

**PREVALENCE OF GROUP B *STREPTOCOCCUS* COLONIZATION  
AMONG PREGNANT WOMEN ATTENDING ANTENATAL  
CLINIC OF HAWASSA HEALTH CENTER, HAWASSA, ETHIOPIA**



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**A Thesis submitted to the School of Graduate studies Addis  
Ababa University in partial fulfillment of the requirements of  
Masters degree in Medical Microbiology**

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## **Acknowledgements**

I would like to thank my supervisors, Dr. Daniel Asrat (MD, M.Sc, PhD), and Dr. Yimtubezenash W/Amanuel (MD, M.Sc, PhD) for their spectacular help starting from topic selection, proposal development, and the actual project work and final write up.

I am also grateful to my collaborator Ato Demisse Assegie and Ato Getahun Hylemeskel for being with me during Laboratory work and providing me with most basic laboratory equipment and basic culture media required.

I also would like to thank Sister Aynelem Chawicha (midwifery) for her assistance during specimen collection.

My special gratitude goes to David William for providing me Lancefield classification antisera and also to Pegah Hafiz for bringing Antisera from United Kingdom to Ethiopia.

I also would like to thank SETEMA PLC for their cooperation in timely arrival of Todd Hiowtii broth with antibiotic which was very important for the study.

I am indebted to AAU, School of graduate studies for the financial support to conduct this project and I also present my gratitude and respect to all staff of Microbiology, Immunology and Parasitology department for basic theoretical background they provided me, which I found strong spring board for my future career.

I would like to thank Hawassa University for the sponsorship.

<b>TABLE OF CONTENTS</b>	<b>PAGE</b>
Acknowledgements.....	I
Table of contents.....	II
List of tables.....	IV
List of figures.....	V
Abbreviations.....	VI
Abstract.....	VII
 <b>CHAPTER I: INTRODUCTION</b>	
1.1. General Introduction.....	1
1.2. Literature review.....	2
1.2.1. Historical perspectives.....	2
1.2.2. Microbiology of Group B <i>Streptococcus</i> .....	3
1.2.3. Epidemiology of Group B <i>Streptococcus</i> .....	4
1.2.4. Pathogenesis of Group B <i>Streptococcus</i> infection.....	7
1.2.5. Maternal colonization.....	9
1.2.6. Clinical features.....	9
1.2.7. Laboratory detection of GBS colonization and infection.....	11
1.2.8. Management of GBS colonization and infection.....	14
1.2.9. Prevention.....	16
1.3. Significance of the study.....	17
1.4. Hypothesis.....	19
1.5. Objectives of the study.....	20
 <b>CHAPTER II: MATERIALS AND METHODS</b>	
2.1. Study Design and Area.....	21
2.2. Study Population.....	21
2.3. Sample Collection, Handling and Transport.....	21
2.4. Culture of GBS using Selective Enrichment broth.....	22
2.5. CAMP test.....	22
2.6. GBS latex agglutination test.....	24
2.7. Antimicrobial Susceptibility Testing.....	24
2.8. Quality control.....	25
2.9. Statistical Analysis.....	26
2.10. Ethical Consideration.....	26

<b>CHAPTER III: RESULTS</b>	
3.1. Study subjects.....	27
3.2. GBS culture.....	29
3.3. Antimicrobial susceptibility .....	33
<b>CHAPTER IV: DISCUSSION.....</b>	<b>34</b>
Limitation of the study.....	38
<b>CAPTER V CONCLUSION AND RECOMENDATION</b>	
5.1 Conclusion.....	39
5.2 Recommendations.....	39
References.....	40
Appendix I.....	48
Appendix II.....	50

<b><u>LIST OF TABLES</u></b>	<b><u>PAGE</u></b>
Table 3.1. Socio-demographic characteristics of pregnant women investigated for Group B Streptococcus, Hawassa Health center, Hawassa, Ethiopia.....	32
Table 3.2b Variables associated/not associated with Group B Streptococcus colonization in pregnant women, Hawassa Health center, Hawassa, Ethiopia.....	33
Table 3.3. Antimicrobial susceptibility pattern of 29 Group B Streptococcus isolated from pregnant women, Hawassa health center, Hawassa, Ethiopia.....	34

<b>LIST OF FIGURES</b>	<b>PAGE</b>
Figure 2.3 Site of rectovaginal specimen collection for GBS isolation.....	25
Figure 2.1. Streaking pattern for the CAMP test.....	27
Figure 2.2. CAMP test for the identification of <i>Streptococcus agalactiae</i> (group B)....	27

## ABBREVIATIONS

ABCs	Active Bacterial Core surveillance/Emerging Infections
ANC	Antenatal care
ATCC	American Type Culture Collection
CAMP	Chrstie, Atkins, Munch, Peterson
CBC	Complete Blood count
CDC	Center for Disease Control
CD14	Cluster differentiation 14
CNS	Central Nervous System
CRP	C - reactive protein
DNA	Deoxyribose Nucleic Acid
EHNRI	Ethiopian Health National Research Institute
EOD	Early Onset Disease
EOGBS	Early Onset Group B <i>Streptococcal</i>
FDA	Food and Drug Administration
GBS	Group B <i>Streptococcus</i>
IAI	Intrapartum Amniotic Infection
IAP	Intrapartum Antibiotic Chemoprophylaxis
IDI	Infectio Diagnostic Inc
IL	Interleukin
LOS-GBS	Late Onset Group B <i>Streptococcal</i>
LOD	Late Onset Disease
NCCLs	National Committee for Clinical Laboratory Standards
PBS	Phosphate Buffer solution
PCR	Polymerase Chain Reaction
PLC	Private Limited Company
PPE	Postpartum Partum Endometritis
<i>S. agalactiae</i>	<i>Streptococcus agalactiae</i>
SNNP	South Nations Nationality and People
TNF $\alpha$	Tumor Necrosis Factor $\alpha$
US	United States
AAU	Addis Ababa University

## ABSTRACT

**Background:** Group B *Streptococcus* (GBS) or *Streptococcus agalactiae* are members of the normal flora of the female genital tract. During labor GBS may infect the newborn, leading to neonatal sepsis and meningitis. GBS emerged in the 1970s as one of the most frequent causes of sepsis and meningitis in neonates and young infants. Since the mid-1960s, GBS has become the major cause of bacterial infections in the perinatal period, including bacteraemia, amnionitis, endometritis, and urinary tract infection in pregnant women. Rates of GBS colonization vary widely throughout the world due to differences in laboratory investigation methods, regional variations and racial differences. Report on prevalence of GBS among pregnant women in Ethiopia is very limited.

**Objectives:** The objective of this study was to determine the prevalence of group B Streptococcal (GBS) colonization among pregnant women attending antenatal clinic at Hawassa Health centre, Hawassa Ethiopia and analyze risk factors related to GBS colonization.

**Methods:** A total of 139 pregnant women were screened for GBS colonization between May and June 2010. Standard microbiological methods were used to isolate and identify GBS from vaginal and anorectal swabs obtained from study subjects. Antimicrobial susceptibility test was performed for all GBS isolates according to the criteria of the National Committee for Clinical Laboratory Standards by disk diffusion method.

**Results:** A total of 29 out of 139 pregnant women studied (20.8%) were colonized by GBS. No statistically significant association was observed for GBS colonization with any of sociodemographic characteristics of the study subjects including age, occupation, type of contraceptive used, types of gravida, number of antenatal clinic visits etc. All GBS strains were susceptible to penicillin, ampicillin, vancomycin and gentamicin, Low level of resistance (<60%) were observed against erythromycin, tetracycline, ceftriaxone, chloramphenicol, ciprofloxacin and norfloxacin.

**Conclusion and recommendations:** this study including 139 pregnant women, confirmed the prevalence of GBS colonization to be around 21%. This prevalence was compared with findings reported from developed and developing countries and have reached comparable level. However, further epidemiological investigations should be conducted in different parts of the country in order to know the actual GBS colonization rate in pregnant women and consider implementation of prevention plans using intrapartum antibiotics prophylaxis to prevent early onset GBS-neonatal diseases.

Key words: Group B *Streptococcus* (GBS), risk factors, Antibiotic susceptibility, Ethiopia.

## CHAPTER I: INTRODUCTION

### 1.1. General Introduction

Since the mid-1960s, group B *Streptococcus* (GBS) has become the major cause of bacterial infections in the perinatal period, including bacteraemia, amnionitis, endometritis, and urinary tract infection in pregnant women as well as focal and systemic infections in newborns (Samuel, 2002). It is a relatively rare cause of infection in older children and non-pregnant adults (Apgar *et al.*, 2002). Initial case series reported case-fatality ratios as high as 50%. In the early 1980s, clinical trials demonstrated that administering antibiotics during labor to women at risk of transmitting GBS to their newborns could prevent invasive disease in the first week of life (Boyer and Gotoff, 1986).

Since the 1970s, GBS has been recognised as the most important infectious cause of morbidity and mortality in newborn infants. Despite decrease in mortality during the last decades, early onset GBS disease remains a serious neonatal condition, which may cause severe neurological damage (Valkenburg-van *et al.*, 2006). Today, it is also the leading cause of early invasive infections in new-borns worldwide (CDC, 1996) and can also cause life-threatening infections in pregnant women, in immunocompromised adults, and apparently in the general population as a whole (CDC, 1996). Group B *Streptococcus* transmission is vertical from mother to child. The gastrointestinal tract is the source of vaginal GBS colonisation and many adults are colonised with GBS without showing symptoms. Approximately 10–30% of women of child bearing age carry GBS in the rectovaginal compartment (Regan *et al.*, 1991).

The publication of treatment and prevention guidelines by the U.S. Centers for Disease Control and Prevention in 1996 (CDC, 1996) led to a significant decline in the incidence of early-onset neonatal disease in institutions that adopted and followed these guidelines strictly (Heather and Lahra, 1998). The CDC recommendations are to screen the entire maternal population proximal to labor (35-37 weeks of gestation) and to administer intrapartum prophylactic antibiotics to all carriers. In addition, if the maternal carrier status is not known at labor, chemoprophylaxis should be administered to all patients with one or more major risk factors indicated by the CDC and others as being

significantly related to higher rates of maternal transmitted neonatal disease (CDC, 1996).

Treatment of colonized mothers succeeded in temporarily eradicating the organism, but most of the women were re-colonized within 6 weeks. At birth, 50 to 65% of infants who are born from colonized mothers have positive GBS cultures from mucus membranes and skin (external ear canal, throat, umbilicus, anorectal sites) (Shet and Ferrieri, 2004) Approximately 98% of colonized newborns remain healthy, but 1 to 2% developed invasive GBS infection (Baker and Edwards, 2001).

Published information on prevalence of colonization of GBS among pregnant women in Ethiopia is scarce (Schmidt *et al.*, 1989). Therefore the present study was conducted to determine the magnitude of colonization of GBS among pregnant women attending antenatal clinic of Hawassa health center Hawassa, Ethiopia.

## **1.2. Literature Review**

### **1.2.1. Historical Perspectives**

In 1933, Rebecca Lancefield noted that many *Streptococci* could be serologically identified and grouped according to their cellular carbohydrates or so-called C substances. Initially, from 106 strains of  $\beta$ -hemolytic *Streptococci* studied, 5 antigenically distinct *streptococcal* groups were identified and were designated as A, B, C, D, and E. Since then, the number of *streptococcal* groups has steadily increased (A through H, K through V (Nandyal, 2008).

Although the Lancefield grouping was originally utilized to distinguish among the  $\beta$  hemolytic *streptococci*, it should be noted that hemolysis and Lancefield serogrouping are not mutually exclusive. For example, group C *streptococci* can be  $\alpha$ ,  $\beta$ , or nonhemolytic. Even though group B *streptococci* are frequently referred to as “ $\beta$ -*Strep*” actually group A *streptococci*, group B *streptococci*, group D *streptococci*, group G *streptococci*, and other groups of *streptococci*, all belong to the collective term “ $\beta$ -Hemolytic *Streptococci*” or “ $\beta$ -*Strep*.” (Nandyal, 2008).

In 1935, Lancefield and Hare first isolated group B *streptococci* from the birth canal of postpartum women, and in 1938 Fry found group B *streptococci* in vaginal cultures from both symptomatic and asymptomatic women at the time of delivery (Nandyal, 2008). Despite these early observations, there was a hiatus of interest in GBS, and for many years it was considered the primary organism responsible for mastitis in cows and was therefore named *Streptococcus agalactiae*. Eickhoff stressed the importance of GBS

as a cause of neonatal infection in 1964. By the late 1970s, GBS surpassed *Escherichia coli* as the leading cause of early-onset neonatal sepsis in the United States (Nandyal, 2008).

### **1.2.2. Microbiology of Group B *Streptococcus***

Group B *Streptococcus* (GBS) is an encapsulated gram-positive diplococcus that usually produces a narrow zone of beta-haemolysis on blood agar. Most strains are resistant to bacitracin. Group B *streptococci* belongs to Lancefield group B. Group B *streptococci* can be sub typed into at least 9 capsular polysaccharide serotypes; type Ia, Ib, I a/c, I b/c, II, III, IV, V, VI. Among these, Ia, Ib/c, Ia/c, II, III, and V being the most common serotypes in the United States (O'Brien *et al.*, 2007).

The serotype distribution of isolates causing both neonatal and adult disease has shifted in the past decade. The earlier dominance of serotype III in early and late onset neonatal disease has given way to a more balanced distribution between serotype Ia (35%-40%), type III (30%), and type V (15%-20%) in early onset neonatal GBS disease (O'Brien *et al.*, 2007). Types Ia, III, and V are also currently the most common serotypes in adult disease, in nearly equal proportions (O'Brien *et al.*, 2007).

