on diagnostic samples, type of infection or research work being done. And these may include the following: blood, amniotic fluid, cerebrospinal fluid (CSF),breast milk, urine, rectal, vaginal, ears, nose and umbilicus swabs (Skoff *et al.*, 2009; Dore *et al.*, 2003). Sometimes bone marrow cultures may also be useful. The contribution of GBS to clinical sepsis and pneumonia for which cultures are negative is not known.

2.9.1 Specimen Collection

According to CDC, Swabbing both the lower vagina (vaginal introitus) and anorectum (through the anal sphincter) increases the culture yield substantially compared with sampling the cervix or the vagina without also swabbing the rectum (CDC, 2010).

The use of appropriate transport media can be helpful, especially where laboratory personnel are not readily available to process the sample immediately. And this can

sustain the viability of GBS for several days at room temperature; however, the recovery of isolates declines from 1 to 4 days, particularly at high temperatures. Even when appropriate transport media are used, the sensitivity of culture is greatest when the specimen is stored at 4°C before culture and processed within 24 hours of collection (CDC, 2010).

2.9.2 Culture of Specimen and the Medium

Since GBS is a fastidious organism its isolation involves culturing the samples in an enriched medium to improve the viability of the GBS in the sample as opposed to the other naturally occurring bacteria and this usually takes 24–48 hours. Following this, the sample is plated on a solid media as before, to determine presence of GBS. This enriched culture medium (ECM) method according to CDCD, is the "Gold Standard" for GBS testing and is the best GBS test currently available (CDC, 2010; Peltroche-Llacsahuanga *et al.*, 2009; Schrag *et al.*, 2002).

The inclusion of antibiotic in the selective medium suppress the over growth of other micro-organism that are co inhabiting the area. Broth (enrichment media) containing the antibiotic is used to inhibit the growth of other intestinal bacteria and permit the multiplication of GBS in the sample. Examples of selective enrichment broths recommended by CDC include; Todd-Hewitt broth supplemented with colistin (10 μg/ml) and nalidixic acid (Lim broth) or Todd-Hewitt broth supplemented with gentamicin (8 μg/ml) and nalidixic acid (15 μg/ml) (TransVag broth) (CDC, 2010). Although TransVag and Lim broth media are often available without blood, the addition of 5% sheep blood can increase the recovery rate of GBS (CDC, 2010).

Culturing of specimen can also be done using chromogenic or pigmented media which work as differential media and indicate growth of GBS by development of a

specific color (CDC, 2010). Such media include Columbia CNA agar (CNA), Group B streptococcus differential agar (GBSDA) Granada Medium, ChromID Strepto B agar (CA) and Northeast Laboratory GBS Screening Medium (NEL-GBS) (Aila *et al.,* 2010; Carvalho *et al.,* 2009; Tazi *et al.,* 2008; Block *et al.,* 2008). Blood agar with and without Lim broth as an enrichment media can also be used in identification of GBS.

StrepB Carrot Broth (SBCB), a derivative of Granada medium, is a selective and differential (contain chromogenic substance) medium for cultivation of *S. agalactiae*. Culture of primary clinical specimens that generates an orange pigment upon overnight incubation is specific for β-hemolytic strains of *S. agalactiae* but non hemolytic isolates will not be detected by these broths alone (Munson *et al.,* 2010; CDC, 2010).

To detect GBS colonization in pregnant women, the CDC recommends isolation of the bacterium from vaginal and anorectal swab samples by growth in a selective enrichment medium, such as Lim broth or TransVag (Todd-Hewitt broth supplemented with selective antibiotics), followed by subculture on sheep blood agar (Aila *et al.,* 2010; CDC, 2010).

2.9.3 Serology Antigen Detection

Serologically, GBS is rapidly identified from cultures using slide agglutination or a capillary precipitation with commercially produced antisera. Using antigen suspension of growth from an agar plate or suspension of broth culture (Martins *et al.,* 2010; Alfa *et al.,* 2010) the serotype is deduced from the specific pattern of agglutination reactions or is by precipitation capillary test tube (Slotved *et al.,* 2007). Antisera polysaccharide antigens and clumping or precipitation occurs within few

seconds after reaction with corresponding antisera (Group B latex agglutination) depending on the group specific antigen to confirm the identity of GBS. (Alfa *et al.,* 2010; Brochet *et al.,* 2009; Smith *et al.,* 2008; Lopardo *et al.,* 2003).

2.9.4 Molecular Identification of GBS

For prenatal screening of GBS the gold standard (culture) is the best method since the direct plating of swabs yields false-negative culture results in as many as 50% of GBS colonized women (Peltroche-Llacsahuanga *et al.,* 2009; Smith *et al.,* 2008; Schrag *et al.,* 2002). Although chromogenic media techniques was introduced to reduce time-to-result (TTR) these methods however, could not meet the need since it still require overnight incubation, and there is still a need for more sensitive and accurate technique (CDC, 2010; Block *et al.,* 2008; Tazi *et al.,* 2008).

Efforts to minimize TTR of the gold standard which require up to 72 hours before a final result is declared have led to the development of molecular based methods. Rapid techniques such as DNA probes and nucleic acid amplification tests (NAAT) such as polymerase chain reaction (PCR) have been developed. A real-time based polymerase chain reaction (PCR) such as Strep B assay, GeneXpert GBS assay, BD GeneOhm assay and peptide nucleic acid fluorescence in situ hybridization (PNA-FISH) which are now being employed for the identify GBS directly from sample, enrichment broth, or after subculture have been developed (CDC, 2010; Ippolito *et al.,* 2010; Radtke *et al.,* 2010; Peltroche-Llacsahuanga *et al.,* 2009; Edwards *et al.,* 2008; Montague *et al.,* 2008).

Although molecular -based techniques usually provide a TTR of less than 3 hours, there usage in an ordinary diagnostic laboratory is hindered by the high costs of the

PCR equipment and its consumables (CDC, 2010; Peltroche-Llacsahuanga *et al.*, 2009). The PNA FISH technique combines the simplicity of traditional staining procedures with the unique hybridization characteristics of PNA probes and provides specific detection of ribosomal RNA targets in bacterial and fungal pathogens.

The PNA FISH method can be considered as a feasible alternative diagnostic method in terms of cost-benefit for intrapatum identification of GBS since it only requires a slide incubator, a water bath, and a fluorescence microscope (Peltroche-Llacsahuanga *et al.*, 2009).

2.9.5 Biochemical Test

There are many biochemical tests for isolation and identification of GBS. Aside the colonial morphology and beta haemolysis on 5-10% blood agar and being negative to catalase test (3% hydrogen peroxide) GBS can be identified by the hippurate reactions and CAMP (Christie, Atkins, Munch-Peterson) test. Together with the unique hemolytic reaction (very small zone of lysis 1-2mm around the colony), these two presumptive tests are very accurate in the identification GBS (Facklam, 2002). The CAMP test is based on the fact that, group B streptococci produce a protein-like compound known as the CAMP factor that acts synergistically with a staphylococcal beta-hemolysin (β-lysin) on sheep erythrocytes to produce an enhanced zone of hemolysis. CAMP it is a presumptuous test used in identification of GBS in a situation whereby the serological method is unavailable (CDC, 2010) as seen in fig.2.2.

In 1919, J. H. Brown first defined the reactions of streptococci on blood agar plates. Ever since, GBS is mostly identified by using a pattern of heamolysis. Hemolysis is used as a guide for managing patients as well as an aid in classification of the bacterium to the species level. Up to 80% of group B *Streptococcus* can grow in

6.5% salt broth (Tang and Stratton, 2006). GBS is chemically identified as being resistant to bacitracin. It also gives a negative reaction in pyrrolidonylarylamidase, Voges-Proskauer reaction, hydrolysis of esculin, hydrolysis of starch, production of acid in sorbitol broth and deamination of arginine but positive reaction in CAMP reaction hydrolysis of hippurate (Facklam, 2002).

