ISOLATION AND ANTIBACTERIAL SUSEPTIBILITY PATTERN OF *STREPTOCOCUS AGALACTIAE* IN PREGNANT WOMEN IN ADIGRAT ZONAL HOSPITAL AND ADIGRAT HEALTH CENTER, TIGRAY, ETHIOPIA

BY

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Abbreviations

AAP…….American Academy of Pediatrics

ACOG……..American College of Obstetricians and Gynecologists.

ACP …….....Alpha C protein

APF……....Addis Pharmaceuticals Factory

ATCC.....American Type Culture Collection

CA…….....ChromID *Strepto* B Agar

CAN……Columbia agar with colistin and nalidixic acid

CDC……....Centers for Disease Control and Prevention

CLSI……Clinical and Laboratory Standards Institute

CPS…….Capsular Poly Sacchraide

CAMP……Christie, Atkins, Munch-Peterson

EHNRI…… Ethiopian Health and Nutrition Research Institute

EOD…….Early onset disease

GBS…….Group B *Streptococcus*

GBSDA ……..Group B *Streptococcus* differential agar

LOB……..Late onset disease

MAC…….....Membrane Attack Complex

ML ……Macroliode

MYD88…….....Myeloid Differentiation Factor 88

MMWR……....Morbidity Mortality Weekly Report

Pbp……....Penicillin binding protein

PROM……...Prolonged Rupture of Membranes

SHT……...*Streptococcal* Histidine Triad

SPSS…….Statistical Package for Social Science

TLR2 ……....Toll like receptor 2

Abstract

Back ground: *S. agalactiae* which are group B *Streptococci* asymptomatically colonize the vaginal or rectal areas of 10 to 30 % of pregnant women. In these women, *S. agalactiae* may cause preterm labor or membrane rupture, as well as urinary tract infections, chorioamnionitis, postpartum endometritis, postpartum wound infection, septic pelvic thrombophlebitis, endocarditis and sepsis. These bacteria is a major cause of invasive disease at all ages and is the most frequent cause of serious bacterial sepsis, including neonatal meningitis.

Objective: This study was undertaken to determine the carriage rate of *S. agalactiae* and to assess their antimicrobial susceptibility pattern. An attempt has been also made to identify the possible risk factors related with *S. agalactiae* colonization.

Methods: Rectal and vaginal swabs were obtained from 150 pregnant women at 35-37 weeks of gestational period that attended anti natal clinic at Adigrat Zonal Hospital and Adigrat Health Center. The specimen was cultured on selective CHROMagar^{TM} *Strep*B and incubated aerobically at 37^{o}c for 18-24 hours. Suspected colony of *S. agalactiae* mauve colony (pink color) was confirmed by gram stain, catalase test, Christie, Atkins, Munch-Peterson (CAMP) testing and latex agglutination (serological) test. In cases of positive cultures obtained, antibiotic susceptibility tests were carried out on all *S. agalactiae* isolates using the disc diffusion technique on Mueller-Hinton agar supplemented with 5% sheep blood and incubated at 37^{0}c for 20-24 hours in 5% co_2. A univariate and multivariate binary logistic regression model was used to ascertain the association between the frequencies of colonization in relation to the different variables.

Results: Seventeen of the study participants (11.3%) were colonized by *S. agalactiae*. Thirteen (76.5%) of the isolates were from health center and 4(23.5%) werfrom hospital. The study revealed a higher colonization rate among the age group 21 to 30 years (76.5%) but one pregnant woman with *S. agalactiae* was identified (5.9%) in women aged lesser than or equal to 20 years. Bacterial resistance was not detected against ampicillin, penicillin G, amoxacilline and vancomycin, whereas 11.8% and 17.6% of the isolates were resistant to

erythromycin and clindamycin respectively. Intermediate susceptibility was also detected in 2 isolates (11.8%) against erythromycin and in 2 isolates (11.8%) against clindamycin. By multi variant logistic regression analysis, Prolonged rupture of membrane was associated with a higher colonization rate of *S. agalactiae* (OR=5.864, 95% CI= 1.395 – 24.643, P-value= 0.016). No significant association was identified between *S. agalactiae* colonization rates with other socio- demographic/gynecological characteristic of the pregnant women.

Conclusion: The carriage rate of *S. agalactiae* in the study area was 11.3%. High *S. agalactiae* isolates were detected from Adigrat Health Center. Prolonged rupture of membrane was strongly associated with the colonization of *S. agalactiae*. Based on the finding, penicillin G was the best antibiotic for the treatment of *S. agalactia*e. Out of the isolates 11.8% were resistance to erythromycin and 17.6% were resistance to clindamycin Common resistance to erythromycin and clindamycin was seen in two isolates.

Keywords: *S. agalactiae*, Antibiotic susceptibility pattern, Pregnancy, Colonization, Ethiopia.

1. Introduction

The word *agalactiae* means want of milk, this is because of the original isolate called *S. mastitidis* was responsible for bovine mastitis. *S. agalactiae* or Lancefield group B *Streptococci* (GBS) is part of the microbiota of the mucous membranes of humans and animals, mainly colonizing the intestinal and genitourinary tracts (CDC, 1996). The great medical importance of this microorganism is the contamination of neonates, causing severe septicemia, pneumonia and meningitis (Schuchat, 1995; Rollins, 2000).

Women colonized with *S. agalactiae* during pregnancy are at increased risk of stillbirths, premature delivery (Schrage *et al.*, 2000), symptomatic and asymptomatic urinary tract infections (CDC, 2002). *S. agalactiae* also have been recognized as an ever-growing cause of severe invasive infections in non-pregnant adults, particularly older adults and immunocompromised patients (CDC, 2007; Schrage *et al.*, 2000; Iannelli, 2004). Women with a complicated pregnancy are more often colonized than those with a normal pregnancy (Strus *et al*., 2009).

S. agalactiae has remained the leading infectious cause of neonatal morbidity and mortality in different part of the world. In Italy an annual *S. agalactiae* disease incidence was 0.50 per 1000 live births (Beradi *et al*., 2007). In Canada the incidence rate was 0.46 per 10,000 adults (Tyrrell *et al*., 2000). In less-developed countries, the incidence of *S. agalactiae* neonatal disease also varies widely: 0.17 per 1000 live births in India (Kuruvilla *et al*., 1999) to 1.8 per 1000 live births in Malawi (Gray *et al*., 2007).