The serotype distribution appears to be continuously evolving, making ongoing surveillance essential to vaccine-development efforts. Minor geographic variations in serotype distribution have been noted in the United States and Canada but more significant differences have been reported from Japan (O'Brien *et al.*, 2007). In the US and Western Europe, types Ia, II, and III accounted for 85% of the isolates from infants (Dillon *et al.*, 1987). Studies in the US have also demonstrated that serotypes Ia, III, and V accounted for 78-87% of early-onset invasive disease in newborn infants and parturient women (Zaleznik *et al.*, 2000). Study from India show a variable distribution of serotypes, but the most common isolates belong to types III, II and Ib (Shet and Ferrieri, 2004). From Study conducted in seven prenatal clinics in six different countries it was shown serotype III and V were most common one (Whitney *et al.*, 2004).

Study from public and private hospital in Greece showed serotype II (26.9%), III (22.4%), Ia (19%), Ib (12%), and V (9%) to be the most common serotypes identified. A considerable proportion of the isolated strains were also found to be resistant to erythromycin (4.5%), clindamycin (6%), or both (6%) (Tsolia *et al.*, 2003).

Study from Malawi showed that Serotype III (56%) and serotype Ia (21%) were the most frequently identified serotypes; they constituted 77% of both early onset disease

(EOD) and late onset disease (LOD) (Gray *et al.*, 2007). Study done in Zimbabwe to investigate the distribution of capsular polysaccharide (CPS) types and subtypes (serovariants) revealed serotype; Ia (15.7%), Ib (11.6%), II (8.3%), III (38.8%), V (24.0%), and non typeable (1.7%) (Mavenyengwa and Maeland, 2008). In study conducted in Gondar, Ethiopia the sero distribution among the 25 isolated strains showed 60% to be type Ib/c and 16% to be type Ia strains (Schmidt *et al.*, 1989).

### **1.2.3. Epidemiology of Group B *Streptococcus***

Vaginal colonization is usually from gastrointestinal tract which serve as the natural reservoir for GBS and is most likely source of vaginal colonization. Colonization is unusual in childhood but becomes more common in late adolescence (CDC, 2002). Approximately 10% to 30% of pregnant women are colonized with GBS in their vagina or rectum. Maternal intrapartum GBS colonization is a major risk factor for early-onset disease in infants, and vertical transmission of GBS from mother to fetus primarily occurs after the onset of labor or membrane rupture. However, colonization early in pregnancy is not predictive of neonatal sepsis (CDC, 2002).

During the 1970s and 1980s, GBS emerged as a significant neonatal and maternal pathogen in the United States (US) and Western Europe with reported mortality rates of 15 to 50% (Shet and Ferrieri, 2004). In the US, 10 to 35% of pregnant women are asymptomatic carriers of GBS in the genital and gastrointestinal tract at the time of delivery (Shet and Ferrieri, 2004). Prevalence of maternal carriage of GBS in developing countries, including populations in tropical Africa, is almost similar to that identified in populations in the United States (Gray *et al.*, 2007). Different Studies from Kenya, South Africa, Zimbabwe and Malawi suggested that GBS is emerging as an important cause of neonatal sepsis in Africa (Gray *et al.*, 2007).

Rates of GBS colonization vary widely throughout the world. Culture methods, including the number and type of sites cultured and type of medium used, have accounted for some of the variations (Shet and Ferrieri, 2004). Despite these differences in technique, real regional variation exists. For example, GBS colonization is prevalent in the United States, 10-35% (Regan *et al.*, 1991), Italy 18%, (Savoia *et al.*, 2008) Trinidad, 32.9% (Orrett, 2003), and Gambia, 22% (Sura *et al.*, 1994). Low prevalence rates have been reported from Turkey, 8% (Bararos *et al.*, 2005), Israel, 12.3% (Marchaim *et al.*, 2003) and Ethiopia, 9% (Schmidt *et al.*, 1989).

In addition to regional differences, some investigators have reported racial differences in GBS colonization rates. In the United States, Anthony and others (Collins *et al.*, 1998) found a lower prevalence of carriage in Mexican-Americans (18.4%) than in whites (40.9%) or blacks (31.3%) (Collins *et al.*, 1998). The multicenter Vaginal Infections and Prematurity Study Group found the prevalence of GBS colonization to be higher in blacks (21.2%) than whites (13.7%) (Whitney *et al.*, 2004).

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

In study conducted to determine the prevalence of GBS and to identify GBS colonisation risk factors in a multicultural population of pregnant women in The Netherlands, it was found 21% GBS carrier rate late in pregnancy. This study indicated, African women were at a higher risk (29%) Compared to Europeans and Asian women were at lower risk (13%) for GBS carriage (Valkenburg-van den Berg *et al.*, 2006). In the Netherlands, the incidence of early onset GBS disease in 1997–1998 was estimated at 1.9 per 1000 live births, with a case fatality rate of 5% (Trijbels-Smeulders *et al.*, 2002).

In study done on 738 students who lived in a first-year dormitory at the University of Michigan, the prevalence of colonization of GBS was found to be 34% among women and 20% among men living in a college dormitory; sexually experienced subjects had twice the colonization rates than sexually inexperienced participants (Manning *et al.*, 2004).

The prevalence of GBS colonization during pregnancy is variable; in one study, among women who had positive GBS cultures between 26 and 28 weeks of gestation, only 65% remained colonized at term, while 8% of those with negative prenatal cultures were positive for GBS at term (Shet and Ferrieri, 2004). Study conducted in seven prenatal clinics in six different countries indicated colonization rate of 11.3% with 5

were heavily contaminated and 6.4 were slightly colonized (Whitney *et al.*, 2004). Study from North-Eastern Italy group B *Streptococcus* was detected in 901 women, corresponding to a prevalence rate of 17.9% (Busetti *et al.*, 2007).

Study from public and private hospitals in Athens and in a city of northern Greece; The overall maternal and neonatal colonization rates with GBS were 6.6% and 2.4%, respectively. The vertical transmission rate was 22.5%. In contrast with other studies, this study indicated middle-class women followed privately were more frequently colonized (10%) than those followed at the public hospital (3.9%). This study also showed that a higher number of prenatal visits were associated with a higher colonization rate. No association was found in this study between colonization and maternal age, previous obstetric history, marital status, nationality, prematurity, caesarean section, or infant birth weight (Tsolia *et al.*, 2003).

Study in Hong-Kong showed the prevalence of group B *Streptococcus* colonisation in antenatal population was found to be 10.4%. The majority of carriers were identified by low vaginal swabs (78%), while high vaginal swabs and rectal swabs only identified 31% and 30% of the carriers, respectively. Pregnant women those who work outside home yielded a higher carrier rate than housewives (21% vs 10%). There was no increase in preterm delivery rate in group B *Streptococcus* carriers indicated in this study (Tsui *et al.*, 2009).

Study conducted in the northeast region of Brazil (São Luís, Maranhão) showed the prevalence of GBS colonization in the mothers was 20.4%. In this study no association was indicated between the socio demographic variables or gynecological-obstetrical antecedents and a larger presence of GBS colonization (Costa *et al.*, 2008). Study done in Lima, Peru from August through October 1991 showed relatively low prevalence of GBS colonization of 6.0% in parturient women and 10.6% in non pregnant women. In this study no association of GBS colonization was made with some risk factors such as age, parity, or birth control practices (Collins *et al.*, 1998).

A study from Maputo, Mozambique indicated low prevalence of colonization of GBS among pregnant women between 35 and 37 weeks of pregnancy (1.8%). The method used was a rectovaginal swab which was taken from women between 35 and 37 weeks of pregnancy who visited the clinic for antenatal consultation (De Steenwinkel *et al.*, 2008).

Study done in Malawi showed the overall colonization rate of GBS to be 16.5 % (Dzowela *et al.*, 2005). In three separate studies in Zimbabwe GBS colonization rate was shown to be 20 %, 31 %, and 32% (Dzowela *et al.*, 2005). In another study conducted in Zimbabwe (2006), Mother Colonization rate was found to be significantly higher in the rural areas (60%) as compared to the urban areas (46%). GBS colonization persistence was showed to be more in rural (48%) than in urban women (12%). Baby colonization was also showed to be more in the rural (23%) than in urban area (5%) (Mavenyengwa *et al.*, 2006).

In Ethiopia, 200 postpartum women and 80 newborn infants were investigated for group B *Streptococcal* carriage at Gondar College of Medical Sciences from January to April 1987, using swabs from the vagina and rectum and from the throat and external ear, respectively. It was found a colonization rate of 9% in the mothers and 5% in the neonates (Schmidt *et al.*, 1989).

#### **1.2.4. Pathogenesis of Group B *Streptococcus* infection**

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

##### **a) Adherence to epithelial surfaces**

Group B *Streptococcus* adheres to a variety of human cells including vaginal epithelium, placental membranes, and respiratory tract epithelium and blood brain barrier endothelium. Optimum adherence occurs at the acidic pH of vaginal mucosa, allowing GBS to occupy a niche that places infants at risk of vertical transmission (Tamura *et al.*, 1994). Low affinity GBS attachment to epithelial cells is mediated by its amphiphilic cell wall associated lipoteichoic acid, while higher affinity interactions with host cells are mediated by a series of size-variable, pronase-sensitive; hydrophobic GBS surface proteins (Wibawan *et al.*, 1992). GBS effectively binds the extracellular matrix components fibronectin, fibrinogen and laminin (Schwartz *et al.*, 2004). Unusually well adapted GBS binds to immobilized fibronectin to facilitate mucosal colonization, but not to soluble fibronectin that may serve as an opsonin for phagocyte recognition (Tamura and Rubens, 1995)

##### **b) Penetration of host cellular barriers**

Group B *Streptococcus* can pass through placental membranes and weaken its strength. As a result of these processes, GBS may access the fetus within the amniotic

cavity, cause placental membrane rupture or trigger premature delivery. After aspiration of infected amniotic or vaginal fluid, the newborn lung is the primary focus of GBS infection. From lung, the organism can enter into the bloodstream and then spreads to various organs and tissues (Doran and Nizt, 2004).

**c) Avoidance of immunologic clearance**

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

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

Majority of GBS associated with human disease are encapsulated. The serotype specific epitopes of each polysaccharide are created by different arrangements of four component sugars (glucose, galactose, *N*-acetylglucosamine and sialic acid) into a unique repeating unit, but all these structures contain a terminal sialic acid (Neu5Ac) bound to galactose in an  $\alpha 2 \rightarrow 3$ -linkage. GBS capsule biosynthesis is encoded in the single long transcript of a 16-gene operon now it is fully sequenced in type Ia, III and V strains (Chaffin *et al.*, 2000).

The GBS terminal  $\alpha 2 \rightarrow 3$  Neu5Ac capsular components are identical to a sugar epitope widely present on the surface of all mammalian cells. The terminal  $\alpha 2 \rightarrow 3$  linked Neu5Ac is overexpressed in humans who in evolution have lost the genes to produce the alternative sialic acid Neu5Gc. The sialylated GBS surface capsule protects GBS by interference with opsonophagocytosis (Doran *et al.*, 2003).

**d) Activation of inflammatory responses**

Resisting phagocytic clearance in the bloodstream, GBS may disseminate to reach end organs such as bones, joints and the central nervous system (CNS). As the result of this the host inflammatory response to invasive infection mounts, and development of the sepsis syndrome and multiorgan dysfunction often occurs. Peptidoglycan and other GBS components associated with the cell wall without the surface polysaccharide capsule,

appear to be the most provocative agents in triggering host cytokine cascades, in particular the proximal mediators tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) and interleukin-1 (IL-1). (Doran and Nizt, 2004).

#### **1.2.5. Maternal Colonization**

Group B *Streptococcus* is a common inhabitant of the gastrointestinal and genitourinary tracts of women. Approximately 15% to 45% of pregnant women are colonized with GBS (at some time during the pregnancy), usually without any symptoms, and 20% of women have intrapartum positive cultures. Some women may have intermittent or transient colonization with GBS (Nandyal, 2008).

Factors that increase the risk of maternal GBS carriage include diabetes, age less than 20 years, and African American race. These factors also increase the risk of preterm delivery, which will further increase the mortality and long-term morbidity. About 50% of vaginally delivered infants of colonized mothers will become colonized, and 1% to 2% of colonized infants develop GBS invasive disease (Nandyal, 2008).

#### **1.2.6. Clinical Features**

##### **a) Maternal Infection**

Group B *Streptococcus* can cause significant morbidity in pregnant women. Manifestations of symptomatic maternal infection include chorioamnionitis, endometritis, cystitis, pyelonephritis and febrile GBS bacteraemia (Dillon *et al.*, 1987). Colonization with GBS was significantly associated with prolonged labour, premature rupture of membranes (PROM) and preterm delivery (Regan *et al.*, 1981). Less commonly, GBS is isolated in cases of post-operative wound infection, pelvic abscess, septic pelvic thrombophlebitis and osteomyelitis. Maternal sepsis is responsible for over 35% of these deaths (Anandalakshmy and Buckshee, 1997).

GBS has been associated with maternal peripartum complications; however, reports associating GBS with intra-amniotic infection (IAI) and postpartum endometritis (PPE) have presented conflicting findings. The evidence that GBS is associated with maternal infections includes isolation of GBS from blood and genital specimens of women with IAI and PPE and association of vaginal colonization with an increased risk of PPE (Krohn *et al.*, 1999). A study examined the risk of high-density GBS vaginal colonization and IAI or PPE by evaluating women who had samples cultured within 2 weeks of delivery without receiving antimicrobial therapy (Krohn *et al.*, 1999). In this study increased concentration of vaginal GBS colonization was associated with a

stepwise increase in the risk of IAI infection but not PPE. GBS identified in mid pregnancy was not shown to be associated with IAI at delivery (Krohn *et al.*, 1999).