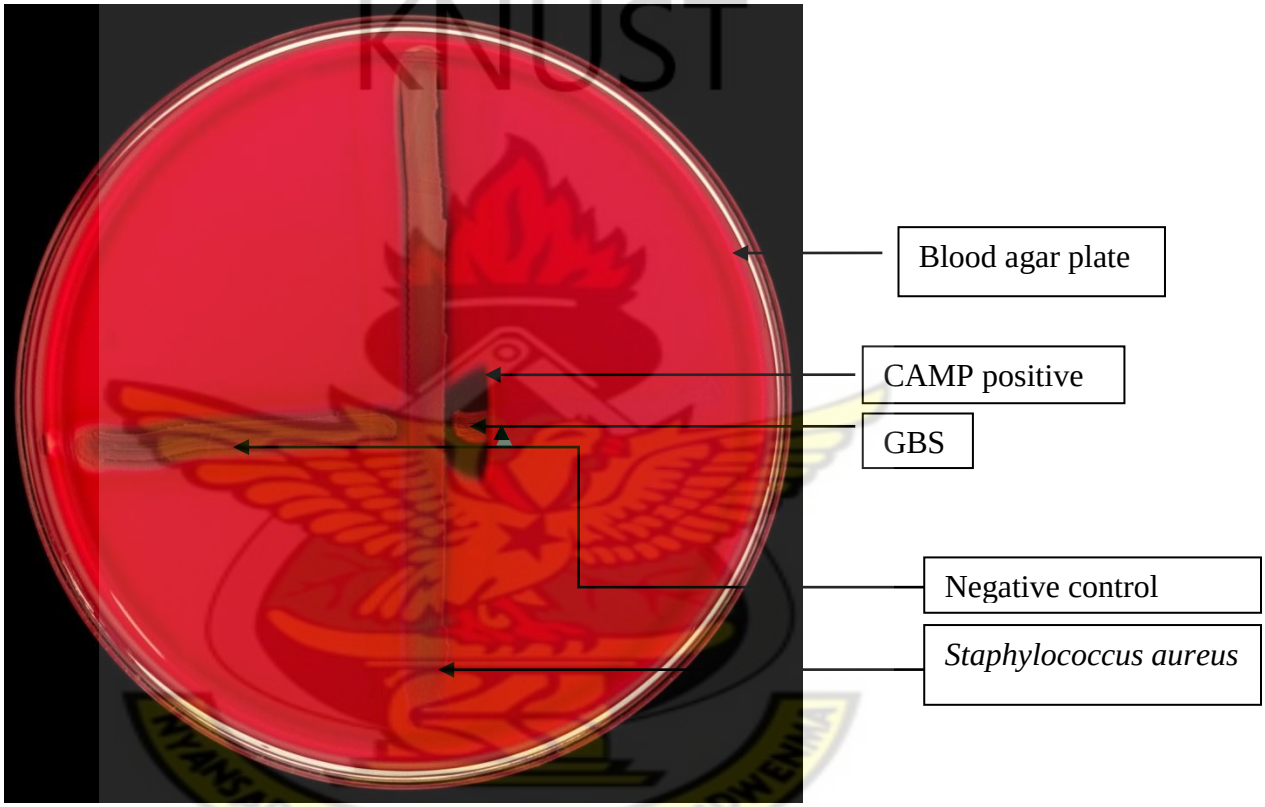


Figure 2.2: A Plate showing CAMP Test Positive Reaction

Source: CDC, 2010

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design and Study Site

The study employed basic quantitative and experimental research techniques to identify and ascertain the prevalence of GBS in pregnant women, their antibiotic susceptibility patterns and the prevailing serotype.

This study was carried out between May 2012 and June 2013 in the Greater Accra region involving two health facilities. Thus, samples were collected from Mamprobi Polyclinic, Accra and Dangme West District Hospital, Dodowa and the laboratory work was done in the National Public Reference Laboratory, Korle-Bu Teaching Hospital.

Greater Accra is situated in the southern part of Ghana, and has a high population density. Its inhabitants form about 15.8% of Ghana's population. Three major hospitals with polyclinic and other types of heath facilities are situated in greater Accra. The collection site Mamprobi polyclinic is situated in Ablekuma Sub-metro which is the largest of the six sub-metropolis of the Accra Metro with a total population of 691,364. The polyclinic is one of the five polyclinics in Accra Metro. This facility serves the people of Ablekuma and its environs.

It is estimated that Mamprobi has a population of about 380, 250, and about 62 percent of the people in the Ablekuma Sub-Metropolitan District area work in the informal sector. Buying, selling and fishing as well as fish mongering are their main occupation. This polyclinic caters for about 65% of pregnant mothers in Ablekuma Sub metropolis and due to the free and focus antenatal care, the clinic has high

patient's turnover. The polyclinic has fifty three (53) beds capacity. It is a Government Facility established in 1992. It is operating as a polyclinic under the management of Ghana Health Service, Ministry of Health (MOH). It recorded a total of 24,254 pregnant women at the antenatal clinic and 4,216 deliveries were attended to in the year 2011(Annual report of Ablekuma sub- metro, 20011).

The second collection site Dangme West District Hospital is an Ultra Modern Hospital with fifty (50) beds capacity. It is Government Facility established in 1970 as a clinic but now upgraded and operating as a hospital under the management of Ghana Health Service, Ministry of Health (MOH). It serves as a major referral center for the people of Dangme West District which have a population of 136,622 with majority of dwellers being farmers. This hospital manages most of the clinical laboratory cases with the only limitation of not performing culture and antimicrobials sensitivity on pathogenic bacteria (Annual report of Dangme West District Hospital, 2010).

The National Public Health Reference Laboratory, Korle-Bu is the head of all Public Health Reference Laboratories in the Ghana. It is a high standard laboratory which controls all the quality assurance procedures for all the laboratories in the country.

3.2 Ethical Clearance

Ethical clearance and approval was obtained from the School of Allied Health Science, University of Ghana (Appendices I). Permission to conduct the study was also from both Dangme West District Hospital and Accra Metropolitan Health Directorates for Mamprobi Polyclinic. And permission for place of work was also sought form National Public Health Reference Laboratory, Korle Bu Teaching Hospital (Appendices II). Written informed consent was obtained from each of the

participating individuals (Appendice III). The study also ensured that no study participant was intimidated or harmed as a result of participating in it.

3.3 *Minimum sample size determination*

The minimum sample size of *pregnant women* that will be screened for GBS carriage will be determined by the formula; $N = \underline{Z^2 (P) (1-P)} / (ERROR)^2$

The prevalence of GSB in Ghana is unknown. The sample size will be based on a population 50%, estimator

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

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

Our minimum sample size, $N = \underline{1.96^2(0.5) (1-0.5)}$

$(5/100))^2$

$= 384.16.$

Minimum sample size = 385 pregnant women.

Granting the critical factors of time and cost, a sample size of 400 pregnant women will be considered and screened for GBS in all. Thus, 200 samples from each study site.

3.4 Inclusion and Exclusion Criteria

All pregnant women in the last trimester of pregnancy (35-37 weeks of gestation) who consented were included in the study. And any person who was not in the last trimester of pregnancy (35-37 weeks of gestation) was excluded.