Ethiopia is one of the developing countries with an estimated infant mortality rate of 77 per 1000 live birth (DHS, 2005). In Tigray analysis of admissions of the hospitals showed that pneumonia is the leading cause of admissions in under-five children which accounts for 30.5% and it is the leading cases of mortality (31%). Neonatal sepsis is also common causes of admissions and deaths in this region (THB, 2010). This clinical condition can be correlated with *S. agalactiae* colonization.

Penicillin is the first line antibiotic for chemoprophylaxis of pregnant women colonized by *S. agalactiae* and ampicillin is the acceptable alternative commonly used antibiotic. In cases of penicillin allergic pregnant women not at risk of anaphylaxis cefazolin is the recommended antibiotic. If the pregnant woman is penicillin allergic and at risk of anaphylaxis: clindamycin or erythromycin is used. If *S. agalactiae* resistance is demonstrated to clindamycin or erythromycin by culture and sensitivity then vancomycin is used (CDC, 2010; AGOC, 2011).

In the study area the carriage rate of *S. agalactiae* in pregnant women and neonatal disease associated with mortality and morbidity was largely unknown. The present study was therefore undertaken to determine the carriage rate of *S. agalactiae* colonization among pregnant women in their third trimester and to evaluate the antimicrobial susceptibility pattern of the isolates.

2. Background and significance of the study

2.1. Background

2.1.1. Morphology and characteristics of *S. agalactiae*

S. agalactiae is grouped under the genus *Streptococci***.** The differentiation of species within the genus is complicated, because three different, overlapping schemes are used to classify the organisms: (1) Serologic properties: Lancefield groupings (originally A to W) each of which is generally correlated with an established species. *S. agalactiae* is termed as group B *Streptococcus* because of the antigenicity of a carbohydrate occurring in their cell walls (Lancefield antigen). *S. agalactiae* is the only species that carries the group B antigen; (2) hemolytic patterns: complete (beta [β]) hemolysis, incomplete (alpha [α]) hemolysis, and no (gamma) hemolysis; and (3) biochemical (physiologic) properties (Rollins, 2000).

S. agalactiae is gram-positive, nonmotile, catalase-negative, facultative anaerobic cocci (Hardie and Whiley, 1997), capsular (Freimer, 1996) that form short chains in clinical specimens and longer chains in culture. They are β-hemolysis or non hemolytic. They grow well on nutritionally enriched media, and in contrast with the colonies of *S. pyogenes*, the colonies of *S. agalactiae* are large, buttery with a narrow zone of β-hemolysis (Rollins, 2000).

S. agalactiae produce several enzymes, including proteases, hippurase, hyaluronidase (Lint *et al*., 1994), neuraminidase (Milligan *et al*., 1977) and Sialidase (Hayano and Tanaka, 1969) and all of *S. agalactiae* isolates contained *cfb* gene coding for Christie, Atkins, Munch-Peterson (CAMP) factor a diffusible heat-stable protein that enhances β-hemolysis of *Staphylococcus aureus* and most of *S. agalactiae* isolates contained one or multiple *bca, bac, rib, alp1, alp3* genes coding for surface protein antigens (Ramaswamy *et al*., 2006).

S. agalactiae has a polysaccharide capsule. The different capsular polysaccharide antigens are used to classify *S. agalactiae* into nine different serotypes. They are– Ia, Ib, II–VIII (Kong *et al.*, 2002; Muller *et al.*, 2006) all of which contain sialic acid in the form of

terminal side chain residues as well as galactose and glucosamine components (Baker and Kasper, 1976; Wessel *et al.,* 1992).

Serotypes III is the most predominant *S. agalactiae* strain that cause infection in most part of the world (Gray *et al*., 2007; Madzivhandila *et al.*, 2011; Murayama *et al*., 2009; Fluegge *et al.,* 2004). However, Serotypes V and VIII are the emerging CPS (capsular) serotype in some part of the world **(**Edwards *et al*., 2005; Paoletti *et al*., 1999). Small numbers of isolates of serotypes IV, VI, and VIII were isolated in Australasia (Kong *et al.*, 2002). Serotypes Ia is also reported as predominant in Minnesota (Hickman *et al*., 1999). And in Ethiopia (Gondar) the *S. agalactiae* isolates were Ib/c and Ia serotype (Schmidit *et al*., 1989). This is different from a finding in other part of Africa (Malawi (Gray *et al*., 2007) and South Africa (Madzivhandila *et al.*, 2011)).

2.1.2. *S. agalactiae* Pathogenesis and virulence factors

The capsule is a critical virulence determinant as it cloaks antigenic proteins allowing the bacteria to evade host immunity (Segura, 2012) and most isolates from invasive disease are encapsulated. The capsular polysaccharide inhibits the binding of the activated complement factor C3b to the bacterial surface; preventing the activation of the alternative complement pathway and this is due to the presence of sialic acid residues on the surface of the organisms (Marques *et al.*, 1992; Wessel *et al*., 1992). The amount of sialic acid in *S. agalactiae* strains isolated from patients with invasive infection is significantly higher than that observed for strains isolated from carriers and its content is N-acetylneuraminic acid (Teixeira *et al.,* 1993). The type III *S. agalactiae* capsular polysaccharide regulates interactions with neutrophil complement receptors (Edwards *et al*., 1993). The *Streptococcal* histidine triad (SHT) surface protein on the surface of *S. agalactiae* type III bind to the host complement regulator, factor H (FH), whereby complement is broken down to help *S. agalactiae* survive in the host by evading complement opsonization (Figure 1) (Maruvada *et al*., 2009).