#### **b) Perinatal Infection**

Group B *streptococcal* genital colonization has been considered a possible cause of premature deliveries and premature rupture of membranes (Dillon *et al.*, 1987) although definite evidence of a causal relationship is still lacking; Several prospective studies have also suggested that GBS colonization may play a causal role in the occurrence of intrauterine deaths, late abortions and low birth weight infants (Dillon *et al.*, 1987). In a prospective study of 325 pregnant women in Vellore (India), 31 % of the GBS-colonized mothers reported a history of foetal losses and neonatal deaths as opposed to only 18% of non-colonized mothers (Kuruvilla *et al.*, 1999).

#### **c) Neonatal Infection**

Two distinct clinical syndromes are recognized, early and late onset disease. Early onset GBS disease occurs within the first 7 days of life, although most cases are evident in the first 24 h after birth. As can be demonstrated by serotyping GBS isolates from colonized mothers and infants, transmission of early-onset disease is vertical (Boyer *et al.*, 1983).

Infection may be acquired by the intra amniotic route, or directly during passage through the birth canal. The initial presentation is respiratory distress in more than 80% of neonates. Pneumonia and septicaemia are the most common manifestations, and 5 to 10% neonates will also have meningitis. The incidence of early-onset disease is about 10 times higher in premature than in term neonates (Boyer *et al.*, 1983).

Late-onset disease develops in infants after 7 days and up to 3 months of age, the median age of onset being 1 month. Transmission can be either horizontal (from other infected infants or health care workers) or vertical (from the mother due to close proximity). These infants almost always have an unremarkable early neonatal history, and later present with meningitis or sepsis. Osteoarticular infections and cellulitis can also occur. The initial signs usually are fever, lethargy, irritability, poor feeding and tachypnoea. Respiratory distress as a presenting feature is less common. Over 20% of survivors of GBS meningitis have permanent neurological sequelae, including sensorineural hearing loss, mental retardation, cortical blindness and seizures (Yagupsky *et al.*, 1991).

### **1.2.7. Laboratory detection of GBS Colonization and Infections**

#### **a) Isolation of GBS using Selective Enrichment Broth**

Group B *Streptococcus* is part of the microbiologically complex vaginal and rectal human flora. Consequently, selective enrichment broths have been developed to improve GBS recovery from vaginal and/or rectal swabs and to inhibit overgrowth of other organisms of the normal flora (Jones *et al.*, 1983).

Consensus prevention guidelines of 1996/1997 from the Centers for Disease Control and Prevention (CDC, 1996), the American College of Obstetricians and Gynecologists (AOG Committee opinion, 1996), and the American Academy of Pediatrics (American Academy of Pediatrics, 1997) recommended that GBS culture should be performed at 35–37 weeks of gestation from swabs collected from both the vagina and the rectum using selective Todd–Hewitt broth supplemented with either colistin (10 µg/ml) and nalidixic acid (15 µg/ml) or with gentamicin (10 µg/ml) and nalidixic acid (15 µg/ml). The inoculated selective medium is incubated for 18–24 hours and then subcultured onto sheep blood agar (CDC, 1996). If GBS is not identified after the incubation of 18–24 hours, the blood agar plate should be reincubated and examined at 48 hours to identify suspected organisms. Suspected colonies may be tested using various slide agglutination tests or GBS antigen detection assays for specific identification of GBS, or alternatively, the CAMP test may be used for presumptive GBS identification (AOG Committee opinion, 1996).

The developed guidelines have led to marked improvements of the screening-based approach using culture methods as revealed by a study showing that the use of standard direct blood agar plating rather than selective enrichment broth leads to false-negative culture results in as many as 50% of pregnant women colonized by GBS. Major factors that influence the accuracy of detecting GBS maternal colonization are the choice of bacteriological media, sample collection sites, and the time of the sampling (CDC, 1999).

#### **b) Immunological Assay**

Group B *Streptococcus* strains are identified reliably by the production of group B Lancefield antigen (Picard and Bergeron, 2004). As a result, many latex agglutination tests and immunoassays that detect this antigen for GBS identification have been developed (Yancey *et al.*, 1992). However, the overall sensitivity of these commercially available immunological assays is low (Yancey *et al.*, 1992). When compared with the

results of selective broth culture, the sensitivity of popular rapid immunoassays to detect GBS colonization in pregnant women directly from vagino-rectal swabs ranged from only 4% to 37%. Rapid antigen detection tests may only be suitable to detect GBS in heavily colonized patients or from overnight cultures in standard selective broth (Yancey *et al.*, 1992).

### **c) Nucleic Acid Testing Assays**

The most popular probe hybridization system for GBS is the AccuProbe Group B *Streptococcus* Culture Identification Test from Gen-Probe (San Diego, CA, USA). This Assay, which targets specifically the GBS ribosomal RNA, is suitable to identify GBS from 18 to 24 hours cultures in selective enrichment broth. Compared with the standard culture method, this probe-based assay had a sensitivity of 94.7–100% and a specificity of 96.9–99.5% for screening for GBS colonization in pregnant women (Williams-Bouyer *et al.*, 2000). Some investigators have also tested shorter incubation periods in selective broth and have found lower sensitivities. More specifically, the sensitivity of the probe assay was 44% for 2.5 hours of incubation, 71% for 3.5 hours of incubation, and 95% for 8.0 hours of incubation (Kircher *et al.*, 1996).

In addition, a DNA-based probe test developed by Microprobe (Bothell, WA, USA) was shown to have a sensitivity of only 81% as compared to standard selective culture (Rosa *et al.*, 1995). Thus, available probe hybridization methods are suitable for GBS identification from overnight cultures in selective enrichment broth but are poorly sensitive for direct detection and identification of GBS from vagino-rectal swabs obtained from pregnant women (Picard and Bergeron, 2004).

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

Polymerase chain reaction (PCR) assay is based on the recently developed and widely used real-time PCR technology (Cockerill and smith, 2002). This <1 hour GBS-specific real-time PCR assay, which targets the *cfb* gene encoding the CAMP factor, relies on a simple and rapid (approximately 10 minutes) vagino-rectal swab sample preparation and nucleic acid extraction and uses a rapid thermal cycling instrument that allows real-time fluorescence monitoring of the PCR reactions (Ke *et al.*, 2000). The instrument measures, at each PCR cycle, the fluorescence signal from probes labelled with fluorophores hybridizing specifically to target DNA sequences of the GBS-specific amplicons generated during the nucleic acid amplification process. Besides its rapidity, this real-time PCR assay also offers the advantage of reducing the risks of carry-over contamination by amplicons because amplification and detection occur within a closed reaction vessel (Cockerill and smith, 2002). This assay has been validated by a clinical study performed with vagino-rectal swab specimens obtained from 112 pregnant women at delivery (Bergeron *et al.*, 2000).

When compared with the standard antepartum selective broth culture method, this real-time PCR assay had a sensitivity of 97.0%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 98.8% for screening for GBS colonization during delivery. One important advantage of using this test for GBS screening is its suitability for testing at the time of admission for delivery, thereby offering better sensitivity and specificity for detecting colonization in women at the time of labor when compared to the currently recommended culture-based screening method performed at 35– 37 weeks gestation. Thus, this PCR assay provides a novel diagnostic tool for GBS detection, potentially allowing more accurate and effective intrapartum antibiotic prophylaxis. Incidentally, a recent cost–benefit analysis study suggested that for a test price of up to 32–33 US dollars, a PCR test performed in less than 1 hour at the time of delivery is more cost-effective than screening using either the selective culture method or the risk-factor approach (Haberland *et al.*, 2002).

A technology transfer of the real-time PCR assay developed by Bergeron and Ke to Infectio Diagnostic led to the development of a diagnostic kit called IDI-Strep B. This product, which has been commercially available since March 2003, is the first and only test for GBS detection approved by the FDA and Health Canada capable of detecting and identifying GBS in pregnant women at the time of delivery or at any other stage of their pregnancy. This test specifically detects GBS DNA directly from vaginal–rectal swabs,

with specimen preparation, analysis, and results all achievable in less than 1 hour (Picard and Bergeron, 2004).

In a multi-site clinical trial, the *IDI-Strep B* test demonstrated a sensitivity of 94% and a specificity of 96% in comparison to reference intrapartum culture-based screening performed at delivery (Davies *et al.*, 2004). In a recent comparative study, the sensitivity of the *IDI-Strep B* test surpassed both standard antepartum selective culture at 35–37 weeks gestation and risk-factor assessment, resulting in fewer false-negative results and potentially lower infant mortality and morbidity. In fact, 13% of women in this trial would have been treated more appropriately with screening using the *IDI-Strep B* as compared to the use of the standard antepartum selective culture method, while 36% of them would have been treated more appropriately with screening using *IDI-Strep B* as compared to the risk-based approach (Davies *et al.*, 2004). Therefore, the *IDI-Strep B* PCR test has high sensitivity for screening of GBS colonization in pregnant women than the standard antepartum selective culture methods.

### **1.2.8. Management of GBS Colonization and Infections**

#### **a) Intrapartum Antibiotic Chemoprophylaxis (IAP)**

The intrapartum antibiotic chemoprophylaxis (IAP) is indicated for all GBS carriers except for those in whom cesarean delivery is planned in the absence of labor or membrane rupture. Penicillin G remains the drug of choice for prophylaxis with ampicillin as the alternative medication. Estimates of the rate of anaphylaxis caused by penicillin range from 4 per 10,000 to 4 per 100,000. In addition; as many as 10% of the adult population have less severe allergic reactions to penicillin. Five cases of severe maternal allergic reactions, due to the administration of antibiotics at late preterm or term, were reported (Nandyal, 2008).

For patients who are allergic to penicillin but do not have a history of anaphylaxis, cefazolin is the preferred antibiotic. Otherwise, vancomycin is recommended for those with a history of anaphylaxis, and when GBS is resistant to erythromycin and clindamycin. If the GBS is sensitive to one of these antibiotics, either erythromycin or clindamycin is recommended for IAP. But, their efficacy in the prevention of EOD is yet to be confirmed. In the presence of maternal chorioamnionitis, early treatment of the mother with broad-spectrum antibiotics is indicated. Criteria for the diagnosis of maternal chorioamnionitis include maternal fever, plus one of the following criteria: uterine tenderness, foetal tachycardia, foul smelling amniotic fluid,

prolonged rupture of membranes of equal to or more than 18 hours, or maternal leukocytosis. It is impractical to administer chemoprophylaxis to all parturient mothers and neonates. The challenge therefore is to identify correctly high risk infants before they are born (Nandyal, 2008).

To prevent early onset disease two preventative approaches were used; a culture screening-based and a risk-based approach. The first approach involved universal screening for GBS colonization of all pregnant women between 35 and 37 weeks of gestation using vaginal and rectal cultures to detect GBS colonization. Properly obtained and processed antenatal cultures correctly identified most women colonized at the time of labour. Intrapartum antibiotics are administered to all those with a positive GBS culture regardless of risk factors (CDC, 2002).

The risk-based approach involved administration of antibiotics based solely on the presence of antenatal or intrapartum risk factors. Maternal risk factors for group B *streptococcal* neonatal sepsis are as follows; (CDC, 2002) preterm labour or premature rupture of membranes (< 37 weeks' gestation); prolonged rupture of membranes ( $\geq 18$  h); intrapartum fever  $\geq 100.4^{\circ}$  F ( $\geq 38.0^{\circ}$ C); history of a previous newborn with GBS disease; and group B *Streptococcus* bacteruria during pregnancy (CDC, 2002).

#### **b) Management of Infant born to mothers who received Intrapartum Antibiotic Prophylaxis**

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

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

and the antibiotics are continued beyond 48 hours, CSF should be obtained for cell count, biochemical analysis, and culture (Nandyal, 2008).

**c) Asymptomatic Preterm Neonates**

For preterm neonates of less than 35 weeks' gestation, limited evaluation should be obtained (because of their increased risk for sepsis and rapid deterioration) in all asymptomatic infants of GBS colonized mothers, regardless of their IAP status (Nandyal, 2008). Limited evaluation includes CBC with differential and blood culture. They need close observation, including frequent vital signs (at least every 2 to 4 hours), frequent diligent assessment of the infant by the staff. Any significant change in vital signs including temperature imbalance, any change in the clinical status including poor feeding, respiratory distress, apnea, abdominal distension, frequent emeses, or lethargy deserves and requires immediate notification to the physician team and reassessment, which may necessitate repeating laboratory tests. Full evaluation including spinal tap and empiric antibiotic therapy seem appropriate, if the infant becomes symptomatic, or in the presence of maternal chorioamnionitis (suspected or proven), or other high-risk factors such as preterm premature rupture of membranes (Nandyal, 2008).

**d) Asymptomatic Term and Late Preterm Neonates**

Asymptomatic neonates born at 35 weeks' gestational age or later and whose mothers received inadequate IAP may require limited evaluation and observation for 48 hours. There is some evidence suggesting that limited evaluation does not add any benefit over close clinical observation. On the basis of newly available data, it is recommended that the neonate should be observed closely for 48 hours without laboratory evaluation, or 2 serial CBCs obtained within the first 24 hours after birth. Blood culture is not necessary. Serial CRP measurements (because of CRP's high sensitivity) are noted to be useful along with serial CBCs (total white blood cell count, band count, and immature/total polymorphonuclear cell ratio or I/T ratio) to evaluate for sepsis in healthy/asymptomatic term and late preterm neonates (Nandyal, 2008).