3.5.1 Collection of Samples

A total number of 400 third trimester pregnant women between 35 - 37 weeks of gestation attending routine antenatal clinics at Mamprobi Polyclinic, Accra and Dangme West District Hospital, Dodowa were involved in the study. In all 400 lower vaginal and 400 rectal swab samples were collected between August 2012 and March 2013. Thus 200 pregnant women's samples were collected from each study site. Two Sterile swab sticks were used to take two different swabs from each person; 1) Swab from the lower vagina (vaginal introitus) and 2) From the rectum (i.e., insert swab through the anal sphincter). The swabs were either taken by qualified health worker or the subject themselves after giving them appropriate instruction (CDC, 2010, Arya *et al.*, 2008). Questionnaires were used to obtain other demographic information such as age, parity, level of education, place of residence and the recent use of antibiotics from the clients.

3.5.2 Specimen Processing and Culturing

The enriched culture medium (ECM) technique (the gold standard) was employed in this study. Immediately samples were collected under aseptic conditions, the swab sticks were transferred into a selective enrichment broth medium thus Todd - Hewitt broth (oxoid LTD) supplemented with gentamicin (8 μg/ml), nalidixic acid (Sigma Aldrich) (15 μg/ml) [TransVag broth] and 5% sheep blood to increase the recovery rate of GBS (CDC, 2010; Joachim *et al.*, 2009). This was transported to the laboratory within three hours of sample collection.

In the laboratory, the inoculated broths were incubated overnight (18-24 hours) at 37ºC. The broths were then sub cultured onto 5% sheep blood agar plate (Liofilchem S.R.L. Bacteriology products) and incubated for 18-24 hours at 37ºC in 5% CO_2. The

plates that had no growth were reincubated for another overnight. Where there were growths the colonies were examined for their characteristic colonial morphology and beta- haemolysis and non haemolytic were also considered. The suspected colonies were Gram stained (Gram positive) and tested for *catalase* (catalase negative) as described by Kaufhold *et al.,* (1989). The suspected colonies were further tested using Group B latex agglutination kit (Latex agglutination test, Statens Serum Institut, Copenhagen, Denmark) to confirm the identity of GBS (Kaufhold *et al.,* (1989).

3.5.3 Gram Staining

Gram staining was performed by making a smear of the specimen to be stained on a slide. The slide was heat fixed for a few seconds until so that bacteria were firmly mounted to the slide. The fixed smear was covered with crystal violet stain for 30-60 seconds and rapidly washed off the stain with clean water. Gram's iodine was added for 30 seconds. The slide was decolourized rapidly with acetone-alcohol. Gram-positive bacteria retained the primary stain whilst Gram-negative bacteria lost the primary stain and appeared colourless. Secondary stain, neutral red was added 15 seconds and wash off stain with water. The smear was examined microscopically, first with the X40 objective to check the staining and distribution of material, and then with the oil immersion objective. Gram-positive bacteria appeared black-violet whilst Gram-negative bacteria appeared red-pink as seen in figure 3.1 below.

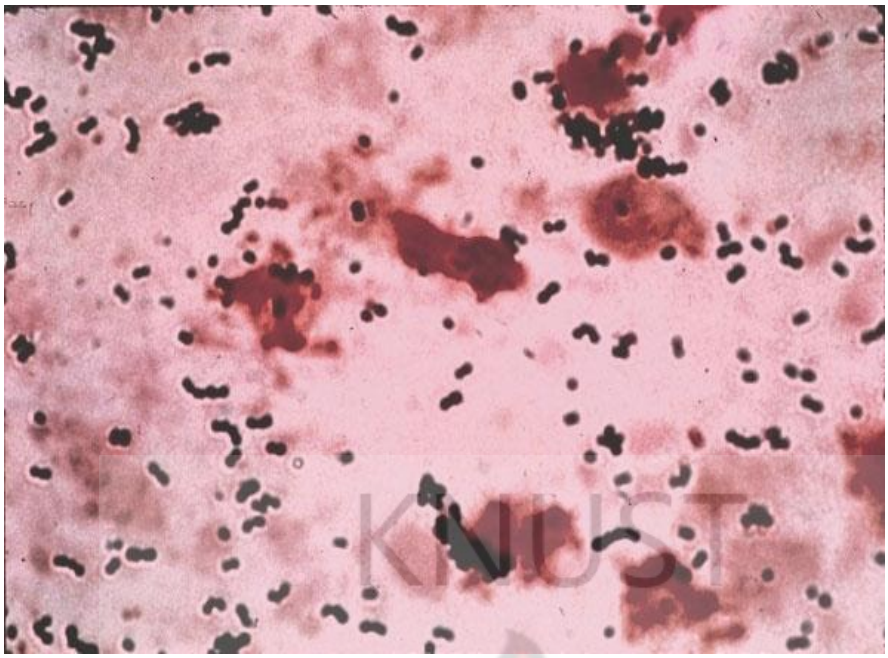


Figure 3.1: Gram Stain Reaction of GBS

Source: ASM Microbe Library CDC, 2010

3.5.4 Catalase Test

Catalase test was performed on the Gram positive cocci to differentiate between *Streptococcus sp.* and *Staphylococcus sp*. This test was used to identify the presence of the catalase enzyme produced by some organisms to decompose the hydrogen peroxide produced as an end product of the aerobic breakdown of sugars. The test was performed by placing a drop of 3% hydrogen peroxide on a glass slide and with the aid of a plastic inoculating loop, touching the bacterial colonies and immersing the loop in the reagent. Catalase positive is indicated by vigorous bubbling which implies the presence of the catalase enzyme as the hydrogen peroxide was converted into water and oxygen. Catalase negative is indicated by the absence of the bubble. A known *Staphylococcus aureus* was used as positive control and *Streptococcus pneumoniae* was used as a negative control.

3.5.5 Species Confirmation and Serotyping

Species confirmation of GBS was performed by Latex agglutination according to the manufacturer's instructions (Statens Serum Institut, Denmark). Briefly, one µl inoculation loopful of bacteria culture from a 24-h culture at 37°C on 5% sheep blood agar (SBA) were suspended in one drop of reagent 1, a drop of reagent 2 was added to the mixture and after waiting for 10 minutes reagent 3 was added. To the reaction card one drop of latest agglutination reagent was added and mixed with 10 µl of the reaction extract from the tube. The mixture on the card was rocked; agglutination within 30 seconds was reported as positive GBS. The confirmed GBS were serotyped. GBS capsular serotyping was performed using the Strep-B-Latex kit (Statens Serum Institut, Denmark). Strains were cultured for 24 h in Todd Hewitt broth. Ten microlitres from this culture was mixed with specific antisera against serotypes Ia, Ib, and II–IX specific to CPS antigens latex agglutination suspension. Agglutination was read after 5 to 10 s as described by Slotved (Slotved *et al.*, 2003).

3.6 Antimicrobial Susceptibility Testing

The GBS that were isolated were tested for their sensitivity to the antibiotics; Ampicillin (10µg), vancomycin (30µg), penicillin (10 IU), cefotaxime (30µg) ceftriaxone (30µg) and erythromycin (15µg) ciprofloxacin (5µg). Kirby-Bauer disc diffusion method was employed (Bauer *et al*, 1966). An inoculums suspension was prepared from a pure culture in a sterile Todd Hewitt broth using an inoculation loop. These were incubated overnight. The turbidity of the inoculums were adjusted and compared with 0.5 McFarland standards so as to obtain a confluent growth. Mueller-Hinton media (Hi Media laboratories Pvt Ltd, India) with 5% sheep blood (from Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon, Accra) plates were inoculated with a loop-full of the prepared inoculums and

streaked by swabbing the surface of the medium three times, rotating the plate through an angle of 60^{O} after each application. Finally, the swab was passed round the edge of the agar surface. The inoculated plates were left for a few minutes at room temperature with the lid closed. This allowed the moisture on the surface to be absorbed into the medium. The antibiotic discs (Abtek Biologicals Ltd) were placed on the inoculated plates using a pair of sterile forceps. Each disc was gently pressed down to ensure even contact with the medium. A control was set up using *S. pneumonia* (ATCC 25923) which was susceptible to all the antibiotics to be tested. The set up was incubated at 35ºC for 20-24 hours in5% CO_2. After overnight incubation, the diameter of each zone inhibition (including the diameter of the disc) of each antibiotic was measured and recorded in millimetres. The results were compared with the zone diameter interpretive standards of the Clinical Laboratory Standards Institute NCCLS, 2006.