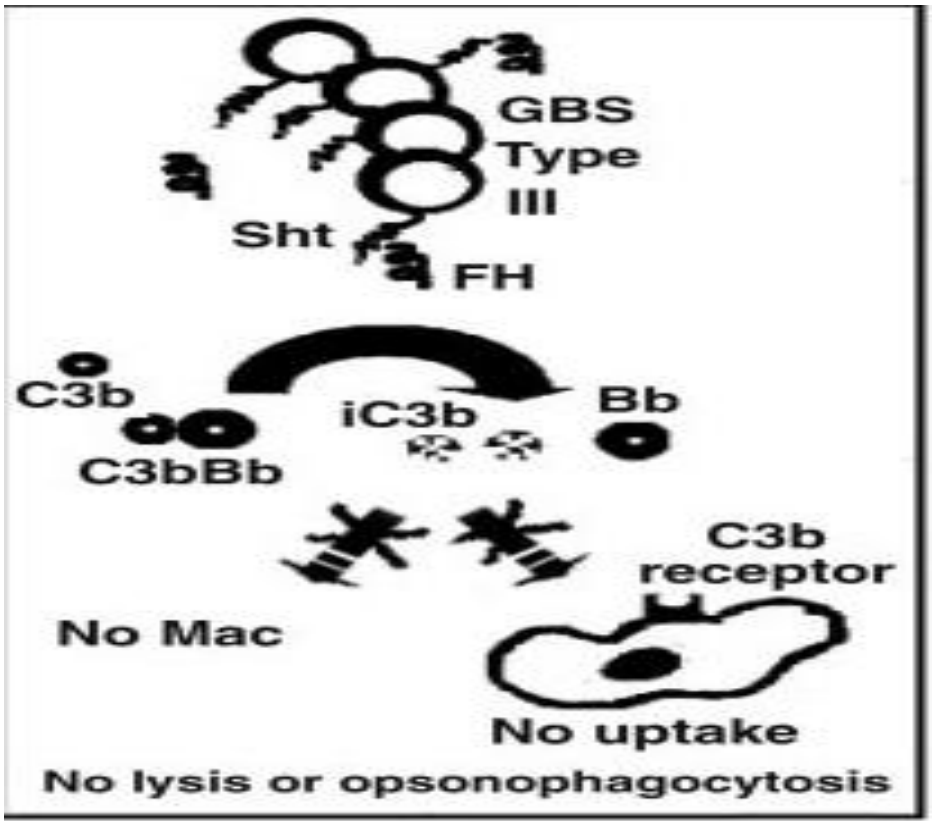

Figure 1: Diagrammatic representation of the role of SHT in the evasion of complement attack by *S. agalactiae* (Picture from Maruvada *et al*., 2009).

S. agalactiae expresses a *cylJ*, encoding a putative glycosyltransferase, and *cylK*, whose product is unknown, are both required for the full hemolytic/cytolytic activity, pigment formation and virulence of *S. agalactiae* (Forquin *et al*., 2007). *S. agalactiae* hemolysin expression correlates with lung epithelial cell injury and may be important in the initial pathogenesis of early-onset disease, particularly when pulmonary surfactant is deficient (Nizet *et al*., 1996). *S. agalactiae* have also been shown to produce a peptidase that inactivates C5a, the major chemo attractant of PMNs (Bohnsack *et al*., 1997).

Adhesion to human epithelial and endothelial cells is a critical step for *S. agalactiae* colonization and invasion. The first surface protein antigen described was the c antigen in 1971, which was found to consist of two fractions. One fraction was sensitive to digestion with trypsin and the other was resistant to digestion with trypsin (Wilkinson and Eagon, 1971). The proteins were called the "Ibc proteins". These proteins were later designed alpha

and beta, where the alpha antigen corresponded to the trypsin resistant protein fraction and beta to the trypsin sensitive fraction (Bevengar and Maeland, 1979). The two proteins have then been designed alpha c protein and beta c protein. C proteins are an immunologically important group of surface-associated antigens in *S. agalactiae*. Alpha C Protein (ACP) binds to human epithelial cells and plays a role in the internalization and translocation of *S. agalactiae* across epithelial cells (Baron *et al.*, 2007).

2.1.3. Epidemiology

The major burden of neonatal sepsis and meningitis occurs in the developing world, but most evidence derives from wealthy countries although the spectrum of disease, etiology and prognosis may differ. Neonatal infections currently cause about 1.6 million deaths annually in developing countries. Sepsis and meningitis are responsible for most of these deaths (Vergnano *et al*., 2005). In the 1970s, the bacterium *S. agalactiae* emerged as the leading infectious cause of early neonatal morbidity and mortality in the United States (CDC, 1996). Greater than 15,000 cases and > 1,300 deaths due to *S. agalactiae* disease in newborns are reported each year (Zangwill *et al*., 1992).

Results collected from different geographic regions indicated heterogeneous carriage rate of *S. agalactiae*. According to the reports, in Norway (34.3%) (Bergseng *et al.*, 2007), in British (28.6%) (Knox, 1979), in Singapore (14.1%) (Chua *et al*., 1995), in Canada (18%) (Church *et al.*, 2008), in Trindad (32.9%) (Orrett, 2003), in Thammasat (16%) (Tor-Udom *et al.*, 2006) and in Brazil (20.4%) (Costa *et al.*, 2008) of pregnant women are carriers of *S. agalactiae*.

The frequency of maternal carriage from vagina and rectum has been reported from some African countries. A literature review study indicates in Sub-Saharan Africa and in Middle East/North Africa *S. agalactiae* colonization rate was 19% and 22% (Stoll and Schuchat, 1998), respectively. Similarly, there are also independent *S. agalactiae* colonization rate studies, in Gambia (22%) (Suara *et al.*, 1994) and in Egypt (26%) (Abdelmoaty *et al.*, 2009) and (17.89%) (Elbaradie *et al.*, 2009). The carriage rate of *S. agalactiae* in Gondar, Ethiopia is 9% in the mothers and 5% in the neonates (Schmidt *et al*., 1989).

Most of early-onset neonatal sepsis was caused by *S. agalactiae* followed by *E. coli* or *S. pneumonia* (English *et al.,* 2003; Gray *et al*., 2007; Mayor-Lynn *et al.*, 2005). In the UK and Republic of Ireland the incidence for early-onset disease was 0·48 per 1000 (0·43—0·53) (Heath *et al.*, 2004) and in Pennsylvania 1.16 per 1000 live births before and 0.14 per 1000 live births after institution of the CDC protocol (Brozanski *et al*., 2000).

In Ethiopia even though the incidence of *S. agalactiae* neonatal disease specifically was not known; pneumonia, sepsis, meningitis were common causes of morbidity and mortality according to reports from Ethio Swedish children's clinic, Black Lion, Addis Ababa and in North Western (Gondar), Ethiopia (Ghiorghis, 1991 and 1997; Muhe *et al*., 1999; Woldehanna and Idejene, 2005). In Ethio Swedish children's clinic the incidence of neonatal sepsis was 11 per 1000 live born babies (Ghiorghis, 1997) and the incidence of neonatal meningitis was 1.37 per 1000 live births in a hospital population-based retrospective study (Gebremariam, 1998) and the most etiologic agent were *Klebsiella* (Ghiorghis, 1997; Gebremariam, 1998) and *S. pneumonia* (Muhe *et al*., 1999). And, the maternal mortality rate, for the period 1994-2000 was 1.3 per 1000 women (DHS, 2005). Puerperal infections and complicated abortion are some of the leading causes of maternal death in Ethiopia (WHO, 2008-2009) which could be due to *S. agalactiae* colonization.