**1.2.9. Prevention and control**

While different strategies for identification of high risk mothers and infants and provision of intrapartum prophylaxis may reduce the rate of neonatal sepsis, they are unlikely to eliminate the problem (Shet and Ferrieri, 2004). Maternal immunization against GBS appears to be a promising and potentially lasting approach for preventing neonatal sepsis, preterm deliveries and low birth weight infants (Shet and Ferrieri, 2004).

Multivalent polysaccharide-protein conjugate vaccines based on serotype-specific capsular polysaccharides are in development for prevention of neonatal GBS disease (Shet and Ferrieri, 2004). Although groups of adults at high risk for invasive GBS disease have been identified, the role of capsular polysaccharide antibodies in the prevention of either localized or invasive GBS disease in non-pregnant adults has not been adequately evaluated (Shet and Ferrieri, 2004).

GBS capsular polysaccharide protein conjugate vaccines for types Ia, Ib and III have been shown to be safe and efficient in inducing type specific antibody levels in healthy vaccinated individuals (Baker *et al.*, 1999). A vaccine formulation of GBS types Ia, II, III and V would be expected to provide protection against over 90% of infections. Therefore epidemiological surveillance of serotype distribution in the population is critical for vaccine studies (Schuchat *et al.*, 2001). The cost of developing and implementing a vaccine strategy, although formidable, is considerably less than that of treating these infections, and for some infants, their life-long sequelae (Dillon *et al.*, 1987).

Among experts in this field, there is considerable discussion regarding whom to immunize non pregnant adolescents or pregnant women in the first trimester. In addition, an argument can be made for vaccinating 'at risk' non pregnant adults (Shet and Ferrieri, 2004). Clinical trials are in progress and research is ongoing for developing a safe and efficacious vaccine against GBS disease (Mohle-Boetani *et al.*, 1993). A major difficulty in developing group B *streptococcal* vaccines is the existence of a multiple serotypes in different geographic locations (Johri *et al.*, 2006).

### **1.3. Significance of the Study**

Group B *Streptococcus* is an encapsulated gram-positive bacteria occurring either in short chains or in pairs that usually produces a narrow zone of beta-haemolysis on blood agar and belong to Lancefield group B (Baker and Edwards, 2001). When neonatal infections caused by GBS appeared in the 1970s, as many as 50% of patients died. During the 1990s, the case-fatality ratio of early- and late-onset disease was 4% because of advances in neonatal care (Baker and Edwards, 2001).

The gastrointestinal tract as the natural reservoir for GBS and is most likely source of vaginal colonization. Colonization is unusual in childhood but becomes more common in late adolescence (CDC, 2002). Approximately 10% to 30% of pregnant women are colonized with GBS in the vagina or rectum. Maternal intrapartum GBS colonization is a

major risk factor for early-onset disease in infants, and vertical transmission of GBS from mother to fetus primarily occurs after the onset of labor or membrane rupture. However, colonization early in pregnancy is not predictive of neonatal sepsis (CDC, 2002).

Before the widespread use of intrapartum antibiotics, the incidence of invasive neonatal GBS disease ranged from 2 to 3 cases per 1,000 live births (Zangwill *et al.*, 1992). Active, population-based surveillance in selected states in 1990, when GBS prevention was still rarely implemented, projected an incidence of 1.8 cases per 1,000 live births in the United States (early-onset disease: 1.5/1,000; late-onset: 0.35/1,000) was found (Zangwill *et al.*, 1992).

Active prevention efforts in the 1990s decreased the incidence of early-onset disease by 70% to 0.5 cases per 1,000 live births in 1999. Projections from active surveillance data for 1999 from the Active Bacterial Core surveillance/Emerging Infections Program Network (ABCs) (CDC, 2000) estimated that intrapartum antibiotics prevented nearly 4,500 early-onset cases and 225 deaths in 1999 (CDC, 2000). Other countries that have adopted perinatal GBS disease prevention guidelines similar to the United States have seen comparable declines in early-onset disease incidence (Davies *et al.*, 2001).

GBS can also cause significant morbidity in pregnant women. Manifestations of symptomatic maternal infection include chorioamnionitis, endometritis, cystitis, pyelonephritis and febrile GBS bacteraemia (Dillon *et al.*, 1987). Infection of new born may be acquired by the intraamniotic route, or directly during passage through the birth canal. The initial presentation is respiratory distress in more than 80% of neonates. Pneumonia and septicaemia are the most common manifestations, and 5 to 10% neonates will also have meningitis. The incidence of early-onset disease is about 10 times higher in premature than in term neonates (Boyer *et al.*, 1983).

Most data on GBS epidemiology over the years has come from the Europe and North America and to date; only Zimbabwe in Africa has an active research program on GBS colonization and burden of disease (Dzowela *et al.*, 2005).

Knowledge about the prevalence of colonization of GBS among pregnant women in Ethiopia is limited. There was only one study which was conducted 23 years ago in Gondar, northwest Ethiopia (Schmidt *et al.*, 1989). In this study conducted in Gondar, 18 women were found to be positive for Group B *streptococcal* carriage among 200

postpartum women investigated (Schmidt *et al.*, 1989). Therefore, the present study was conducted to give baseline and update information about the prevalence of colonization of GBS among pregnant women attending Hawassa Health center, Hawassa, Ethiopia.

#### **1.4. Hypothesis**

The prevalence of GBS among pregnant women in Ethiopia could be similar with the reported prevalence in other developing and/or developed countries.

## **1.5. Objectives of the Study**

### **General Objective**

- To determine the prevalence of group B *Streptococcal* (GBS) colonization among pregnant women attending Hawassa Health center, Hawassa, Ethiopia.

### **Specific Objectives**

- To isolate GBS from vaginal and anorectal specimens from pregnant women at 35 to 37 weeks of gestation.
- To assess risk factors associated with GBS colonization in pregnant women
- To determine the susceptibility pattern of GBS isolates to antimicrobial agents that are commonly used for intrapartum antibiotic prophylaxis and treating GBS infection in newborns.

## CHAPTER II: MATERIALS AND METHODS

### 2.1. Study Design and Area

This prospective cross sectional study was conducted from May 2010 through June 2010 in pregnant women attending the antenatal clinic of Hawassa Health center, Hawassa, Ethiopia. Hawassa town is the capital city of SNNP and it is located 275 km from capital city of Ethiopia, Addis Ababa. The altitude of the town is 1697 km above sea level with mean annual temperature and rainfall of 20.9 °C and 997.6 mm, respectively. The total population of Hawassa town is 130579 with one to one male to female ratio.

### 2.2. Study Population

During the study period a total of 139 pregnant women attending the routine antenatal care follow up were screened for GBS colonization. The screening approach was based on universal screening of all pregnant women for GBS colonization between 35 and 37 of weeks of gestation (CDC, 2002).

**Inclusion criteria:** Pregnant women in their 35 to 37 weeks of gestation.

**Exclusion criteria:** Pregnant women on any antibiotic treatment and those who were not in 37 and 38 weeks of gestations.

Written informed consent was obtained from study participants. Demographic and other relevant data was also obtained by attending health professionals and transferred to the questionnaire prepared for this study (see appendix I).

Sample size was calculated based on the prevalence indicated in the epidemiology part which was 9% from Ethiopia (Schmidt *et al.*, 1989). Expected margin of error (d) will be 0.05 and confidence interval (z) will be 95%. Contingency for the unknown circumstance will be 10%.

$$n = \frac{(Z \alpha/2)^2 * P(1-P)}{d^2}, \quad n = \frac{(1.96)^2 * 0.09(1-0.09)}{(0.05)^2} = 126 + 10\% = 139$$

### 2.3. Sample Collection, Handling and Transport

According to the Centers for Disease Control and Prevention (CDC, 2002) and American College of Obstetricians and Gynecologists (AOG Committee opinion, 1996) guidelines, swab was taken from both the lower one third of vagina and the anal region using sterile cotton swab (figure 2.1) by the attending midwifery and placed in Amies transport

medium (OXOID, UK) and immediately transported to the Microbiology Laboratory of Hawassa Referral Hospital for culture.

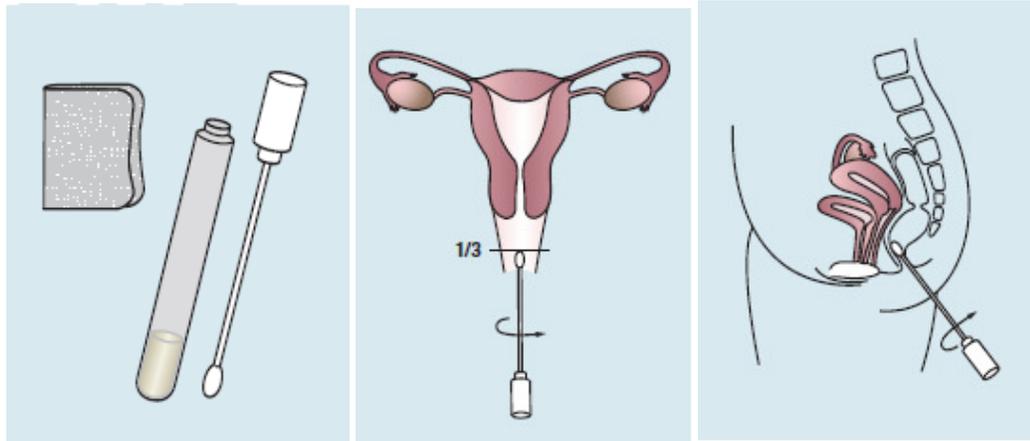


Figure 2.1. Site for collection of rectovaginal specimen for GBS isolation (Adapted from BD diagnostics [www.geneohm.com](http://www.geneohm.com)).

#### 2.4. Culture and Identification of GBS

The vaginal and anorectal swabs were placed into 1 ml Todd-Hewitt broth (OXOID, UK) supplemented with 10 $\mu$ g/ml colistin and 15 $\mu$ g/ml nalidixic acid (BIOMERIEUX, France) to prevent growth of contaminants. The broth was incubated for 18–24 hours at 35-37 °C and subcultured on 5% sheep blood agar (OXOID, UK) and incubated overnight in 5% CO<sub>2</sub> atmosphere for 18-24 hours. All suspected GBS colonies (pin point, with narrow beta-hemolysis) were subcultured on blood agar and subjected for Gram stain and catalase test. All gram positive and catalase negative cocci isolates were tested for CAMP test and latex agglutination assay as a confirmatory testing for GBS.

#### 2.5. CAMP test

The CAMP test was used to identify beta hemolytic streptococci, *Streptococcus agalactiae* (group B) (CAMP positive) from *Streptococcus pyogenes* (group A) (CAMP negative) (Christie *et al.*, 1944). The "lytic phenomenon" between *Staphylococcus aureus* and *Streptococcus agalactiae* (GBS) is the basis for the CAMP test. In brief, *Staphylococcus aureus* (obtained from Addis Ababa University Microbiology) was inoculated onto a sheep blood agar plate by making a narrow streak down the center of the plate with a loop. The test organism (suspect group B *Streptococcus*) was streaked in a straight-line inoculum at right angles to the *S. aureus* as shown in Figure 2.2. The *Streptococcus* streak was within 2 mm without touching the *S. aureus* streak. The plates

were incubated at 35°C for 24 hours. During incubation the beta hemolysin produced by *Staphylococcus aureus* acts synergistically with the CAMP factor produced by *Streptococcus agalactiae* (group B). This synergistic reaction results in an enhanced and very visible zone of hemolysis in the region between the two cultures. A positive CAMP result was indicated by an "arrowhead"-shaped enhanced zone of beta-hemolysis in the area between the two cultures with the "arrow point" toward the *S. aureus* streak (Figure 2.3). No enhanced zone of beta-hemolysis was observed in a CAMP negative reaction.

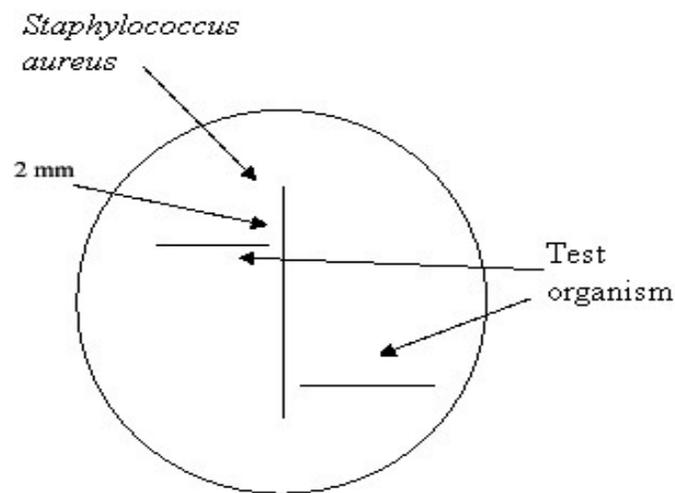


Figure 2.2. Streaking pattern for the CAMP test

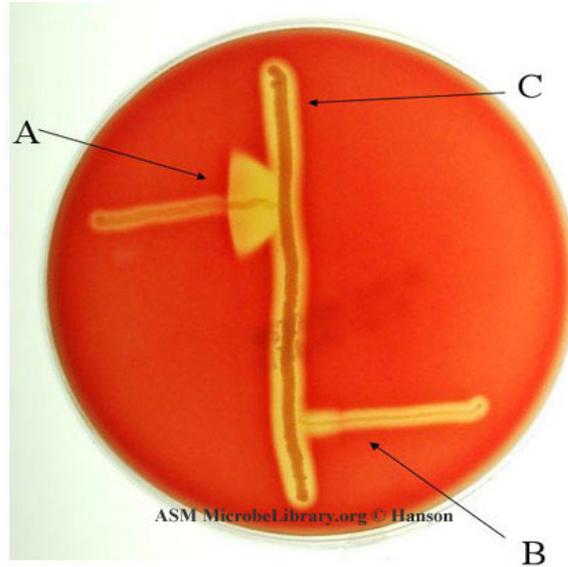


Figure 2.2. CAMP test for the identification of *Streptococcus agalactiae* (group B) (Adapted from ASM MicroLibrary.org). (A) *Streptococcus* (group B) shows a positive CAMP reaction. (B) *Streptococcus pyogenes* (group A) shows a negative reaction when inoculated at a right angle to (C) *Staphylococcus aureus*

## 2.6. GBS latex agglutination assay

CAMP test positive culture results were reconfirmed using a GBS latex agglutination assay using serogrouping kits (PRO LAB DIAGNOSTICS, UK) according to the manufacturer's instructions. In brief the test protocol was as follows in step wise: a) the test tube was labeled for each specimen b) one drop of extraction reagent-1 was added on each tube c) several suspected colonies were transferred to a test tube and mixed with extraction reagent-1 until it became turbid d) one drop of extraction reagent-2 was added on each test tube and mixed e) five drops of extraction reagent-3 were added and mixed f) one drop of anti Group B *Streptococcus* blue latex suspension was added on separate circle on the test card g) using Pasteur pipette one drop of extract was placed beside each drop of latex suspension h) blue latex and extract was mixed using the sticks provided and the card was rotated i) within one minute under normal lightening condition agglutination was observed.