3.7 Storage

The isolates were stored at -80 ºC in a broth containing skim milk, tryptone, glucose and glycerol (STGG) at the National Public Reference Laboratory, Korle-Bu.

3.8 Quality Control

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

3.9 Data Analysis

Data were entered into Microsoft Office Excel, and results analyzed using Statistical Package for Social Sciences (SPSS) Version16.

CHAPTER FOUR

RESULTS

4.1 Characteristics of Study Population

Four hundred pregnant women (from 35-37 weeks of gestation) were enrolled in the study between the months of May, 2012 and May, 2013. In all, 800 swabs were taken from the lower vagina and rectum of 400 pregnant women. In this study population, pregnant women aged between 15 and 44 years were enrolled. The mean age of the entire population was 28.5 ± SD5.1, median age was 28.5 and the modal age was 28.0 years. The age range of pregnant women who attended ANC at Mamprobi Polyclinic was 15 to 42 years, with mean age of 27.99 SD ± 5.70 and that of the pregnant women who attended ANC at Dangme West District Hospital was 21 to 44 years, with mean age of 28.97 SD± 4.45years. There was no statistical difference in the mean ages of subjects in both groups (p value > 0.05). The result is as shown in table 4.1.

Table 4.1: Mean Age Comparison of Mamprobi Polyclinic and Dangme West District Hospital

Sites	Number of patients (400)	Age range (years)	Mean age (years)	P value
DWDH	200	15 – 42	27.99 ± 5.70	> 0.05
MPC	200	21- 44	28.97 ± 4.45	

Key: *MPC:* *Mamprobi Polyclinic*

DWDH: *Dangme West District Hospital*

4.2 General Characteristics of Study Isolates

Among the 400 pregnant women that were enrolled in this study, overall 107 (26.8%) were culture positive for GBS in one or both of the collected swab samples. Vaginal carriage rate was higher than rectal carriage. GBS isolated from vagina of 82 women (20.5%) and in 19 women (4.5%) the rectal swab was culture positive. In 6 women (1.5%) GBS was found in both vagina and rectal swab as summarized in table 4.2. The colonization rate of GBS was 28% in pregnant attending ANC at Mamprobi Polyclinic whereas those Dangme West District Hospital (DWDH) 25.5 % pregnant women, showing little variation in colonization rates among pregnant women in rural and urban setting but in the same range as presented in Table 4.3.

Table 4.2: Colonization and Distribution of *S. agalactiae* Isolate at the Two Anatomical Sites

	Women(n)	S. agalactiae culture	Positive anatomical culture site (%)		
		Positive (%)	Vaginal (%)	Rectal (%)	Both (%)
MPC	200	56 (28.0 %)	42 (75%)	10(17.9%)	4(7.1%)
DWDH	200	51(25.5%)	40 (78.4%)	9 (17.6%)	2 (3.9%)
TOTAL	400	107(26.8%)	82(77.4%)	19(17.9%)	6(5.7%)

P- value of log_{10} transformed variables, Students paired t-test P-value P < 0.795

MPC- Number of pregnant women attending ANC at Mamprobi Polyclinic

DWDH- Number of pregnant women attending ANC at Dangme West District Hospital

Table: 4. 3 Comparison of GBS in the two sampling sites

GBS	Dangme West District Hospital (DWDH)	Mamprobi Polyclinic (MPC)
	NUMBER (%)	**NUMBER (%)**
NEGATIVE	149 (75.5)	144 (72.0)
POSITIVE	51 (25.5)	56(28.0)
TOTAL	200 (100)	200(100)

4.3 Association between Gestational Age and GBS Colonization

Table 4.4.1 and table 4.4.2 summarized the rate of GBS colonization by gestational age as obstetric factors. The gestational age of the women ranging from 35-37 weeks was chosen according to CDC guide line. Generally, GBS colonization did not appear to be influenced by gestational age since there was no statistical association between the gestational age and the rate of colonization at the two study sites as the p -values were all greater than 0.05. A high incidence rate was seen among gestational age 35weeks (31%) at MPC and 36 weeks at (36%) at DWDH $P < 0.05$ at both sites**.**

Table 4.4.1: Association between Gestational age and GBS Colonization among Pregnant Women Attending ANC at MPC (n = 200)

Gestational age	Total (%)	Number of GBS isolated	Percentages positive
35	75(37.5)	23	30.7
36	71(35.5)	19	27.8
37	54(27.0)	14	25.9
Total	200(100.0)	56	28.0

Table 4.4.2: Association between and GBS Colonization among Pregnant Women Attending ANC DWDH (n = 200)

Variable: Gestational age	Total (%)	Number of GBS isolated	Percentages
35	55(27.5)	15	27.3
36	76(38.0)	23	30.3
37	69(34.5)	13	18.8
Total	200(100)	51	25.5

Table 4.5 Serotype Distribution of GBS from the Two Sample Collection Sites

Serotypes	DWDH		MPC		Average Percentage colonization
	Number	Percentage	Number	Percentage	
Ia	0	0	1	1.8	0.9
II	0	0	1	1.8	0.9
III	4	7.7	3	5.5	6.6
IV	6	11.5	4	7.3	9.4
V	4	7.7	3	5.5	6.6
VII	20	38.5	23	41.8	40.2
VIII	4	7.7	3	5.5	6.6
IX	14	26.9	18	32.7	29.8

4.4 Comparison between Serotype Distributions from the Two Sample Collection Sites

In this study it has been observed that serotype Ia , II,III,IV, V, VII, VIII, IX were identified as indicated in the figure 4.5 above. The dominant two serotypes identified were VII and IX (40.2% and 29.8%).