The likelihood of colonization at birth is higher if the mother is heavily colonized. Colonization with subsequent development of disease in the neonate can occur in utero, at birth, or during the first few months of life. Approximately 60% of infants born from colonized mothers are believed to become colonized with their mothers' organisms (Namavar *et al*., 2008).

2.1.4. Risk factors

Potential risk factors that predispose infants to invasive *S. agalactiae* diseases include: premature delivery (<37 weeks' gestation), intraparatum fever (temperature $>38^0$C), prolonged rupture of membranes (PROM) (>18 h), a previous infant with *S. agalactiae* disease, a high colonization of *S. agalactiae* bacteria (Schuchat *et al*., 1994; Law *et al*., 2005; CDC, 1996) and intrauterine fetal monitoring (Adair *et al.*, 2003). Preterm labor and

delivery before 37 weeks of gestation are related to an incomplete transfer of maternal antibodies resulting in low levels of anti-capsular antibodies homologous to the maternal *S. agalactiae* colonizing strain. Other factors that can contribute to a newborn's risk of contracting *S. agalactiae* infection include age, ethnicity, and medical criteria (Jackson *et al*., 1995; Iannelli, 2004), such as the following: being born to an older mean maternal age (Kovavisarach *et al*., 2007), being African American (Chohan *et al*., 2006).

Infants born from *S. agalactiae* colonized mothers were more likely to be of low birth weight, to have been delivered preterm or to have a mother with ammonites, intraparatum fever or premature rupture of the membranes (Adair *et al.*, 2003).

2.1.5. Types of disease caused by *S. agalactiae*

I Neonatal *S. agalactiae* Disease

S. agalactiae is the most common cause of sepsis (blood infection) and meningitis (infection of the fluid and lining around the brain) in newborns. *S. agalactiae* is a frequent cause of newborn pneumonia and is more common than other, more well-known, newborn problems such as rubella, congenital syphilis and spina bifida (Iannelli, 2004; Schuchat, 1998; Rollins, 2000).

Meningitis with bacteremia (89%) or without bacteremia (10%) was the most common types of neonatal disease in USA (CDC, 1997). Infants who have *S. agalactiae* disease can require prolonged hospitalization and expensive supportive therapy, and survivors may suffer permanent disability (e.g., hearing or visual loss or mental retardation) (CDC, 1996; Edwards *et al*., 1985). There are 2 main types of Neonatal *S. agalactiae* disease according to the time of onset of the disease: they are known as EOD and LOD.

A) Early onset infection

Early-onset (EOD) is transmitted by vertical (acquired before and during delivery) and usually occurs during the first week (early form), within the first 12 hours of life in approximately 90% of cases (AAP, 1997; CDC, 2010; Iannelli 2004). The most common route of EOD in infants is via an ascending amniotic infection. Members of the maternal

genital flora, such as *S. agalactiae* and *E. coli*, the organisms responsible for the majority of cases of EOD (English *et al.,* 2003; Mayor-Lynn *et al.*, 2005; Handsfield *et al*., 1973), may ascend through the birth canal to the amniotic fluid either through intact amniotic membranes (Neri *et al*., 1984) or, more commonly, after rupture of membranes (Krasnianin *et al*., 2009). Once infected amniotic fluid is aspirated or swallowed by the fetus, pathogens may penetrate through immature mucosal barriers, resulting in pneumonia or bacteremia, and may penetrate the blood-brain barrier, leading to meningitis (Law *et al*., 2005).

The symptoms for early-onset *S. agalactiae* can seem like other problems in newborns. Some symptoms are fever, difficulty feeding, irritability, or lethargy (limpness or hard to wake up the baby) (Iannelli, 2004). Sepsis was the most common manifestation of EOD (Gray *et al*., 2007).

The mortality rate has decreased as a result of rapid diagnosis and better supportive care; One eight-state surveillance program in USA reported a 65% reduction in the prevalence of early-onset disease from 1993 to 1998 (Scharge *et al*., 2000) and the introduction of neonatal screening programmes has had a substantial impact on the incidence of neonatal *S. agalactiae* disease in the United States (CDC, 2004; Schuchat *et al*., 1994).

B) Late onset of infection

Late-onset (LOD) appears after the first week and half of the cases are transmitted via the vertical (development of late infection after colonization acquired at birth), in the remaining cases by horizontal (for transmission nosocomial or asymptomatic carriers). It is generally not associated with maternal complications it might be in the nursery or in the community after leaving the hospital. Colonizing organisms may enter the bloodstream through breaks in the skin or mucosa or by gastrointestinal translocation or may be introduced through invasive devices such as vascular catheters, endotracheal tubes, or feeding tubes. And it results in meningitis, sepsis, or more rarely, focal infections. Some infants are healthy at birth but develop sepsis 1 to 3 months later (Law *et al.*, 2005; Iannelli, 2004; Rollins, 2000).

Meningitis is more common with LOD B *Streptococci* disease than with EOD B *Streptococci* disease (Iannelli, 2004; Schrage *et al.*, 2000; Gray *et al*., 2007). Rates of late-

onset disease have remained fairly stable since 1990. At this time, a strategy has not yet been identified for preventing late-onset *S. agalactiae* disease (CDC, 2010; Schrage, 2004).

II Adult disease caused by *S. agalactiae*

Although adult disease caused by *S. agalactiae* may be serious, they are usually not fatal unless patients are immunocompromised. Conditions that predispose to the development of adult disease include diabetes mellitus, cirrhosis, stroke, breast cancer, decubitus ulcer and neurogenic bladder (Jackson *et al*., 1995). While the rates of serious *S. agalactiae* infections are much higher among newborns than among any other age group, serious *S. agalactiae* infections occur in other age groups in both men and women. The most common problems caused by *S. agalactiae* in adults are:

- Bloodstream infections
- Pneumonia (infection in the lungs)
- Skin and soft-tissue infections
- Bone and joint infections and rarely, *S. agalactiae* can cause meningitis in adults (Rollins, 2000). Recurrent *S. agalactiae* infection is common among adults and in most cases appears to be caused by relapse (Harrison *et al*., 1995).