## 2.7. Antimicrobial susceptibility testing

All procedures for disk susceptibility were performed according to the methodologies described in the National Committee for Clinical Laboratory Standards

(NCCLS approved standard, 2002). Fresh subcultures of GBS were used after overnight growth on blood agar plate (OXOID, UK). The inoculum was standardized by suspending colonies in sterile phosphate buffered saline (pH 7.2) to achieve a turbidity of 0.5 McFarland standards. A sterile cotton swab was dipped into the bacterial suspension, elevated above the liquid and rotated several times against the inside wall of the tube to remove excess inoculum. Then the swab was inoculated on Mueller–Hinton agar plate (OXOID, UK) supplemented with 5% defibrinated sheep blood to obtain confluent growth, antibiotic disks were placed and incubated at 35°C with 5% CO<sub>2</sub> atmosphere for 20 hours.

Ten antibiotic disks (Oxoid) were used as follows: - penicillin G (P) (10 IU), erythromycin (E) (15µg), ceftriaxone (CRO) (30µg), gentamicin (CN) (10µg), norfloxacin (NOR) (10µg), chloramphenicol (C) (30µg), vancomycin (VA) (30µg), tetracycline (TE) (30µg), ampicillin (AMP) (10µg) and ciprofloxacin (CIP) (5µg).

The zones of growth inhibition were measured to the nearest whole millimeter using a sliding caliper. The sizes of the inhibition zones were graded according to the (NCCLS approved standard, 2002). Each isolate was classified as susceptible, intermediate or resistant to each antibiotic tested.

## **2.8. Quality control**

As quality control, sterility of sheep blood agar and Todd-Hewitt broth were checked by incubating overnight at 34-37°C without specimen inoculation. The proficiency of Todd-Hewitt broth were checked by inoculating the broth with known Gram negative bacteria (*Escherichia coli*) and GBS isolates to see if it can really inhibit Gram negative bacteria and allow growth of Gram positive bacteria. The proficiency of GBS latex agglutination grouping kit was checked by positive control within the kit and by GBS isolates stored in Hawassa referral Hospital microbiology laboratory (Positive control) and *Staphylococcus aureus* and *Streptococcus pyogenes* as negative controls. The proficiency of catalase reagent (hydrogen peroxide) was checked by known *Staphylococcus aureus* (positive control) and *Streptococcus pyogenes* (negative control). For Gram staining reagents *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative) were used as quality control. Before use of any reagents and culture media any physical change was assessed and expiration date was also checked. Temperature of incubator and refrigerator was monitored daily. *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 24923), *Streptococcus pyogenes* (ATCC 19615) (obtained from EHNRI) were

used as a quality control throughout the study for culture and antimicrobial susceptibility testing.

### **2.9. Statistical Analysis**

Data entry and analysis was done using SPSS version 11.5 software. Prevalence figures were calculated for the total study population and separately by age groups. Chi-square test was used to compare results between the pregnant women with different age groups and with the previous findings from the literature. P-value less than 0.05 were considered statistically significant.

### **2.10. Ethical Consideration**

This M.Sc. research project proposal was approved by the Department Research and Ethical Review Committee of DMIP and ethically cleared by Institutional Review Board (IRB), Faculty of Medicine; Addis Ababa University. Official permission from the study site was obtained. Written informed consent was obtained from study participants (see Appendix II). For those pregnant women who were found to be colonized with GBS; the result was given to them in case they give birth in another health institution so that they can inform their attending physician and the results was also given to the midwifery in charge at Hawassa health center for the better management of colonized pregnant women.

## **CHAPTER III: RESULTS**

### **3.1. Study subjects**

The socio-demographic characteristics of 139 pregnant women screened for GBS colonization is presented in Table 3.1a. The mean age of the participants was 25.6 years (range 17-40 years). The majority of the participants were between the ages of 25-29 years (57%). All pregnant women who participated in this study were married.

The study participants were from 18 different kebeles of Hawassa town and Hawassa town area, with the majority of participant were from higher 1, kebele 1 (23.7%) followed by higher 2, kebele 07 and with few participant from Algie (0.7%), higher 1, kebele 06 (0.7%) (Table 3.1a).

The majority of the study participants were protestants (63.3%) followed by Orthodox (30.9%) and Muslim (5.8%).The ethnic group of participants were; Sidama (51.8%), Wolayta (18%), Silte (4.3%), Amhara (20.1%), Tigre (4.3%) and Gurage (1.4%). Most study participants are house wives (91.4%) the rest are merchants, students, teacher, accountant and lawyer (Table 3.1).

**Table 3.1a. Socio-demographic characteristics of prengnanat women investigated for GBS, Hawassa Health center, Hawassa, Ethiopia (May 2010-June 2010).**

<b>Socio-demographic characteristics</b>	<b>Category</b>	<b>Frequency</b>	<b>Percent (%)</b>
<b>Age groups in years (n=139)</b>	15-19	9	6.47
	20-24	51	30.69
	25-29	57	41.01
	30-34	17	12.3
	35-39	3	2.2
	40-44	2	1.4
<b>Address (n=139)</b>	Algie	1	0.7
	Hawassa	132	94.96
	Tikur wuha	4	2.9
	Wondo	2	1.4
<b>Religion (139)</b>	Protestant	88	63.3
	Orthodox	43	30.9
	Muslim	8	5.8
<b>Ethnicity (n=139)</b>	Sidama	72	51.8
	Wolayta	25	18
	Silte	6	4.3
	Amhara	28	20.1
	Tigre	6	4.3
	Gurage	2	1.4
<b>Occupation (n=139)</b>	House wife	127	91.4
	Teacher	3	2.2
	Student	6	4.3
	Lawyer	1	0.7
	Accountant	1	0.7
	Merchant	1	0.7

### 3.2. GBS culture

#### a. Overall prevalence

A total of 29 out of 139 pregnant women studied (20.86%) were colonized with GBS.

#### b. Variables associated/not-associated with GBS colonization

Different variables associated/not-associated with GBS colonization are outlined in Table 3.2. Among 29 GBS isolated from pregnant mothers, 13.8%, 27.6%, 41.4%, 13.8 %, and 3.5 % were recovered from age group of 15-19, 20-24, 25-29, 30-34, and 35-39 years, respectively (Table 3.2a). No GBS was isolated from the age group of 40-44. The GBS colonization rate was higher in 25-29 age group and the colonization rate was lower in 35-39 age group. The frequency of GBS colonization among different age groups was not statistically significant ( $p=0.48$ ) (Table 3.2).

**Table 3.2a. Distribution of 29 GBS isolated among different age interval of the study participants**

Age interval	No of GBS isolated	Percentages
15-19	4	13.8%
20-24	8	27.6%
25-29	12	41.6%
30-34	4	13.8%
35-39	1	3.5%

Colonization rate of GBS was found to be high 25/29 (82.2%) in those who were house wives compared to those who have some kind of occupation such as teacher, lawyer, accountant, merchant and also students (17.8%). However, the difference in GBS colonization rate among pregnant women who were house wives and those who have jobs outside the home was not statistically significant ( $p=0.12$ ) (Table 3.2).

The colonization rate of GBS among pregnant women screened in this study was high in certain ethnic groups including sidama 15 (51.7%) followed by Amhara 8 (27.6%), Wolayta 5 (17.2%) and Tigre 1 (3.5%). No GBS was isolated among participants from Gurage and Silte ethnic groups. However, the difference in GBS colonization was not statistically significant among different ethnic groups ( $p=0.45$ ) (Table 3.2). No statistical significant difference was observed in the rate of colonization

with GBS in pregnant women living in different localities of Hawassa ( $p=0.50$ ) (Table 3.2).

Out of 29 GBS isolates, 17 (58.6%) were from pregnant women who had a history of using injectable contraceptive followed by 3 (10.4%) and 1 (3.5%) from women using pills as well as injectable and pills, respectively. No GBS was detected from those who were using loop as contraceptives and those not using contraceptives as a whole. No statistical significant difference was observed in the rate of colonization with GBS in pregnant women who were using different contraceptives ( $p=0.85$ ) (Table 3.2).

Out of 29 GBS isolates, the highest colonization rate was detected from pregnant women who visited antenatal clinic four times (55.2%) compared to those who visited three (27.6%), two (13.8%) and one (3.5%) time, respectively (Table 3.2). No statistical significant difference was observed in GBS colonization rate regarding the number of antenatal clinic visit ( $p=0.05$ ) (Table 3.2).

Among 29 GBS isolated, 75.9% were isolated from multigravida (pregnant for the second time and more) and 24.1% were from primigravida (pregnant for the first time) (Table 3.2). However the difference in GBS colonization between multigravida and primigravida was not statistically significant ( $p=0.59$ ) (Table 3.2). Overall, no statistically significant association was observed for GBS colonization in the study subjects with any of sociodemographic characteristics mentioned above.

**Table 3.2. Variables associated/not associated with Group B *Streptococcus* colonization in pregnant women, Hawassa Health center, Hawassa, Ethiopia (May 2010 -June 2010).**

<b>Variables</b>	<b>Total</b>	<b>GBS colonization</b>	<b>Percentages (%)</b>	<b>P value</b>
<b>Age (in years)</b>				
15-19	9	4	44.4	0.48
20-24	51	8	15.7	
25-29	57	12	21.1	
30-34	17	4	23.5	
35-39	3	1	33.3	
40-44	2	0	0	
<b>Occupation</b>				
House wife	127	25	19.7	0.12
Teacher	3	0	0	
Student	6	2	33.3	
Lawyer	1	1	100	
Accountant	1	1	100	
Merchant	1	0	0	
<b>Ethnicity</b>				
Sidama	72	15	20.8	0.45
Wolyta	25	5	20	
Silte	6	0	0	
Amhara	28	8	28.6	
Tigre	6	1	16.7	
Gurage	2	0	0	
<b>Religion</b>				
protestant	88	17	19.3	0.08
Orthodox	43	12	27.9	
Muslim	8	0	0	
<b>Address</b>				
Algie	1	0	0	0.49
Higher 1,Kebele 03	11	4	36.4	
Higher 2, Kebele 06	6	0	0	
Higher 1,Kebele 01	33	7	21.2	
Higher 2, Kebele 01	4	1	25	
Tikur wuha	4	1	25	
Higher 2,Kebele 07	21	2	9.5	
Higher 2,Kebele 04	9	2	22.2	
Higher 1,Kebele 02	14	3	21.4	
Wondo	2	0	0	
Higher 1,Kebele 04	3	1	33.3	

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Higher 2, Kebele 02	5	2	40	
Higher 1,Kebele 05	5	2	40	
Dato	7	1	14.3	
Higher 2,Kebele 05	2	1	50	
Higher 2, Kebele 03	5	0	0	
Higher 1,Kebele 06	1	1	100	
Higher 1,Kebele 07	6	1	16.7	
<b>Type of Contraceptive use</b>				
Injectable	85	17	20	
Pills	11	3	27.3	0.84
Injectable & pills	6	1	16.7	
loop	2	0	0	
none	35	8	22.9	
<b>Number of ANC Visit</b>				
one times	1	1	100	0.05
two times	19	4	21.1	
three times	21	8	38.1	
four times	98	16	16.3	
<b>Type of Gravida</b>				
Primigravida	39	7	17.95	0.59
Multigravida	100	22	22	

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### 3.3. Antimicrobial susceptibility

The susceptibility pattern of 29 GBS isolated from pregnant women against 10 antimicrobial agents is presented in Table 3.3. All strains were susceptible to penicillin, ampicillin, vancomycin and gentamicin, Low level of resistance (<60%) were observed against erythromycin, tetracycline, ceftriaxone; Chloramphenicol, ciprofloxacin and norfloxacin.