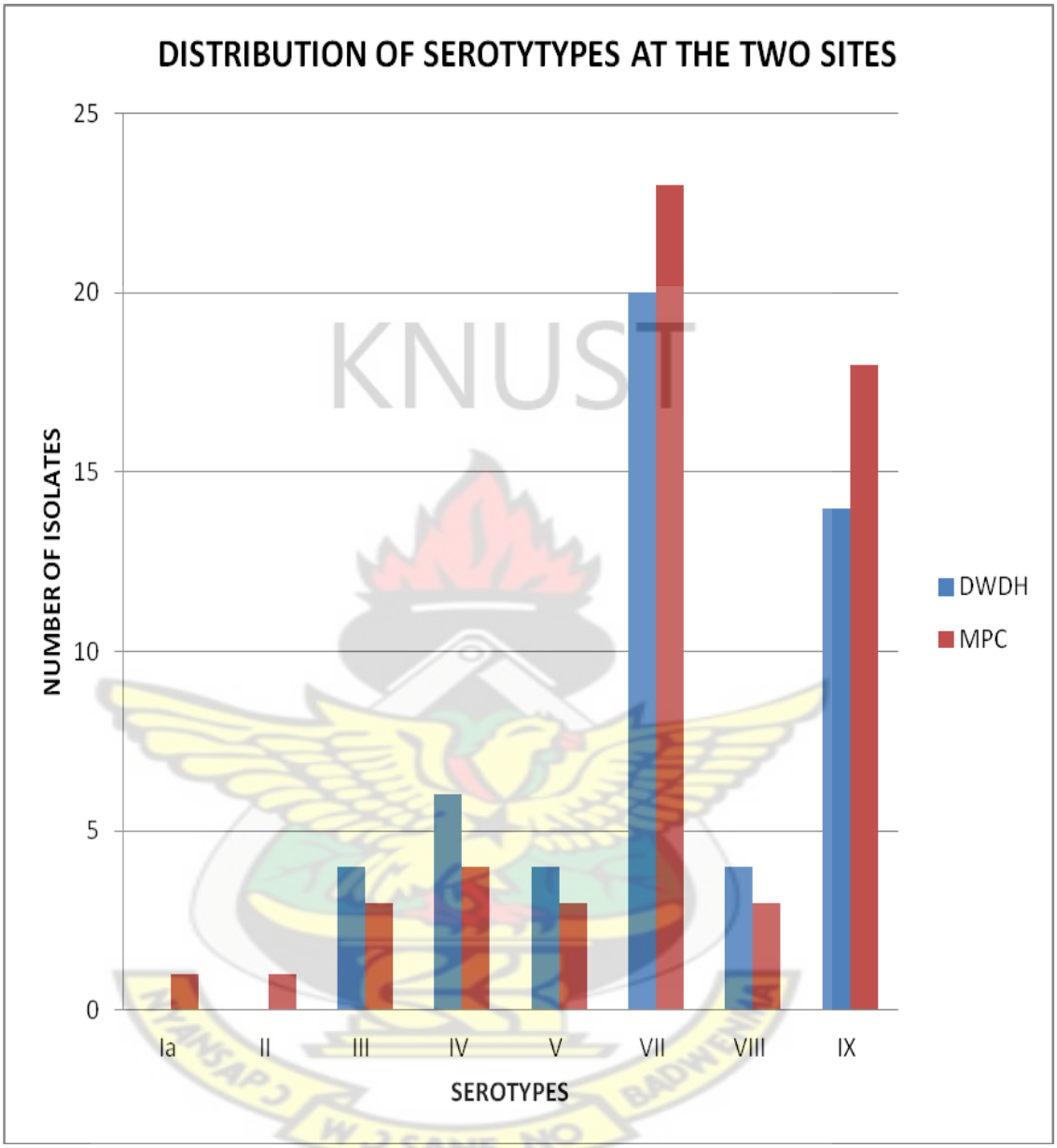


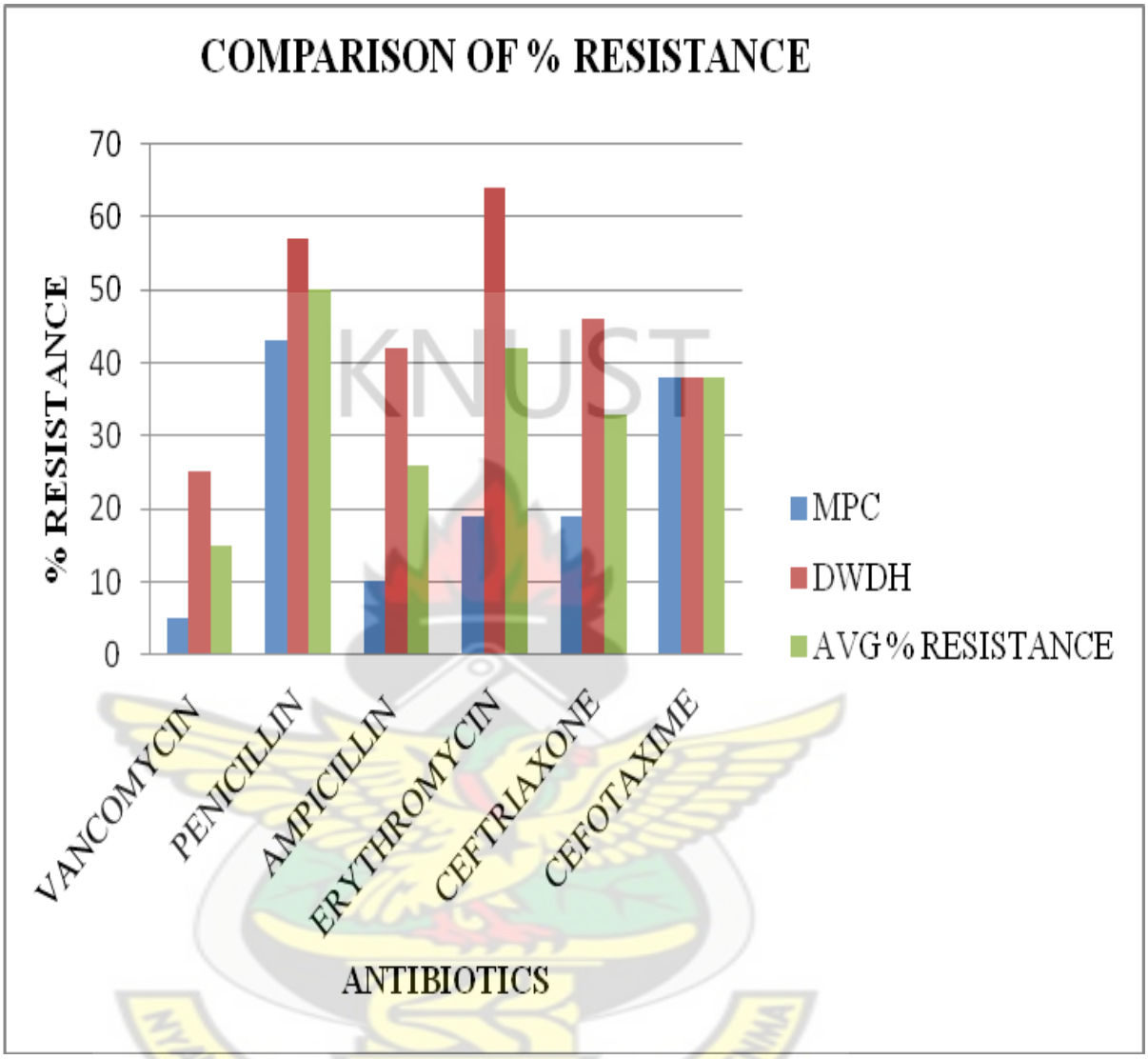
Figure 4.1: Comparison of GBS Serotype Distributions from Sample Collection Sites.

Table 4.5.1: Antibiogram for GBS isolate from Mamprobi Polyclinic (N=60)

ANTIBIOTIC	NUMBER	PERCENTAGE
VANCOMYCIN	3	5
PENICILLIN	27	43
AMPICILLIN	6	10
ERYTHROMYCIN	12	19
CEFTRIAXONE	12	19
CEFOTAXIME	24	38

Table 4.5.2: Antibiogram for GBS isolate from Dangme West District Hospital (n = 53)

ANTIBIOTICS	NUMBER	PERCENTAGE
VANCOMYCIN	13	25
PENICILLIN	30	57
AMPICILLIN	22	42
ERYTHROMYCIN	33	64
CEFTRIAXONE	24	46
CEFOTAXIME	19	38



Key: avg- average

Figure 4.2 Antimicrobial Susceptibility of *Streptococcus agalactiae* Isolated from MPC and DWDH Study Sites.