2.1.6. Immunity

Innate immunity is responsible in the defense of *S. agalactiae* infection. Macrophages sense spurious amounts of TLR2/6 ligands released from *S. agalactiae*, such as lipoteichoic acid. Activation of TLRs will activate both the cytoskeleton, via phosphatidylinositol 3-kinase, and the release of cytokines and chemokines, via MyD88, which results in the recruitment and activation of other phagocytes (Henneke and Berner, 2006). *S. agalactiae* attach to the surface of peritoneal macrophages and are ingested through a process of phagocytosis with the formation of a membrane-bound vacuole. Some ingested bacteria presented significant morphological changes, clearly indicating that they were killed by the macrophages (Teixeira *et al*., 2001).

Acquired immunity is also responsible in the defense of *S. agalactiae* infection. Studies on mice show MyD88- and TLR2 are responsible for the production of Antibody against *S. agalactiae* infection (Mancuso *et al.*, 2004). Antibody directed against the type III polysaccharide has been shown to be protective in experimental animals and to promote opsonophagocytosis of *S. agalactiae* in vitro (Baltmore, 1979). Antibody to be protective against *S. agalactiae* disease it must be specific to the infecting type of *S. agalactiae*. *S. agalactiae* strains expressing the alpha antigen were less readily killed in the absence of specific antibody than were alpha-negative strains as demonstrated in vitro (Madoff *et al*., 1991). Antibody is acquired by *S. agalactiae* infection, and specific IgG may be transmitted transplacentally to the fetus, providing protection in the perinatal period. In the presence of type-specific antibody, classical pathway C3b deposition, phagocyte recognition, and killing proceed normally.

2.1.7. Laboratory diagnosis and identification

The colonization of *S. agalactiae* is transient i.e. the bacteria can grow back quickly (Iannelli, 2004). Because of this, results at 35-37 weeks correlate more closely with *S. agalactiae* colonization at term delivery (Chua *et al*., 1995), the Centers for Disease Control and Prevention (CDC) and others have recommended that all pregnant women be screened for carriage of *S. agalactiae* at between 35 and 37 weeks of gestation, so that *S. agalactiae* positive women can receive antibacterial treatment (chemoprophylaxis) prior to delivery, to reduce mother-to-child transmission (CDC, 2010; Money and Dobson, 2004; ACOG, 2011).

Culture

Culture is the standard method used to identify *S. agalactiae*. Methods that maximize the likelihood of *S. agalactiae* recovery are required, and specific media are needed (El Aila *et al.*, 2010). Critical factors that influence the accuracy of detecting *S. agalactiae* maternal colonization include sampled anatomic sites (Bergseng *et al*., 2007), timing in pregnancy (Chua *et al*., 1995), transport condition of swabs (AAP, 1997) and culture procedures (CDC, 2010). In addition, failure to culture *S. agalactiae* may be caused by maternal factors such as use of oral antibiotics or a variety of feminine hygiene products, since *S. agalactiae*

originates from intestinal flora and colonizes individuals, hygienic condition of the perineal and vaginal regions before specimen collection can reduce the colonization rate.

a) Sampling site

Vaginorectal swabbing was most sensitive compared to separate swabbing of rectum and vagina (Bergseng *et al*., 2007). From a previous study (El Aila *et al.*, 2010), the *S. agalactiae* detection rate on the basis of rectovaginal samples were significantly higher than the detection rate on the basis of vaginal samples, but not significantly higher than that on the basis of rectal samples. However, according to another study (Orrett, 2003), vaginal colonization was not significantly different from rectal colonization. For *S. agalactiae* isolation CDC and others recommend vaginorectal swabbing by double or single swab collected either by health professional or by pregnant women herself (CDC, 2010; ACOG, 2011).

b) Culture media

Culture swabs may be placed in a transport medium (such as Amies' medium, Difco, West Molesey, Surrey, UK) at environmental temperature for up to 96 hours (CDC, 2010; AAP, 1997).

To isolate *S. agalactiae* CDC recommends inoculation of the swab on selective modified Todd-Hewitt broth with gentamycin (8µg/ml) & nalidixic acid (15µg/ml) at 37°C for 18–24h, sub cultured onto sheep blood agar plates (SBAP) and incubated aerobically at 37°C for 18–24h with 5-10% $C0_2$ (CDC, 2010). However researches show its detection rate is lesser than the Chromogenic agar (Craven *et al*., 2010). The ability to detect non-haemolytic strains of *S. agalactiae* from a mixed culture is greatly enhanced when using a chromogenic plate. CHROMagar^{TM} *Strep*B works in aerobic conditions. And the availability of its mauve (pink) colonies (Figure 2) allow for easier visualization of the pathogen (Smith *et al*., 2010). And these selective modified broth culture methods can be labor-intensive and may require up to 72 h before procurement of a final result.

A recent study on Belgium shows Group B *Streptococcus* differential agar (GBSDA) and ChromID *Strepto* B Agar (CA) detected more positive women than Columbia agar with colistin and nalidixic acid (CNA). In addition, detection of *S. agalactiae* from rectovaginal specimens by direct plating onto GBSDA or CA was equally sensitive as detection by Lim broth enrichment with subculture on this agar (El Aila *et al.*, 2010).

Other study on France indicates CHROMagar^{TM} *Strep*B was the most sensitive on day 1. Sensitivity of CHROMagarTM *Strep*B was 79% on day 1 and 92% on day 2. Blood agar medium was significantly less sensitive than CHROMagar^{TM} *Strep*B Chromogenic media: 40% on day 1 and 58% on day 2 (Poisson *et al*., 2011).