**Table 3.3. Antimicrobial susceptibility pattern of 29 GBS isolated from pregnant women, Hawassa Health center, Hawassa, Ethiopia (May 2010-June-2010).**

<b>Antibiotics</b>	<b>Susceptible</b>	<b>Intermediate</b>	<b>Resistant</b>
Penicillin G	100%(29/29)	-	0
Erythromycin	89.7%(26/29)	3.4%(1/29)	6.9%(2/29)
Tetracycline	39.3%(11/29)	14.3(4/29)	44.8%
Ampicillin	100%(29/29)	-	0
Vancomycin	100%(29/29)	-	0
Ceftriaxone	89.7%(26/29)	-	10.3(3/29)
Chloramphenicol	20.7%(6/29)	27.6%(8/29)	51.7%(15/29)
Ciprofloxacin	86.2%(25/29)	-	13.8%
Gentamicin	100%(29/29)	-	0
Norfloxacin	89.5%(26/29)	-	10.3%(3/29)

## CHAPTER IV: DISCUSSION

The gastrointestinal tract as the natural reservoir for GBS and is most likely source of vaginal colonization. Colonization is unusual in childhood but becomes more common in late adolescence (CDC, 2002). Approximately 10% to 30% of pregnant women are colonized with GBS in the vagina or rectum. Maternal intrapartum GBS colonization is a major risk factor for early-onset disease in infants, and vertical transmission of GBS from mother to fetus primarily occurs after the onset of labor or membrane rupture. However, colonization early in pregnancy is not predictive of neonatal sepsis (CDC, MWR, 2002).

Neonatal sepsis is estimated to cause 26% of all neonatal deaths worldwide. Estimates of the incidence of neonatal sepsis are all from single-facility studies, and vary in their findings. A study from Malawi is the most specific, considering the incidence of early onset neonatal sepsis caused by *S agalactiae* alone, which was reported as 0.92 cases per 1000 live births. Regarding neonatal sepsis as a whole, 5.46 cases of neonatal bacteraemia per 1000 live births were recorded in Kilifi, Kenya, through blood-culture surveillance of all hospital admissions. In Nigeria, 6.5 cases of neonatal sepsis per 1000 live births occurring in a referral hospital were recorded. Twenty one cases of neonatal sepsis per 1000 live births were reported from a referral hospital in Zimbabwe (Seale *et al.*, 2009).

Group B *Streptococcus* (GBS) remains a leading cause of neonatal sepsis in the United States and other countries. The first consensus guidelines for the prevention of neonatal GBS disease were published in 1996, recommending intrapartum antibiotic prophylaxis on the basis of screening-based or risk-based strategies. Since then, there has been a 70% decrease in the rate of early-onset GBS disease. On the basis of evidence-validating superiority of this screening-based strategy, new national guidelines (for USA) were released in 2002. Data from the Centers for Disease Control and Prevention in 2005 showed a continued decrease in the annual incidence of early-onset GBS infection (Nandyal, 2008).

Intrapartum vaginal presence of Group B *Streptococcus* can cause severe neonatal infections including sepsis, pneumonia and meningitis, which generally occur within the first week of life or after seven days. Infections with GBS have been recognized as the leading cause of early-onset neonatal sepsis with significant mortality. Epidemiologic studies in the pre-prevention era revealed an incidence of 1–3 cases of early-onset

neonatal GBS per 1000 with a case-fatality rate of 20–50%. The risk of colonization of a neonate born to a colonized mother is between 40 and 70%, and 1–2% of these colonized infants will develop an early onset disease. At any given time, between 10–30% of women in the United States and between 5–15% in Europe are colonized with GBS (Rausch *et al.*, 2009). In Ethiopia similar to several other sub-Saharan countries in Africa, the rate of GBS colonisation among pregnant women and early onset neonatal disease due to GBS has not been well studied. Therefore, the present study was conducted to give baseline and update information about the prevalence of colonization of GBS among pregnant women attending Hawassa Health center, Hawassa, Ethiopia.

In the present study the overall prevalence of Group B *streptococcus* colonization among pregnant women was 20.86% (Table 3.2). This finding is high compared to the previous study conducted about 30 years ago among postpartum women in Gondar, Ethiopia, which was 9% (Schmidt *et al.*, 1989). The difference in colonization rate might be due to difference in geographic location, genetic differences, the study participant (pregnant versus postpartum) and laboratory diagnosis method including time and site of sample collection.

The high colonization rate of GBS among pregnant women in this study (20.86%) is consistent with findings from other developing African countries; Blantyre, Malawi, 16.5% (Dzowela *et al.*, 2005), Egypt, 17.89% (Elbaradie *et al.*, 2009), Zimbabwe, 21% (Mavenyengwa *et al.*, 2010), Gambia, 22% (Sura *et al.*, 1994), and Dare salaam, Tanzania, 23% (Joachim *et al.*, 2009). But the finding of this study is higher when compared to colonization rate from some other African countries like Mozambique which reported colonization rate of 1.8% (De Steenwinkel *et al.*, 2008). The difference in colonization rate reported from Mozambique and the present study may be due to source of blood used for culture for isolation of GBS and geographic difference can also contribute for the difference.

Findings from different studies conducted in Latin American are also similar with finding of the present study. Several studies conducted in Brazil indicated a colonization rate of 18.2% (Benchetrit *et al.*, 1982), 27.6% (Nomur *et al.*, 2006) and 17.9% (Zusman *et al.*, 2006). A study from Trinidad also found a higher colonization rate of pregnant women with GBS, 32.9% (Orrett, 2003). However some studies from Latin America found low colonization rate, for instance a study conducted in Lima, Peru showed a colonization rate of 6% (Timothy *et al.*, 1998) and another study from Rosario,

Argentina showed a colonization rate of 3.2% (Toresani *et al.*, 2001). The discrepancy between the above study and the present study may be due to geographical difference, racial difference, time of sample collection, and laboratory diagnosis method used.

In this study, the rate of GBS colonization is almost similar to the findings reported in some European countries. Two studies from Italy found a GBS colonization rate of 17.9% (Busetti *et al.*, 2007), and 18% (Savoia *et al.*, 2008). Studies from Poland and Switzerland also found a colonization rate of 17.2% (Strus *et al.*, 2009) and 21% (Rausch *et al.*, 2009), respectively. Another study in a multicultural population of pregnant women in the Netherlands also showed a colonization rate of 21% (Valkenburg-van den Berg *et al.*, 2006). However, lower GBS colonization rate have been reported in some Mediterranean countries, e.g. studies from Istanbul, Turkey and Elazig, Turkey found a colonization rate 8% (Bararos *et al.*, 2005) and 8.7% (Ayata *et al.*, 1994) respectively. Study conducted in a city of Northern Greece also found a low colonization rate of GBS among pregnant women 6.6% (Tsolia *et al.*, 2003).

Among 21 studies reported on 24,093 women from 13 European countries, GBS vaginal colonization rates ranged from 6.5 to 36%, with one third of studies reporting rates of 20% or greater. The regional carriage rates were as follows: Eastern Europe 19.7–29.3%, Western Europe 11–21%, Scandinavia 24.3–36%, and Southern Europe 6.5–32%. (Barcaite *et al.*, 2008). The result of the present study (20.86%) is within the range of GBS colonization rate reported in different parts of Europe as mentioned above.

The result of this study is also consistent with findings reported from most Asian countries. Studies conducted in Thailand, Thammasat Hospital and Thailand, Rajavithi Hospital found a colonization rate of 16% (Tor-udom *et al.*, 2006) and 18.12% (Kovavisarach *et al.*, 2007) respectively. Study from Tehran, and Hamadan, Iran showed a colonization rate of 20.6% (Fatemi *et al.*, 2008) and 26.7% (Rabiee *et al.*, 2006), respectively. Colonization rate of GBS in Saudi Arabia was reported to be 27.6% (El-Keysh *et al.*, 2002). However, the result of the present study is high when compared to the following two Asian countries; Hong Kong and Korea with colonization rate of 10.4% (Tsui *et al.*, 2009), 3.9% (Uh *et al.*, 1994), respectively. The result of the present study is also high when compared to the colonization rate of GBS from Australia 12.9% (Garland *et al.*, 2000). The difference in colonization rate between some Asian countries mentioned above and this study can be due to site of specimen collection (lower vaginal

swab vs. higher vaginal swab), time of sample collection, racial difference and geographical differences.

Knowledge about risk factors contributing for GBS colonization in pregnant women is relevant to minimize the morbidity, mortality associated with maternal and neonatal GBS infections. In the presents study, no statistically significant association was observed for GBS colonization in the study subjects with any of sociodemographic characteristics as outlined in Table 3.2. Similar findings have been reported in studies conducted elsewhere (Collins *et al.*, 1998, Zusman *et al.*, 2006, Costa, *et al.*, 2008). However studies conducted in Athens and Hong Kong showed that GBS colonization rate was high among pregnant women who work outside home and those who had frequent visits of antenatal clinic (Tsolia *et al.*, 2003, Tsui *et al.*, 2009).

In the present study, the susceptibility pattern of 29 GBS isolated from pregnant women against 10 antimicrobial agents is presented in Table 3.3. All strains were susceptible to penicillin, ampicillin, vancomycin and gentamicin. However resistance was observed against chloramphenicol (51.7%), tetracycline (44.8%), erythromycin (6.9%), ceftriaxone (10.3%), ciprofloxacin (13.8%) and norfloxacin (10.5%). Resistance to chloramphenicol and tetracycline might be explained by wide and indiscriminate use of these antibiotics in the country. On the other hand a study from Tanzania showed that all GBS isolates were sensitive to ampicillin, vancomycin and most isolates (90% to 98%) were sensitive to ciprofloxacin, and penicillin G and (10-20%) were resistant to erythromycin and ceftriaxone (Joachim *et al.*, 2009).

Study from Michigan, USA (Manning *et al.*, 2003) reported a higher resistance to erythromycin (29%) and no resistance to penicillin, ampicillin and vancomycin, which is in agreement with the findings of the present study. Another study form USA found all GBS isolates were sensitive to vancomycin, ampicillin, and penicillin and 31% of the isolates were resistant to ceftriaxone (Simos *et al.*, 2004).

Study from Canada (Azavedo *et al.*, 2001) has reported resistance rate of 18% and >80% for erythromycin and tetracycline, respectively. Another study from Lebanon (Hannoun *et al.*, 2009) reported resistance to erythromycin (11.8%) and tetracycline (86.8%), which was higher than the findings reported in the present study (6.9% for erythromycin and 44.8% for tetracycline).

The use of intrapartum antibiotics to prevent perinatal vertical transmission of GBS and early onset neonatal sepsis has increased significantly since the Center for

Disease Control (CDC) published guidelines in 1996 and subsequently released revised guidelines in 2002. The antibiotic of choice is either penicillin G or ampicillin (Simos *et al.*, 2004).

The current CDC guidelines recommend that patients not allergic to penicillin should receive penicillin or ampicillin. Individuals with a minor allergy to penicillin should receive cefazolin and individuals with a major allergy (rash or a history of difficulty in breathing) should receive clindamycin or vancomycin if the isolate is known to be resistant to clindamycin. However, vancomycin has not been shown to cross the placenta and achieve suitable concentrations in amniotic fluid as well as the fetal blood. This tremendous use of  $\beta$ -lactam antibiotics coupled with the exposure to  $\beta$ -lactams for other reasons can potentially induce the emergence of resistant strains among the resident vaginal microflora. Emergence of resistance has been documented, especially to ampicillin (Simos *et al.*, 2004).

#### **Limitation of the Study**

- Serotyping of GBS was not performed due to lack of group specific antisera. GBS can be subtyped into 9 serotypes; type Ia, Ib, I a/c, I b/c, II, III, IV, V and VI based on their capsular polysaccharide using specific antisera. It is believed that prevention of GBS-early onset neonatal disease strategy based on the administration of chemoprophylaxis is not the best approach to reduce the infection because of allergy to penicillin and wide spread of antibiotic resistance among the community. The best way to prevent GBS-early onset neonatal disease is developing effective multivalent vaccine. Knowledge about the prevalent GBS-serotypes in a given country is very important to develop and implement effective vaccine. However there is a major difficulty in developing group B *streptococcal* vaccines because of the existence of a multiple serotypes in different locations and geographic variations in serotype distribution (Johri *et al.*, 2006).

## **CHAPTER: V CONCLUSION AND RECOMMENDATION**

### **5.1. Conclusion**

Group B *streptococcus* (GBS) is one of the leading causes of morbidity and mortality among newborns, resulting in sepsis, pneumonia, and meningitis. In addition, GBS can cause significant morbidity in pregnant women. Manifestations of symptomatic maternal infection include chorioamnionitis, endometritis, cystitis, pyelonephritis and bacteraemia. In this study, a total of 139 pregnant women attending the routine antenatal care follow up at Hawassa Health center were screened for GBS colonization. A total of 29 out of 139 (20.8%) pregnant women studied were colonized by GBS. No statistically significant association was observed for GBS colonization with any of sociodemographic characteristics of the study subjects including age, occupation, type of contraceptive used, types of gravida, number of antenatal clinic visits etc. All GBS strains were susceptible to penicillin, ampicillin, vancomycin and gentamicin, Low level of resistance (<60%) were observed against erythromycin, tetracycline, ceftriaxone, chloramphenicol, ciprofloxacin and norfloxacin.