4.5 Association between Social Demographic Factors and GBS Colonization

Table 4.6.1and Table 4.6.2 summarized the rate of GBS colonization by social demographic characteristics. The age of the women ranged from 16-44 years with mean age of 28.5 ± SD5.1 years. Generally, GBS colonization did not appear to be influenced by maternal age, and education level. The study revealed a higher colonization rate among the age group 35-39years (65% in MPC and 53% in DWDH) but lower (0 %) in women aged 40-45 years, however the difference was not statistically significant (P > 0.05) in both facilities. GBS colonization did not differ significantly with educational status of the women, being 8(25%), 8(32%), 21(26%), 15(30 **%)**and 4(25%) in MPC and 12 (33%), 0(0%),27(31%),8(29%), and3(11 %) in DWDH among None, Primary, Junior high school, Senior high school and Post secondary respectively (P > 0.05).

Table 4.6 Association between Social Demographic Factors and GBS Colonization among Pregnant Women Mamprobi Polyclinic

Variable	Total	Number of GBS isolated	Percentages
Age Group			
<20	14	5	36
20-24	45	7	16
25-29	64	18	28
30-34	47	9	19
35-39	26	17	65
40-45	3	0	0
Education level			
None	32	8	25
Primary	25	8	32
JHS	81	21	26
Secondary	45	15	30
Post secondary	16	4	25

Table 4.7: Association between Social Demographic Factors and GBS Colonization among Pregnant Women DWDH

Variable	Total	Number of GBS isolated	Percentages
Age Group			
<20	0	0	0
20-24	36	4	1
25-29	75	12	16
30-34	68	23	34
35-39	17	9	53
40-45	4	0	0
Education Level			
None	36	12	33
Primary	20	0	0
JHS	88	27	30.7
Secondary	28	8	28.6
Post secondary	28	3	11

4.6 Association between Obstetric Factors and GBS Colonization

Tables 4.8.1 and 4.8.2 summarized maternal obstetric factors and GBS colonization. The parity of the women ranged from zero to nine. Colonization rate was higher (64.3% in MPC and 100% in DWDH) in women who had delivered four or more times and lower in women who had not delivered before or delivered once (16.4, 25.6% in MPC and 0.0, 17.6 % in DWDH). However, this difference was not statistically significant for the people of Mamprobi ($P > 0.05$) but significant for DW DH ($p<0.05$)

Table 4.8.1 Association between obstetric factors and GBS colonization among pregnant women attending ANC at MPC (n = 200)

Variable	Total	Number of GBS isolated	Percentages
Parity			
0	43	11	25.6
1	55	9	16.4
2	41	13	31.7
3	19	6	31.6
4	14	9	64.3
≥ 5	3	1	33.3
Spot abortion			
0	113	45	39.8
1	30	10	33.3
2	11	3	27.3
3	1	0	0.0
4	1	0	0.0
≥ 5	2	0	0.0
Dysuria			
Yes	18	9	50
No	182	9	4.9
SIGN OF STI (DISCHARGE)			
Yes	11	3	
No	187	52	
No Response	2	1	

Table 4.8.2: Association between obstetric factors and GBS colonization among pregnant women attending ANC DWDH **(n = 200)**

Variable	Total	Number of GBS isolated	Percentages
Parity			
0	24	0	0.0
1	68	12	17.6
2	44	12	27.3
3	44	15	34.1
4	8	8	100.0
≥ 5	12	4	33.3
Spot Abortion			
0	132	28	21.2
1	48	16	33.3
2	16	4	25
3	0	0	0.0
4	0	0	0.0
≥ 5	4	3	75
Dysuria			
Yes	12	0	0.0
No	188	51	27.2
SIGN OF STI (DISCHARGE)			
Yes	120	3	
No	12	28	
No response	4	0	

Table 4.8.3: Association between other factors and GBS colonization among pregnant women attending MPC (n = 200)

Vaginal douching	Total	Number of GBS isolated	Percentages
Yes	34	10	29.4
No	165	46	27.9
Total	199	56	28.1
Use of enema	**Total**	**Number of GBS isolated**	**Percentages**
Yes	49	14	28.6
No	144	41	28.5
Toilet			
Yes	107	32	29.9
No	93	24	25.8
Type			
KVIP	117	33	28.2
WC	78	22	28.2
No response	5	1	20

Table 4.8.4: Association between Other Factors and GBS Colonization among Pregnant Women Attending ANC DWDH (n = 200)

Vaginal douching	Total	Number of GBS isolated	Percentages
Yes	36	12	33.3
No	164	24	14.6
Use of enema			
Yes	84	35	41.7
No	116	16	13.8
Toilet			
Yes	128	31	24.2
No	72	20	27.8
Types			
KVIP	151	35	23.2
WC	33	12	36.4
Bush	16	4	25

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION.

5.1 Discussion

Morbidity and mortality due to GBS have been in global focus for some decades now. Several strategies have evolved in attempt to decrease the menace of the GBS disease (EOD and LOD of GBS). Maternal GBS vaginal colonization at delivery is a prerequisite for EOD, vertical transmission is prevented through the administration of antibiotics during labour (intrapartum antibiotic prophylaxis, IAP) (Berardi *et al.,* 2013).

This study sought to determine the carriage and antibiotic susceptibility profile of Group B *Streptococcus* during late pregnancy among two sites in Greater Accra.The investigation indicates an overall high prevalence of 26.8 % *S. agalactiae* colonization in Greater Accra and confirms that GBS colonization is a common finding in pregnant women. The data is similar to studies performed in Tanzania and other European countries that report colonization rates between 10% and 36% (Brimil *et al*., 2006). It is also consistent with reports from other developing countries but slightly higher than Ghana (23%) in the year 2000, Tanzania (23%) and Nigeria (7%) (Enweronu-Laryea *et al.,* 2011; Joachim *et al.,* 2009; Nsagha *et al.,* 2000). The result is higher than the prevalence rates described for some Southern European countries, such as Portugal (6.2%), Turkey (6.5%) and Greece (6.6%) (Florindo *et al.,* 2010). The data is however lower than the data reported from Zimbabwe (31.6%) and Trinidad (32.9%) indicating country variations (Joachim *et al.,* 2009). These variations could possibly be due in part, to differences in sampling

techniques and sites as well as populations investigated. For instance, in this study samples were collected from pregnant women in 35-37 weeks of gestation and the sites of sample collection were the rectum and lower vaginal in accordance with CDC requirement while other investigators took sample in varied weeks and high vaginal swabs (Enweronu-Laryea *et al.,* 2011; Joachim *et al.,* 2009). Other variations in isolation frequency could also be due to differences in type of culture media and culturing techniques used.

Comparing the result of the two study site as in terms of urban and rural, as in table 4.3, it was observed that urban people has a higher colonization than rural inhabitant. Though there was relatively low number of participants, there was no statistical difference in colonization rate between the two areas ($p > 0.05$). The colonization rate in the urban area (pregnant attending ANC at Mamprobi Polyclinic) was 28% and rural (Dangme West District Hospital pregnant women) was 25.5 %. This finding seems to be contrary to what was observed in Zimbabwe by Mavenyengwa *et al.,* which reported that women who live most of the time in rural areas were likely to be significantly more often colonized with GBS than those who lived in urban areas. The risk was attributed to poor hygiene and increased association with animals such a cattle (Mavenyengwa *et al.,* 2010). But this was not observed in this rural setting (Dangme West District); moreover this area is not noted so much for rearing of such animals identified in the other countries like Zimbabwe. Notwithstanding this study could not find out how long the people had stayed in the area of research. Further study including larger number of sample size may be conducted to check the colonization rate of GBS in such animals and the association between human and the animal in Ghana.

In this study vaginal carriage rate (22%) was higher than rectal colonization rate (6.3%), the rectal colonization rate is comparable to study done in Tanzania and Zimbabwe (5% and 6.3% respectively), but that of vaginal swab is comparatively higher than the two (12.3% and 12.6% respectively) (Joachim *et al.,* 2009). When screening for GBS carriage in pregnant women it is important to sample both vagina and rectum since GBS was at times isolated from one and not the other site.