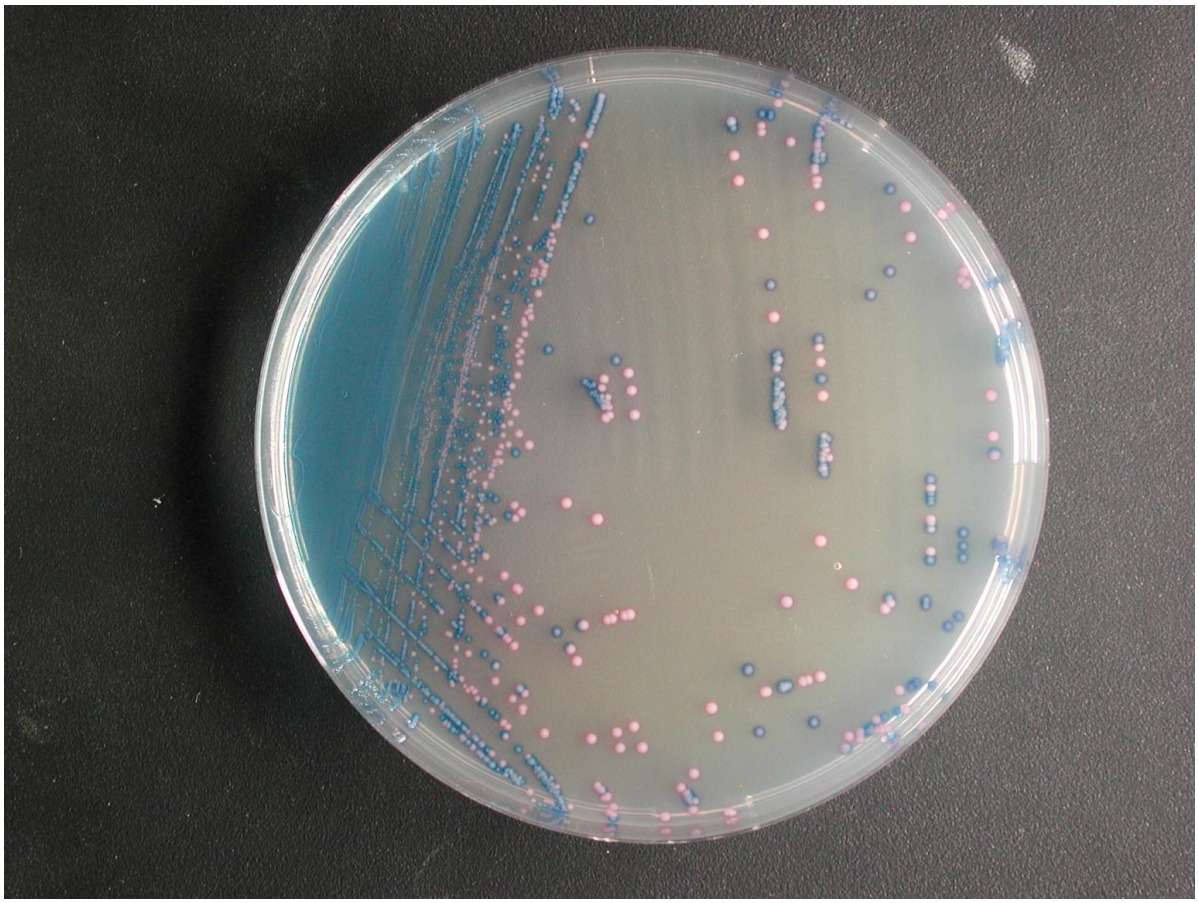

Fig. 2: *S. agalactiae* - the mauve colored isolate on CHROMagar Colorex ™ *Strep* B plate (Picture from Smith *et al*., 2010).

c) Biochemical test

Colonies of suspected *S. agalactiae* are confirmed by either serological methods or by CAMP testing (Park *et al.,* 2001; CDC, 2010). Catalase test, CAMP test and Hippurate hydrolysis test are the most biochemical tests used to differentiate *S. agalactiae* from *Staphylococci* and other *Streptococci* species.

d) Antibacterial susceptibility

To select appropriately the antibiotic prophylaxis for penicillin allergic women at high risk of anaphylaxis, antimicrobial susceptibility testing of *S. agalactiae* isolates is essential. In addition, the double-disk diffusion method (D-zone test) is recommended for testing erythromycin-resistant and clindamycin-susceptible *S. agalactiae* for detection of inducible clindamycin resistance (CDC, 2010; CLSI, 2010). The emerging resistance to erythromycin and clindamycin among *S. agalactiae* strains is of concern suggesting that the currently recommended antibiotic therapy for women with penicillin allergy may need modification.

Antigen Detection

Commercial tests for the direct detection of the organism or antigen in clinical specimens have been developed. A variety of methods are used to detect the group-specific antigen, including *Streptococcal* coagglutination, latex agglutination, genetic probe and fluorescent antibody (CDC, 1996).

Nucleic Acid-Based Tests

S. agalactiae -specific polymerase chain reaction (PCR)-based assays have demonstrated better sensitivity but they require complicated procedures that are not applicable to clinical use. More promising results have emerged from the development of a rapid assay using the Light Cycler technology (Bergeron *et al*., 2000).

Advances in PCR and fluorescence labeling technologies have provided new detection platforms for bacterial identification. Recent data suggest that available real-time PCR-based tests (or Nucleic Acid Amplification Test (NAAT)) can equal or surpass the sensitivity of antenatal culture at 35–37 weeks of gestation and compare favorably with standard culture

methods for the detection of *S. agalactiae* colonization at presentation for delivery (CDC, 2010). Therefore, the commercialization of rapid detection of *S. agalactiae* through real-time PCR offers the potential for *S. agalactiae* detection among women without prenatal care or among those in whom no antenatal culture was collected (Bergeron *et al*., 2000; El Helali *et al*., 2009). The real-time PCR assay is fast, highly sensitive and specific for detecting *S. agalactiae* colonization in pregnant women at delivery, and has the potential for intraparatum detection of *S. agalactiae* colonization (Bergseng *et al*., 2007). Multiplex PCR assay is also developed that offers a rapid and simple method of subtyping *S. agalactiae* based on surface protein genes (Creti *et al*., 2004).

2.1.8. Treatment

The recommended treatment by the CDC and ACOG is intravenous antibiotics during labor for all women who swab positive during their pregnancies without regard to the concentration of colonization present (CDC, 2010; ACOG, 2011). The recommended intraparatum prophylaxis for *S. agalactiae* is Penicillin in the concentration of 5 million units IV initial dose, then 2.5 to 3 million units every 4 hours until delivery or ampicillin, 2g IV initial dose, then 1 g IV every 4 hours until delivery are commonly used in the chemoprophylaxis and in cases of allergy but not at risk of anaphylaxis: cefazolin 2 g IV then 1 g every 8 hours or If the woman is penicillin allergic and at risk of anaphylaxis: clindamycin 900mg IV every 8 hours or erythromycin 500 mg IV every 6 hours are used. If *S. agalactiae* resistance is demonstrated to clindamycin or erythromycin by culture and sensitivity, then vancomycin 1 g IV every 12 hours are used (Money and Dobson, 2004; CDC, 2010; ACOG, 2011).