### **5.2. Recommendations**

Based on the findings of the present study and the above mentioned limitations the following recommendations are made: -

- The role of group B *streptococcus* in neonatal sepsis and meningitis should be investigated.
- This study was conducted in small sample size; extensive epidemiological investigations should be conducted in different parts of the country in order to know the actual GBS colonization rate in pregnant women. As more data regarding GBS in Ethiopia become available, it is important to consider implementation of prevention plans using intrapartum antibiotics prophylaxis to prevent early onset GBS-neonatal diseases.
- As documented in several studies as well as in the present study, GBS is universally sensitive to the penicillins; therefore, it should be the primary antibiotic for intrapartum prophylaxis. If the woman is allergic to penicillins, other antibiotics should be considered based on culture and sensitivity results.
- Serotyping of GBS should be performed. Knowledge about the prevalent GBS-serotypes in a given country is very important to develop and implement effective vaccine for prevention of neonatal GBS disease

## REFERENCES

- American Academy of Pediatrics. (1997) Revised guidelines for prevention of early-onset group B *streptococcal* (GBS) infection. American Academy of Pediatrics Committee on Infectious Diseases and Committee on Fetus and Newborn. *Pediatrics* **99**:489–496.
- Anandalakshmy PN and Buckshee K. (1997) Maternal mortality in a referral hospital of northern India: a sixteen-year review. *J Fam Welf* **43**:1-4.
- AOG Committee opinion. (1996) Prevention of early onset group B *Streptococcus* disease in new born. Committee on Obstetric practice, American College of Obstetrics and gynecologist. *Int J Gynacol Obstet* **54**:205.
- Apgar BS, Greenberg G and Yen G. (2002) Prevention of group B *Streptococcal* infection in newborns. *Can Med Assoc J* **166**:928-30.
- Ayata A, Güvenc H, Felek S, Aygün A, Kocabay K, and Bektas S. (1994) Maternal carriage and neonatal colonization of group B *streptococci* in labour are uncommon in Turkey. *Paediatr Perinat Epidemiol.* **8**:188-92.
- Azavedo SD, McGavin M, and Duncan C. (2001) Prevalence and mechanisms of macrolide resistance in invasive and noninvasive group B *streptococcus* isolates from Ontario, Canada. *Antimicrob Agents and Chemother* **45**:3504–08.
- Baker CJ and Edwards MS. (2001) Group B *Streptococcal* infections, Infectious diseases of the fetus and newborn infant. *Philadelphia: W.B. Saunders* **10**: 91-156.
- Baker CJ, Paoletti LC, and Wessels MR. (1999) Safety and immunogenicity of capsular polysaccharide–tetanus toxoid conjugate vaccines for group B *Streptococcal* types Ia and Ib. *J Infect Dis* **179**:142-15.
- Bararos I, Murat C, Mehmet V, Ismet T, Can K, Sukufe D, Ismail C and Yildiz P. (2005) The colonization incidence of group B *streptococcus* in pregnant women and their newborns in Istanbul. *Pediatr int* **47**:64-6.
- Barcaite E, Bartusevinius A, Tameliene R, Kliucinskas M, Maleckiene L and Nadisauskiene R. (2008) Prevalence of maternal group B *streptococcal* colonization in European countries. *Acta Obstetricia et Gynecologica Scandinavica* **87**:260-71.
- Benchetrit LC, Fraacalanzza EL, Peregrno H, Camelo AA and Sanches LR. (1982) Carriage of *Streptococcus agalactiae* in Women and Neonates and Distribution of Serological Types: a Study in Brazil. *J Clin Microbiol* **15**:787-90.

- Bergeron MG, Ke D, Ménard C, Picard FJ, Gagnon M, Bernier M, Ouellette M, Roy PH, Marcoux S, and Fraser WD. (2000) Rapid detection of group B *streptococci* in pregnant women at delivery. *N Engl J Med* **343**:175–179.
- Boyer KM and Gotoff SP. (1986) Prevention of early-onset neonatal group B *Streptococcal* disease with selective intrapartum chemoprophylaxis. *N Engl J Med* **314**:1665-69.
- Boyer KM, Gadzala CA, Kelly PD, Burd LI, and Gotoff SP. (1983) Selective intrapartum chemoprophylaxis of neonatal group B *Streptococcal* early-onset disease. Predictive value of prenatal cultures. *J Infect Dis.* **148**:802-9.
- Busetti M, D'Agaro P, and Campello C. (2007). Group B *Streptococcus* prevalence in pregnant women from North- Eastern Italy: advantage of screening strategy based on direct plating plus broth enrichment. *J Clin Pathol* **60**:1140-43.
- Center for Disease Control and Prevention. 2000 Early-onset group B *Streptococcal* disease, United States. *MMWR* **49**:793-96.
- Centers for Disease Control and Prevention. (1999) Laboratory practices for prenatal group B *streptococcal* screening and reporting—Connecticut, Georgia, and Minnesota, 1997–1998. *MMWR Morb Mortal Wkly Rep* **48**:426–428.
- Centers for Disease Control and Prevention. Prevention of Perinatal Group B *Streptococcal* Disease. 2002 *MMWR Morb Mortal Wkly Rep* **51**:1-24.
- Centers for Disease Control and Prevention. 1996 Prevention of perinatal group B *Streptococcal* disease: a public health prospective. *MMWR* **45**:1-24.
- Chaffin DO, Beres SB, Yim HH, and Rubens CE. (2000) The serotype of type Ia and III group B *streptococci* is determined by the polymerase gene within the polycistronic capsule operon. *J Bacteriol* **182**: 4466–4477.
- Christie NE, Atkins NE and Munch-Petersen E. 1944 A note on a lytic phenomenon shown by group B *Streptococcus*. *Aust J Exp Biol Med Sci* **22**:193–95.
- Cockerill FR and Smith TF. (2002) Rapid-cycle real-time PCR: a revolution for clinical microbiology. *ASM News* **68**:77–83.
- Collins TS, Calderon M, Gilman RH, Vivar A, and Charache P. (1998) Group B *Streptococcal* colonization in a developing country: its association with sexually transmitted disease and socioeconomic factors. *Am. J. Trop. Med. Hyg* **59**:633- 36.
- Costa AL, Lamy F, Chein MB, Brito LM, Lamy ZC, and Andrade KL. (2008) Prevalence of colonization by group B *Streptococcus* in pregnant women from a public maternity of Northwest region of Brazil. *Rev Bras Ginecol Obstet* **30**:274-80.

- Davies HD, Adair CE, Schuchat A, Low DE, Sauve RS, and McGeer A. (2001) Physicians' prevention practices and incidence of neonatal group B *Streptococcal* disease in 2 Canadian regions. *Can Med Assoc J* **164**:479-85.
- Davies HD, Miller MA, Faro S, Gregson D, Kehl SC, and Jordan JA. (2004) Multi-center study of a rapid, molecular-based assay for the diagnosis of group B *streptococcal* colonization in pregnant women. *Clin Infect Dis* **94**:420-29.
- De Steenwinkel DO, Tak HU, Muller AE, Nouwen JL, Oostvogel PM and Mocumbi SM. (2008) Low carriage rate of group B *Streptococcus* in pregnant women in Maputo, Mozambique. *Trop Med Int health* **13**:427- 29.
- Dillon HC, Khare S, and Gray BM. (1987) Group B *Streptococcal* carriage and disease: a 6-year prospective study. *J Pediatr* **110**:31-6.
- Doran KS and Nizet V. (2004) Molecular pathogenesis of neonatal group B *Streptococcal* infection: no longer in its infancy. *J Mol Biol* **54**:23-31.
- Doran KS, Liu GY, and Nizet V. (2003) Group B *streptococcal* beta hemolysin/cytolysin activates neutrophil signaling pathways in brain endothelium and contributes to development of meningitis. *J Clin Invest* **112**: 736–744.
- Dzowela T, Komolafe OO, and Lgbigbia A. (2005) Prevalence of group B *Streptococcus* Colonization in antenatal women at the Queen Elizabeth Central Hospital Blantyre-a preliminary study. *Malawi Med J* **17**:97-9.
- Elbaradie SM, Mahmoud M, and Farid M. (2009) Maternal and neonatal screening for Group B *streptococci* by SCP B gene based PCR: A preliminary study. *Indian J Med Microbiol* **27**:17-21.
- El-Keysh T, Al-Nuaim L, Kharfy T, Al-shammary F, Al-saleh S and Al-Zamel F. (2002) Detection of genital colonization of group B *streptococci* during late pregnancy. *Saudi Med J* **23**:56-61.
- Fatemi F, Chamani-Tabriz L, Pakzad P, Zeraati H, Rabbani H, and Asgari S. (2008) Colonization Rate of Group B *Streptococcus* (GBS) in Pregnant Women Using GBS Agar Medium. *Acta Medica Iranica* **47**: 25-30.
- Garland SM, Kelly N, and Ugoni AM. (2000) Is Antenatal Group B *Streptococcal* Carriage a Predictor of Adverse Obstetric Outcome? *Infect Dis in Obstet Gynecol* **8**:138-42.
- Gray KJ, Bennett SL, French N, Phiri AJ, and graham SM. (2007) Invasive Group B *Streptococcal* Infection in Infants, Malawi. *Emerg Infect Dis* **13**:223-28.

- Hannoun A, Shehab M, Khairallah MT, Sabra A, Abi-Rachid R, Bazi T, Yunis KA, Araj GF, and Matar GM. (2009) Correlation between Group B *Streptococcal* Genotypes, Their Antimicrobial Resistance Profiles, and Virulence Genes among Pregnant Women in Lebanon. *Int J Microbiol* **2009**:796512.
- Heather EJ and Lahra MM. (1998) Eight-year outcome of universal screening and intrapartum antibiotics for maternal group B *Streptococcal* carriers. *J Pediatrics* **101**:2.
- Joachim A, Matee M, Masswawe FA, and Layamuya EF. (2009) Maternal and neonatal colonization of group B *streptococcus* at Muhimbili National Hospital in Dares Salaam, Tanzania: prevalence, risk factors and antimicrobial resistance. *BMC Public Health* **9**:437-45.
- Johri AK, Paoletti LC, Glaser P, Dua M, Sharma PK, Grandi G, and Rappuoli R. (2006) Group B *Streptococcus*: global incidence and vaccine development. *Nat Rev Microbiol* **4**:932-42.
- Jones DE, Friedl EM, Kanarek KS, Williams JK, and Lim DV (1983) Rapid identification of pregnant women heavily colonized with group B *streptococci*. *J Clin Microbiol* **18**:558–560.
- Ke D, Ménard C, Picard FJ, Boissinot M, Ouellette M, Roy PH, and Bergeron MG. (2000) Development of conventional and real time PCR assays for the rapid detection of group B *streptococci*. *Clin Chem* **46**:324–331.
- Kircher SM, Meyer MP, and Jordan JA. (1996) Comparison of a modified DNA hybridization assay with standard culture enrichment for detecting group B *streptococci* in obstetric patients. *J Clin Microbiol* **34**:342–344.
- Kovavisarach E, Sa-adying W, and Kanjanahareutai S. (2007) Risk Factors related to Group B *Streptococcal* Colonization in Pregnant Women in Labor. *J Med Assoc Thai* **90**:1287-92.
- Krohn MA, Hillier SL, and Baker CJ. (1999) Maternal Peripartum Complications Associated with Vaginal Group B *Streptococci* Colonization. *J Infect Dis* **179**:1410-15.
- Kuruvilla KA, Thomas N, Jesudasan MV, and Jana AK. (1999) Neonatal group B *Streptococcal* bacteraemia in India: ten years' experience. *Acta Paediatr* **88**:1031-32.
- Manning SD, Betsy F, Carl L, Patricia T, Carol J, and Mark D. (2003) Correlates of Antibiotic-Resistant Group B *Streptococcus* Isolated From Pregnant Women. *Am J Obstet and Gynecol* **101**:74 –9.

- Manning SD, Neighbors K, Tallman PA, Gillespie B, Marrs CF, Borchardt SM, Baker CJ, Pearlman MD and Foxman B. (2004) Prevalence of Group B *Streptococcus* Colonization and Potential for Transmission by Casual Contact in Healthy Young Men and Women. *Clin infect Dis* **39**:381-87.
- Marchaim D, Hallak M, Gortzak-Uzan L, Peled N, Reisenberg K, and Schlaeffer F. (2003) Risk Factors for Carriage of Group B *Streptococcus* in Southern Israel. *Isr Med Assoc J* **5**:646-48.
- Mavenyengwa RT and Maeland J. (2008) Distinctive features of surface-anchored proteins of *Streptococcus agalactiae* strains from Zimbabwe revealed by PCR and dot blotting. *Clin Vaccine Immunol* **15**:1420-24.
- Mavenyengwa RT, Afset JE, Schei B, Berg S, Caspersen T, Bergseng H, and Moyo S. (2010) Group B *Streptococcus* colonization during pregnancy and maternal-fetal transmission in Zimbabwe. *Acta Obstetricia et Gynecologica Scandinavica* **89**:250-55.
- Mavenyengwa RT, Masunga P, Meque E, Kudinha T, Moyo S R, Bevanger L, Bergh K, Nziramasanga P, and Mapako T. (2006) *Streptococcus agalactiae* (group B *Streptococcus* (GBS) colonization and persistence, in pregnancy; a comparison of two diverse communities (rural and urban). *Cent Afr J Med* **52**:38-43.
- Mohle-Boetani JC, Schuchat A, Plikaytis BD, Smith JD, and Broome CV. (1993) Comparison of prevention strategies for neonatal group B *Streptococcal* infection. A population-based economic analysis. *JAMA* **270**:1442-48.
- Nandyal RR. (2008) Update on Group B *Streptococcal* infections. *J periant Neonatal Nurs* **22**:230-37.
- National Committee for Clinical Laboratory Standards (NCCLS). (2002) Performance standards for antimicrobial susceptibility testing. *NCCLS approved standard* **12**: M 100 -59.
- Nomur ML, Junior RP, and Oliveira UM. (2006) Selective versus Non-selective Culture Medium for Group B *Streptococcus* Detection in Pregnancies Complicated by Preterm Labor or Preterm-Premature Rupture of Membranes. *Braz J Infect Dis* **10**:247-50.
- O'Brien KL, Hochman M, and Goldblatt D. (2007) Combined schedules of *Pneumococcal* conjugate and polysaccharide vaccines: is hypo responsiveness an issue? *Lancet Infect Dis* **7**:597-606.