Table 4.4 and table 4.5 summarized the rate of GBS colonization by gestational age as obstetric factors. The gestational age of the women ranged from 35-37 weeks according to CDC with mean age of 36 weeks (SD ± 5.1). Generally, GBS colonization did not appear to be influenced by gestational age since there was no statistical association between the gestational age and the rate of colonization at the two study sites as the p -values were all greater than 0.05. A high incidence rate was seen among gestational age 35 weeks (31%) at MPC and 36 weeks at (36%) at DWDH $P < 0.05$ at both sites.

This study also looked at the prevailing serotypes in Ghana. From this study serotypes; Ia, II, III, IV, V, VII, VIII and IX were isolated whilst serotype Ib and VI were not identified in the study. As indicated in the table 4.6 serotype VII (43; 40.2 %) and IX (32; 29.8%) showed the highest prevalence (70%), the lowest were Ia and II (2; 1.9%). This confirms that serotype distribution varies with geographical region and ethnic grouping within a country (Mavenyengwa *et al.,* 2010; Barcaite *et al.,* 2008).

From other studies performed in Africa, serotype Ia, III and V were more prevalent while types VI, VII, VIII and IX occur rarely if at all in the Zimbabwean population. In Gambia low frequency of serotype III and high frequency of type II and type V strains were observed (Moyo *et al* 2002). However data from US and Europe have shown that the serotypes Ia, II, III, and V are found in 80–90% of all clinical isolates, while serotypes IV, VI, VII, and VIII are only rarely observed (Diedrick *et al.,* 2010) and in Japan serotypes VI and VIII predominated. Other studies conducted in Europe and South Africa indicated that, serotype III is more virulent than others. Here in Ghana a further studies needs to be conducted to check the virulence of the VII and IX as the highest prevailing serotypes.

From the two sites there was no much difference in seroprevalence. The only slight difference was; serotype Ia and II were found in the urban area but not in the rural area and this may be due to the cosmopolitan nature of the urban area. A vaccine containing serotype IV, VII and IX could become effective for about 80% of the population in Ghana if the virulence is known to be high.

This study also sought to investigate the antibiogram of the bacteria isolated. From fig. 4.2, GBS showed some form of resistance to all the antibiotics tested. The GBS isolates on average showed resistance to vancomycin, penicillin, Ampicillin, erythromycin, ceftriaxone and cefotaxime. Comparing the antibiogram (Figure 4.2) from the two study site in all it appears the rural site has higher resistance as compared to the urban site. This could be due to the high usage of other local medicine which might contain some residues of the antibiotics used in the study.

However, 31.9% of the isolates were resistant to erythromycin, which is similar to the prevalence of resistance among invasive GBS isolates in the United States, which ranged from 25% to 32% for erythromycin in reports published during 2006–2009 (CDC, 2010) but a higher proportion than that reported from the United Kingdom (4%**),** Zimbabwe (14%), France (21.4%), and Malawi (21%) (Gray *et al.,* 2007). GBS (50.4%) resistance to penicillin in this study is higher than observed by Enweronu-Laryea *et al*, and the difference may be due to increased use and misuse of the antibiotic as chemoprophylaxis(Enweronu-Laryea *et al.*, 2011).

Generally, there seems to be varied association between social demographic factors such as age, educational status and GBS colonization among the pregnant women. The study revealed a higher colonization rate among the age group 35-39 years (65% in MPC and 53% in DWDH). This finding is similar to what was identified in Tanzania by Joachim *et al.* where GBS was more frequently isolated from women of age group 30-34 (32.1%) compared with women aged less than 20 years. Also, no GBS was isolated in women aged 40-45 years in both districts. These could be due to the fact that only few (7) pregnant women were recorded for the ages of 40-45 years enrolled in the study. GBS colonization did not differ significantly with educational status of the women at both sites. Colonization with GBS was slightly higher in the uneducated 33% in DWDH and 32% primary level of education. This showed that women with less formal education were more likely to be colonized with GBS, a finding that is consistent with observations by Joachim et *al*. The association could be partly explained by the difference in personal hygiene, which is more likely to be better among educated than the less educated women. These disparities in social demographic factor are hard to explain but possibly be due to the fact that GBS

colonization might be influenced by multiple factors which may vary from one geographical location to another.

Tables 4.8 and 4.9 summarized the association between maternal obstetric factors and GBS colonization among pregnant women. The parity of the women ranged from zero to nine. For obstetric factors colonization rate was higher in women who had delivered four or more times and lower in women who had not delivered before or delivered once. However, this difference was not significant for the women of Mamprobi but significant for DW DH. This shows that more work needs to be done to analyze the relationship between the parity, other obstetric (abortion, dysuria and sign of UTI) and factor such as vaginal douching, use of enema, and availability of personal toilet facility.

5.2 Conclusion

In conclusion, the results of the study have indicated a high prevalence (26.8%) of GBS colonization among pregnant women. Colonization rates in both urban (28.0%) and rural (25.5%) areas were found to be high with no significant differences between the rates in the urban and rural localities. Apart from serotype Ib and VI, all other serotypes (Ia, II, III, IV, V, VII, VIII, IX) were identified with the dominant serotype being VII (40.2%) and IX (29.8%).

The GBS isolated were highly resistant to the commonly used antibiotics; penicillin, erythromycin, ceftriaxone and cefotaxime and less resistant to vancomycin and Ampicillin.

There were varied associations between social demographic factors (age, educational status and place of residence), obstetric factors (parity, abortion and STI) other factors (used of enema, toilet facility and vagina douching) and GBS colonization.

The study indicates that there is a need for screening of pregnant women attending antenatal care and to know their antibiotic susceptibility so that appropriate intrapartum antimicrobial prophylaxis can be offered to all women identified as carriers. Further studies are required to investigate the presence of antibodies to the serotype and their immunobiological function in pregnant women.

5.3 Recommendation

1. Since GBS has acquired resistance to several of the commonly used antibiotics, such as erythromycin and penicillin, prevention protocols other than intrapartum chemoprophylaxis must be utilized to prevent neonatal disease.

2. Further, the prevalence in pregnancy is high and currently, there is no established prevention protocol for this population. Therefore, future works should focus on gaining a better understanding of the genetic profiles of the more virulent GBS strains in circulation nationwide.

3. Women should be educated on GBS infection and the need to improve on personal hygiene.

4. Also screening should be incorporated in the ANC screening package for the women with termed pregnancy.

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APPENDIX I

SCHOOL OF ALLIED HEALTH SCIENCES
COLLEGE OF HEALTH SCIENCES
UNIVERSITY OF GHANA
ACADEMIC AFFAIRS

Phone: +233-0302-687974/5
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My Ref. No. SAHS/BC/AA/26

Your Ref. No.

P. O .Box KB 143
Korle Bu
Accra
Ghana

9th May, 2012

Ms. Josephine Afi Nukpedu Banini,
Dept. of Microbiology,
KNUST,
Kumasi.

Dear Ms. Banini,

ETHICS CLEARANCE

Ethics Identification Number: SAHS – ET./SAHS/BC/AA/26/2012-2013.

Following a meeting of the Ethics and Protocol Review Committee of the School of Allied Health Sciences held on Tuesday 8th April, 2012, I write on behalf of the Committee to approve your research proposal as follows:

TITLE OF RESEARCH PROPOSAL: **"Carriage and antibiotic susceptibility profile on group B streptococcus during late pregnancy among selected hospital in Greater Accra".**

This approval requires that you submit six-monthly review reports of the protocol to the Committee and a final full review to the Committee on completion of the research. The Committee may observe the procedures and records of the research during and after implementation.