Antibiotics help to kill some of the *S. agalactiae* that are dangerous to the baby during birth. The antibiotics help during labor only and they can't be taken before labor, because the bacteria can grow back quickly. Any pregnant woman who had a baby with *S. agalactiae* disease in the past or who now has a bladder (urinary tract) infection caused by *S. agalactiae* should receive antibiotics during labor (Iannelli, 2004; CDC, 1996).

This practice has been successful in reducing the rate of early-onset infection. In the US, 3900 early-onset infections and 200 neonatal deaths were prevented in 1998 by the use of intraparatum antibiotics (Schrag *et al.,* 2000). And similarly in 1999, 4500 cases and 225 deaths due to early-onset *S. agalactiae* disease were prevented (Schuchat, 2001).

The CDC guidelines recommend that all newborn babies showing signs of sepsis, or if born to women with chorioamnionitis, should undergo a full diagnostic evaluation and receive empiric antibiotic therapy pending culture results irrespective of whether their mothers had received intraparatum antibiotics (CDC**,** 2002).

2.1.9. Antimicrobial Resistant

There are two principal mechanisms of macrolide resistance in *S. agalactiae* (Leclercq, 2002). The most prevalent of these is based on the alteration of the target-binding site i.e. methylation of a single adenine at the 50S ribosomal binding site, a ribosomal modification mediated by erythromycin ribosome methylase encoded by *erm* genes known as the macrolide lincosamides and streptogramin B (MLS_B) resistance phenotype. The presence of an *erm* methylase confers resistance to erythromycin and inducible or constitutive resistance to lincosamides and streptogramin B (Betriu *et al*., 2003; leclercq, 2002).

The second mechanism is *mefA* and *mefE* genes, which are 90% identical, encode 14- and 15-member macrolide efflux pumps and lead to the macrolide only (M) resistance phenotype (Arpin *et al*., 1999). Most of the macrolide resistance among *S. agalactiae* was found among serotype V strains (Diekema *et al*., 2003; Andrews *et al*., 2000; Manning *et al*., 2003).

However different studies indicate there is rarely resistance by different mechanism: *S. agalactiae* strain containing the *linB* gene, encoding a lincosamide nucleotidyl transferase, which confers the L phenotype (lincosamide) resistance, was identified in the study of Gygax *et al*. (Gygax *et al*. 2006). *S. agalactiae* strain containing levofloxacin-resistant which had two amino acid substitutions, Ser_{81}Leu in the *gyrA* gene and Ser_{79}Phe in the *parC*

gene, and which showed similar pulsed-field gel electrophoresis patterns was identified from a study of Murayama *et al.* (Murayama *et al.,* 2009).

S. agalactiae remain fully susceptible to penicillin (Gray *et al*., 2007; Orrett, 2003) as well as to most β-lactams, even though they have penicillin minimal inhibitory concentrations (MIC). However, recently identified very rare isolates with decreased susceptibility to penicillin have been reported in Japan (Kimura *et al*., 2011) and in USA (Nagano *et al*., 2009; Gaudreau *et al*., 2010). A point mutation in the *S. agalactiae pbp*2x gene conferring this decrease in susceptibility was identified.

Antibiotic combinations such as a penicillin plus gentamicin are often used to manage severe *S. agalactiae* infection (Naor *et al*., 1982). However, one case report indicates there is a strain of *S. agalactiae* that was highly resistant to gentamicin (MIC > 500 mg/L) (Liddy and Holliman, 2002).

2.1.10. Prevention

There are two strategies proposed to screen for the risk of perinatal *S. agalactiae* disease, a risk-based strategy and a culture-based strategy (CDC, 1996; Schuchat, 2001). Both strategies significantly reduced the incidence of *S. agalactiae* disease in the 1990s. However revised and updated 2002 CDC guidelines and others concluded that routine culture screening for *S. agalactiae* during pregnancy prevents more cases of early onset disease than the risk-based approach (CDC**,** 2002; Schrage *et al*., 2002; ACOG, 2011).

2.1.11. Vaccine and Prophylaxis

Although maternal intraparatum antibiotic prophylaxis is clearly effective and has reduced the incidence of early-onset *S. agalactiae* neonatal disease substantially in the United States (Schrag *et al*., 2000), it cannot prevent late-onset *S. agalactiae* disease (CDC, 2010). Vaccination of women of childbearing age against *S. agalactiae* could prevent both early-onset and late-onset *S. agalactiae* disease in the neonate, in addition to preventing *S. agalactiae* disease in pregnant women.

Opsonic antibodies directed against the capsular polysaccharide of *S. agalactiae* confer serotype-specific protection. Initial vaccines developed focused on capsular type III. An oligosaccharide tetanus-toxoid conjugate vaccine against *S. agalactiae* type III polysaccharide was developed by Paoletti *et al* (Paoletti *et al*., 1990). But the emergence of type V isolates in recent years has prompted the development of a polyvalent *S. agalactiae* vaccine (Baker and Edwards, 2003).

Modern technologies such as those involving proteomics and genomic sequencing are likely to hasten the development of a universal vaccine against *S. agalactiae* (Koenig and keenan, 2009). One experiment suggest that inclusion of C5a peptidase in a vaccine will both add another level to and broaden the spectrum of the protection of a polysaccharide vaccine (Cheng *et al*., 2002).

2.2. Significance of the study

S. agalactiae asymptomatically colonize the vaginal or rectal areas of 10 to 30 percent of pregnant women (Chua *et al*., 1995; Tor-Udom *et al.*, 2006; Elbaradie *et al.*, 2009; Church *et al.*, 2008; Stoll and Schuchat, 1998; Costa *et al.*, 2008; Suara *et al.*, 1994; Abdelmoaty *et al.*, 2009; Knox, 1979). In these women, *S. agalactiae* may cause preterm labor or membrane rupture, as well as urinary tract infections, chorioamnionitis, postpartum endometritis, postpartum wound infection, septic pelvic thrombophlebitis, endocarditis and sepsis (CDC, 2010). *S. agalactiae* is a major cause of invasive disease at all ages and is the most frequent cause of serious bacterial sepsis, including neonatal meningitis (Iannelli, 2004; Synnott *et al*., 1994).