- Orrett FA. (2003) Colonization with Group B *streptococci* in pregnancy and outcome of infected neonates in Trinidad. *Pediatric Intl* **45**:319-23.
- Picard FJ and Bergeron MG. (2004) Laboratory diagnosis of group B *Streptococcus* for prevention of perinatal disease. *Eur J clin Microbiol Infect Dis* **23**:665-71.
- Pulver LS, Hopfenbeck MM, Young PC, Stoddard GJ, Korgenski K, Daly J and Byington CL. (2009) Continued early onset group B *Streptococcal* infections in the era of intrapartum prophylaxis. *J of Perinatol* **29**:20-5.
- Rabiee S, Arab M, and Mashouf YR. (2006) Epidemiologic Pattern of Vaginal Colonization by Group B *Streptococcus* in Pregnant Women in Hamadan, Central West of Iran. *Iran J Med Sci* **31**:106-8.
- Rausch AV, Gross A, Droz S, Bodmer T and Surbek DV. (2009) Group B *Streptococcus* colonization in pregnancy: prevalence and prevention strategies of neonatal sepsis. *J Perinat Med* **37**:124–9.
- Regan JA, Chao S, and James LS. (1981) Premature rupture of membranes, preterm delivery, and group B *Streptococcal* colonization of mothers. *Am J Obstet Gynecol* **141**:184-86.
- Regan JA, Klebanoff MA, and Nugent RP. (1991) The epidemiology of group B *Streptococcal* colonization in pregnancy. Vaginal Infections and Prematurity Study Group. *Obstet Gynecol* **77**:604-10.
- Rosa C, Clark P, and Duff P. (1995) Performance of a new DNA probe for the detection of group B *streptococcal* colonization of the genital tract. *Obstet Gynecol* **86**:509–511.
- Samuel PG. (2002) Group B *Streptococcal* Infections. *Pediatr Rev* **23**:381-85.
- Savoia D, Gottimer C, Crocilla C, and Zucca M. (2008) *Streptococcus agalactiae* in pregnant women: Phenotypic and genotypic characters. *J Infect* **56**:120-25.
- Schmidt J, Halle E, Halle H, Mohammed T, and Gunther E. (1989) Colonization of pregnant women and their newborn infants with group B *Streptococci* in the Gondar College of Medical Sciences. *Ethiop Med J* **27**:115-19.
- Schuchat A, Hilger T, and Zell E. (2001) Active Bacterial Core Surveillance of the Emerging Infections Program Network. *Emerg Infect Dis.* **7**:92-9.
- Schwartz-Linek U, Hook M, and Potts R. (2004) The molecular basis of fibronectin-mediated bacterial adherence to host cells. *Mol Microbiol* **52**: 631–641.

- Seale AC, Mwaniki M, Newton JC, and Berkley JA. (2009) Maternal and early onset neonatal bacterial sepsis: burden and strategies for prevention in sub-Saharan Africa. *Lancet Infect Dis* **9**:428–38.
- Shet A and Ferrieri P. (2004) Neonatal & maternal group B *Streptococcal* infections: A comprehensive review. *Indian J Med Res* **120**:141-50.
- Simos JA , Aroutcheva AA , Ira H and Sebastian F. (2004) Antibiotic resistance patterns of group B *streptococcal* clinical isolates. *Infect Dis Obstet Gynecol.* **12**:1–8.
- Strus M, Pawlik D, Brzychczywoch M, Gosiewski T, Rytlewski K, Lauterbach R and Heczko PB. (2009) Group B *streptococcus* colonization of pregnant women and their children observed on obstetric and neonatal wards of the University Hospital in Krakow, Poland. *J Med Microbiol* **58**:228–33.
- Sura RO, Adegbola RA, Baker CJ, Secka O, Mulholland EK, and Greenwood BM. (1994) Carriage of Group B *Streptococcus* among pregnant Gambian mothers and their infants. *J Infect Dis* **170**:1316-323.
- Tamura GS, and Rubens CE. (1995) Group B *streptococci* adhere to a variant of fibronectin attached to a solid phase. *Mol Microbiol* **15**: 581–589.
- Tamura GS, Kuypers JM, Smith S, Raft H, and Rubens CE. (1994) Adherence of Group B *Streptococci* to cultured epithelial cells: Role of environmental factors and bacterial surface component. *Infect Immun* **62**:2450-58.
- Toresani I, Limansky A, Bogado I, Guardati MC, Viale A, Sutich EM and pregnancy disease group. (2001) phenotypic and genotypic study of *Streptococcus agalactiae* in vagina of pregnant women in Argentina. *MEDICINA* **61**:295-00.
- Tor-udom P and Hirrote W. (2006) The Prevalence of *Streptococcus agalactiae* (Group B) Colonization in Pregnant Women at Thammasat Hospital. *J Med Assoc Thai* **89**:411-4.
- Trijbels-Smeulders M, Gerards LJ, de Jong P, and M PC. (2002) Epidemiology of neonatal group B *Streptococcal* disease in The Netherlands. *Paediatr Perinat Epidemiol* **16**:334-41.
- Tsolia M, Psoma M, Gavrili S, Petrochilou V, Michalas S, Legakis N, and Karpathios T. (2003) Group B *Streptococcus* colonization of Greek pregnant women and neonates: prevalence, risk factors and serotypes. *Clin Microbiol Infect* **9**:832-38.
- Tsui HY, Ip M, Ng P, Sahota DS, Leung T and Lau T. (2009) Change in prevalence of group B *Streptococcus* maternal colonization in Hong Kong. *Hong Kong Med J* **15**:C1-66.

- Uh Y, Kwon JY, Jang IH, Yoon KJ, and Kim HG. (1994) Colonisation rate of Group B *Streptococcus* in pregnant women and neonates. *Korean J Clin Pathol* **14**:447-53.
- Valkenburg-van den Berg AW, Sprij AJ, Oostvogel PM, Mutsaers JA, Renes WB, Rosendaal FR, and Dörr PJ. (2006) Prevalence of colonization with group B *Streptococci* in pregnant women of a multi-ethnic population in The Netherlands. *Eur J Obstet Gynecol Reprod Biol* **124**:178-83.
- Whitney CG, Daly S, Limpongsanurak S, Festin MR, Thinn KK, Chipato T, Lumbiganon P, Sauvarin J, Andrews W, and Tolosa JE. (2004) The international infections in pregnancy study: group B *Streptococcal* colonization in pregnant women. *Journal of Matern Fetal Neonatal Med* **15**:267-74.
- Wibawan IT, Lammler C, and Pasaribu FH. (1992) Role of hydrophobic surface proteins in mediating adherence of group B *streptococci* to epithelial cells. *J Gen Microbiol* **138**: 1237–1242.
- Williams-Bouyer N, Reisner BS, and Woods GL. (2000) Comparison of Gen-Probe AccuProbe group B *Streptococcus* culture identification test with conventional culture for the detection of group B *streptococci* in broth cultures of vaginal-anorectal specimens from pregnant women. *Diagn Microbiol Infect Dis* **36**:159–162.
- Yagupsky P, Menegus MA, and Powell KR. (1991) The changing spectrum of group B *Streptococcal* disease in infants: an eleven-year experience in a tertiary care hospital. *Pediatr Infect Dis J* **10**:801-8.
- Yancey MK, Armer T, Clark P, and Duff P. (1992) Assessment of rapid identification tests for genital carriage of group B *streptococci*. *Obstet Gynecol* **80**:1038–1047.
- Zaleznik DF, Rench MA, Hillier S, Krohn MA, Platt R, and Lee ML. (2000) Invasive disease due to group B *Streptococcus* in pregnant women and neonates from diverse population groups. *Clin Infect Dis* **30**:276-81.
- Zangwill KM, Schuchat A, and Wenger JD. (1992) Group B *Streptococcal* disease in the United States, report from a multistate active surveillance system. *MMWR CDC Surveill Sum* **20**:25-32.
- Zusman AS, Baltimore RS, and Fosica NS. (2006) Prevalence of Maternal group B *Streptococcal* Colonization and Related Risk Factors in a Brazilian Population. *Braz J Infect Dis* **10**:242-6.

## APPENDIX I

Questionnaire for investigation of colonization rate of GBS among pregnant women in Hawassa health center, Hawassa, SNNP, Ethiopia

### a. Participant Identification

1. Serial No.....
2. Card no.....
3. Address .....
4. Participant name.....
5. Age.....
6. Ethnicity.....
7. Marital status.....
8. Occupation.....
9. History of primigravida or multigravida: Primigravida .....
- Multigravida... ..
- Gestational age (weeks).....
10. Number of prenatal visit.....
11. History of recent any antibiotic treatment      Yes/No  
    If yes, mention antibiotic/s and  
    time taken.....
12. History of any contraceptive use      Yes/No  
    If yes, mention type of contraceptive used.....
13. Date and time of rectovaginal specimen collection .....

**b. Laboratory data**

1. Growth of GBS on Todd Hiowtii selective media.....
2. Growth of GBS on Sheep blood agar and hemolysis pattern.....
3. Result of Gram stain.....
4. Catalase test result:                    positive/Negative
5. Result of CAMP test:                    positive/Negative
7. Result of Slides of Agglutination with antisera: positive/Negative
8. Antimicrobial susceptibility testing    S (mm)            I (mm)            R (mm)
  - Penicillin (10IU)                    .....            .....            .....
  - Erythromycin (15µg)                .....            .....            .....
  - Tetracycline (30µg)                .....            .....            .....
  - Ampicillin (10µg)                    .....            .....            .....
  - Vancomycin (30µg)                    .....            .....            .....
  - Ceftriaxone (30µg)                    .....            .....            .....
  - Chloramphenicol (30µg)                .....            .....            .....
  - Ciprofloxacin (5µg)                    .....            .....            .....
  - Gentamicin (10µg)                    .....            .....            .....
  - Norfloxacin (10µg)                    .....            .....            .....

**c. Comments** \_\_\_\_\_  
\_\_\_\_\_

Name of principal investigator \_\_\_\_\_

Signature \_\_\_\_\_ Date \_\_\_\_\_

## APPENDIX II

### INFORMATION SHEET

You are kindly invited to participate in this study, which involves about 139 pregnant women from Hawassa Referral Hospital and Health centers. The title of the study is “prevalence of colonization with Group B *Streptococcus* among pregnant women attending antenatal clinic, in Hawassa Health center, Hawassa, Ethiopia. The aim of this study is to determine prevalence rate of colonization with Group B *Streptococcus* in pregnant women. Pregnant women with GBS colonization during 37 and 38 weeks of gestations will pose a risk to their children during delivery. Infection with this organism can cause different disease in new born and therefore this study will identify colonization rate in pregnant women so that those who are colonized with GBS will receive prophylaxis before delivery to keep their baby safe.

- a. **Purpose:** the purpose of this research study is to assess the prevalence of colonization of group B *Streptococcus* among pregnant women attending antenatal clinic in Hawassa Referral Hospital, Hawassa, Ethiopia.
- b. **Duration:** the duration of this study depend upon the availability of study subjects it can probably take about three months or more.
- c. **Procedures to be carried on:** the procedure of sample collection is easy and straight forward; sample will be collected from vagina and anorectal area using cotton swab by attending physician and then it will be analyzed in the microbiology laboratory of Hawassa Referral Hospital for the presence of group B *Streptococcus*.
- d. **Risk and discomfort:** almost there will no be any risk associated during sample collection without little discomfort.
- e. **Expected benefits:** from this study you are not directly benefited however; the new born will be benefited, as screening and administering chemoprophylaxis to group B *Streptococcus* carrier pregnant women reduce the risk of vertical transmission of GBS to infants.
- f. **Confidentiality:** All your personal information collected for the purpose of the present study will be kept confidential.
- g. **Compensation:** No compensation will be provided by participating in this study.
- h. **Termination of the study:** Participation in the study is voluntary, and refusal to

participate involves no penalty or loss of benefits to which you are otherwise entitled. The study participants have a right to

- Withhold information
- Decline to cooperate in the study
- To refuse provision of specimens

I would also like to inform you that this study is approved by Department Ethical and Review Committee and ethically cleared by Institutional Review Board (IRB), Faculty of Medicine Addis Ababa University. If you have any question about the right of the study participant the address is:

Faculty of Medicine Addis Ababa University  
Office of Associate Dean, Postgraduate Programs and Research  
P.O. Box 9086. Addis Ababa, Ethiopia  
Tel. 251-011-551-28-765

If you have question about the study the address of the principal investigator is:

Musa Mohammed  
Department of Microbiology, Immunology and Parasitology  
Faculty of Medicine, Addis Ababa University  
P.O. Box. 9086, Addis Ababa, Ethiopia  
Tel: 0911765175



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## **CONSENT FORM**

(To be translated in to Amharic)

Serial no.....

Card no.....

Name of study participant: \_\_\_\_\_

I have been requested to participate in this study which involves collecting of rectovaginal specimen. During collection of the specimen I have told that there is no harm without little discomfort I have also read the information sheet (or it has been read to me); I have understood that this study is about prevalence of colonization with Group B *Streptococcus* among pregnant women attending antenatal clinic Hawassa Referral Hospital, Hawassa, Ethiopia in which my baby will be protected from Group B *Streptococcal* infection. I have asked some questions and clarification has been given to

me. I have given my consent freely to participate in the study, and I hereby approve my agreement with my signature.

Participants signature \_\_\_\_\_ Date \_\_\_\_\_

Investigator signature \_\_\_\_\_ Date \_\_\_\_\_

Witness signature 1. \_\_\_\_\_ Date \_\_\_\_\_

2. \_\_\_\_\_ Date \_\_\_\_\_

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