Please note that any significant modification of the research must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this research to the Committee within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this research. You will therefore, be required to furnish the Committee with any manuscript for publication.

Please always quote the ethical identification number in all future correspondence in relation to this protocol.

Thank you.

Yours sincerely,

Dr. (Maj. Rtd.) George Asare
(Chairman, Ethics and Protocol Review Committee)

cc Ag. Dean
Senior Assistant Registrar

APPENDIX 2

In case of reply the Number and date of this letter should be quoted.

My Ref. :AM/99
Your Ref. No.

Metro Health Directorate
Ghana Health Service
Private Mail BagTUPM 14
TUC Post Office
Accra

Tel: (Main Line) 233-21-665879
(Direct Line) 233-21-687000
(Fax) 233-21-680575
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accrametrohealthdirectorate@yahoo.com

16th January 2012

The Specialist i/c
Mamprobi Polyclinic
Accra

Dear Sir/Madam,

LETTER OF INTRODUCTION
Mrs Josephine Afi Nukpedu Banini- STUDENT

This is to introduce to you the above mentioned student of the Kwame Nkrumah University of Science and Technology College of Health Sciences, Accra.

She has given permission to collect data for her research on the topic **"Vaginal Carriage and Antibiotic Susceptibility Profile of Group B Streptococci (GBS)".** This is to help her complete her project work.

Kindly give her the necessary assistance.

Thanks in advance

Yours faithfully

Dr. George Mensah
(Deputy Director of Metro Health– Accra)

APPENDIX I

QUESTIONAIRE

VAGINAL CARRIAGE AND ANTIBIOTIC SUSCEPTIBILITY PROFILE OF GROUP B STREPTOCOCCUS DURING LATE PREGNANCY AMONG SELECTED HOSPITAL IN ACCRA METROPOLIS

1. Identification number…… Date ……..
2. Age…….
3. Level of education……Illiterate, Primary, JHS, SHS, Tertiary.
4. Occupation…… Income……
5. Income of spouse ……
6. Gestational Age…… (3rd trimester: 35,36, 37, 38, 39, 40, 41……weeks)
7. Number of Pregnancies (gravid)……Para… Abortion….. Spot….. Induced….
8. Parity…….
9. Area of Residence……
10. Ethnicity (Ga / Ewe / Asanti / Fanti /Dagomba / other
11. Do you do vaginal douching? (Yes /No)
12. With what?
13. If yes how often (a.Within the week, b. last week , c. a month, d. three month, e. six month,f. a year or more)
14. Do you use enema(Yes /No)
15. If yes how often (Within the week, last week, a month, three month, six month, a year or more)

16. Is the apparatus (personal /public)

17. Are you on any form of antibiotic (Yes/No) If yes then give name

18. Have you been diagnosed of having UTI during pregnancy before?

    Yes/No

19. Have you been treated for any UTI in the recent past? Yes/No

20. Are you diabetes? Yes/No

21. Do you have the following conditions? A. Urgency in passing urine? Yes/

    No b. Dysuria? Yes/ No c. Frequency of urination? Yes /No

22. Do you have a toilet facility in the house? Yes/No

23. If yes, what type of toilet facility? WC/KVIP

APPENDIX II

GBS RESEARCH CONSENT FORM

Name(s) and affiliation(s) of researcher(s): Prof. E.H. Frimpong Department of Clinical Microbiology, KNUST, PHD, Senior Scientist Hans-Christian Slotved ,Department of Microbiological Surveillance and Research, Statens Serum Institut, Denmark. And Banini Josephine Afi Nukpedu , Department of Clinical Microbiology, KNUST.

Research title: Carriage and Antibiotic Susceptibility Profile of Group B Streptococcus during Late Pregnancy among Selected Hospital in Greater Accra.

Background: *Streptococcus agalactiae* is a bacterium that causes a lot of serious diseases in neonates and adults with under lying medical conditions. They are found in the vagina and rectum of some people, from where they can spread and course infection. During birth this organism can be transfer to the baby thereby coursing GBS early onset infections in neonates. It is therefore important to investigate the carriage and antimicrobial susceptibility of these bacteria in Ghana. The collection process will involve a routine clinical procedure by qualified medical personnel.

Purpose(s) of research: The purpose of this work is to determine the incidence of rectovaginal carriage and antibiotic susceptibility profile of Group B Streptococcus and prevailing serotypes during late pregnancy among selected hospital in Greater Accra.

Procedure of the research, what shall be required of each participant and approximate total number of participants that would be involved in the research: We will pick consecutive participants who agreed to enrol in this study you should be in the third trimester of pregnancy (35 - 37 weeks of pregnancy). Each

participant will give us two swabs one vaginal and the other one rectal swab. Qualified medical personnel will be available to take the sample for you. You will be asked to answer questionnaire with a few questions. And this is only one time sample collection study. In total we expect to enroll 400 participants into this study.

Risk(s): There is no risk to you personally other than the minor and temporary discomfort you will have during swabbing. You unborn child is safe since the swab will be taken at the lower part of the vagina and the rectum

Benefit(s): There may be no personal benefit to you. However, data gathered from this study will inform health policies makers regarding the introduction of GBS screening during antenatal clinic visit. The antimicrobial susceptibility of the organisms to penicillin which is the drug of choice.

Knowledge of local susceptibility patterns will guide clinicians in choosing the right antibiotics for treatment of suspected GB*S* infections because once resistant strains develop they are transmitted to new carriers and maintained in the population.

Confidentiality: All collected materials and information from you will be coded using numbers and letters. Your privacy will be maintained in all published and written data resulting from the study.

Voluntariness: Participating in this study will be out of your own free will. You are not oblige to enrolled Participants after giving consent are at liberty to refuse to answer any question or withdraw from the study completely at any stage without having to give reasons for the withdrawal.

Alternatives to participation: If you choose not to participate, there is no penalty for withdrawal from the study.

Duration of Study: Samples will be collected only once within a two year period of study.

Confidentiality: All information gathered in this study will be kept confidential. When results of this study are published you will not be identified.

Consent for inclusion: If you agree to the inclusion of yourself in this study, please complete the form below;

I …..…… on this day …… (Day/Month/Year) attest that I understood the explanations given in the consent form and thus give permission to **Mrs. Josephine A. N. Banini** to include me in the research study entitled

"Carriage And Antibiotic Susceptibility Profile of Group B *Streptococcus* during Late Pregnancy among Selected Hospital in Greater Accra region".

Signature of client……

Contact Address……

Phone number: ……

Thumb Print (Where required)

Investigator's Name and Signature_____

Date._____

Problems and Question: If you have any problem or question about this study, please contact me or my supervisor

Phone number: 0208187460

Phone number: (supervisor) 0208124866

APPENDIX III

Preparation of 0.5McFarland Standard

i.Composition Barium Chloride (BaCl2) 1.7g, Sulphuric acid (H2SO4) 0.35M Spectrophotometer, Distilled water 4ml.

ii.*Preparation* 1.17% weight per volume of hydrated Barium Chloride (BaCl2. 2H2O) was prepared to make a concentration of 0.048M BaCl2. A 1% volume per volume of sulphuric acid (H2SO4) was also prepared to a final concentration of O.18M H2SO4. 0.5ml of the Barium Chloride preparation was added to 99.5ml the O.18M H2SO4 solution with constant stirring. The turbidity standard was vigourously shaken on vortex mixer. The turbidity of the final preparation was measured with a spectrophotometer to confirm a 0.5McFarland standard. Absorbance at 625nm was between 0.008 to 0.01. The standard was distributed into screw cap tubes and tightly sealed to prevent loss by evaporation. The prepared 0.5McFarland standard was stored at room temperature protected from light.