Neonatal infections currently cause about 1.6 million deaths annually in developing countries. Sepsis and meningitis are responsible for most of these deaths (Vergnano *et al*., 2005). Ethiopia is one of the developing countries with an estimated infant mortality rate of 77 per 1000 live birth (DHS, 2005). In Tigray analysis of admissions of the hospitals showed that pneumonia is the leading cause of admissions in under-five children which accounts for 30.5% and it is the leading cases of mortality (31%). Neonatal sepsis is also common causes of admissions and deaths in this region (THB, 2010). And these clinical conditions can be associated with *S. agalactiae* infection.

CDC recommends to screen pregnant women routinely for colonization of *S. agalactiae* and to treat those colonized pregnant in order to prevent transmission to neonate (CDC, 2010). Different studies showed a dramatic fall in the incidence of early-onset disease as increasing numbers of hospitals implemented antimicrobial prophylaxis, with little change in late-onset disease (CDC, 2010; CDC, 2004; Brozanski *et al*., 2000). Intraparatum prophylaxis, alone or combined with postnatal prophylaxis for the infants, reduces the early-onset *S. agalactiae* attack rate by 80% or 95%, respectively (Benitz *et al*., 1999).

Several studies have shown the appearance of *S. agalactiae* strains resistant to clindamycin and erythromycin antibiotics in different countries making them unsuitable as an option of

choice for both prophylaxis and for treatment of infections (Abdelmoaty *et al.,* 2009; Castellano- Filho *et al.,* 2010; De Mouy *et al*., 2001).

Currently, although excellent data are available from the developed world and in some developing countries, to our knowledge little epidemiologic information is available on carriage rate of *S. agalactiae* and its antimicrobial resistance in pregnant women in Ethiopia especially in Adigrat Zonal Hospital and Adigrat Health Center.

Therefore, the above mentioned reports due to *S. agalactiae* are worrying and continuing surveillance and susceptibility pattern is required to assess the area. Therefore the primary intent of this study is to determine the carriage rate of *S. agalactiae* among pregnant women and determine the drug of choices for individual who are allergic to penicillin to reduce transmission of *S. agalactiae* from colonized women to her neonate.

Findings from this study will help to assess changes in the carriage rate and their sensitivity pattern through time by comparing the results of the previous study done in Ethiopia and elsewhere in the world. Results from this study will also provide update information for appropriate management of *S. agalactiae* infection and it can be used as a base line data for other scholars for further study.

3. Objectives

3.1. General objective

The general objective of this study is to determine maternal *S. agalactiae* carriage rate and antibiotic susceptibility pattern of isolates prior to the time of delivery at Adigrat Zonal Hospital and Adigrat Health Center from December 2011 until March 2012.

3.2. Specific objectives

- To determine the carriage rate of *S. agalactiae* in pregnant women from 35-37 week of gestation
- To assess risk factors of *S. agalactiae* colonization in pregnant women from 35-37 week of gestation
- To determine antibiotic susceptibility pattern of *S. agalactiae* isolates in pregnant women from 35-37 week of gestation.

4. Materials and Methods

4.1. Study area

The study was conducted in Adigrat at Adigrat Zonal Hospital and Adigrat Health Center which are located in Adigrat city**,** the administrative town of the Eastern Zone of Tigray region, Ethiopia. Adigrat town is about 891kms to north direction away from the Ethiopian capital city Addis Ababa. The population of the town is about 130,000. Adigrat Zonal Hospital is one of the 12 Hospitals in Tigray region (5 zonal, 6 district and 1 referral). Totally the hospital has 178 beds and there are 32 B.Sc nurses, 49 diploma nurses, 3 health officers, 5 general practitioners, 1 internist, 1 surgeon and 1 gynecologist and obstetrician. The hospital gives outpatient department service (OPD) each day to the various sections (general OPD, gynecological OPD, dental clinic, ophthalmic clinic and HIV counseling and screening unit). The inpatient section gives services to medical, pediatric, gynecological, obstetrical and surgical patients. Adigrat Health Center is under Adigrat Woreda Health Bureau and gives outpatient service (ANC follow up, adult OPD, pediatric OPD, delivery service, TB patient follow up, HIV counseling and screening unit and Health package service) and there are 2 Health officers, 1 B.Sc nurse, 4 mid wives and 7 diploma nurses.

4.2. Study design and period

A cross-sectional study was conducted at ANC clinic of Adigrat Zonal Hospital and Adigrat Health Center from the period of December 2011 to March 2012.

4.3. Study population

The study populations of our study were pregnant women who attended ANC follow up in Adigrat Zonal Hospital and Adigrat Health Center and who were in their 35 to 37 weeks of gestational period and had the following inclusion and exclusion criteria.

Inclusion criteria

All women who are pregnant and are voluntary to participate in the study and their gestation period was from 35 to 37 week are included in the study.

Exclusion criteria

- Pregnant women who took antibiotics within one week before they came to the antenatal clinic service
- Pregnant woman with gestation period other than 35 to 37 week

4.4. Study variable

 Dependent variable

- *S. agalactiae* Colonization
- Antimicrobial susceptibility profile of isolates

 Independent variables

- Age
- Marital status
- Gravida
- Gestational age
- Preterm labor
- Neonatal death
- Prolonged Rupture of Membrane

4.5. Sample collection and processing

After explaining the procedure and aim of the work demographic data was collected by using questionnaire **(Annex II)**. Using aseptic technique by applying 70% alcohol at the site of rectum and vagina, rectal and vaginal swabs were collected for detection of *S. agalactiae*, according to CDC recommendations (CDC, 2010). For collection of vaginal and anal samples, first a swab from the mucosal secretions of the lower-third part of the vagina was obtained. Thereafter, the second swab was carefully inserted to the anal sphincter and gently rotated to touch the anal crypts. All swabs were collected by the clinical staff by the use of

sterile double swabs and placed in Amies transport medium (Oxoid, England) and was sent to the microbiology laboratory of Addis Pharmaceuticals Factory SC.

4.6. Laboratory procedures

4.6.1 Culture and Biochemical test

For the isolation of *S. agalactiae* we used Chromogenic medium (CHROMagar^{TM} *Strep*B, France). CHROMagar^{TM} *Strep*B contain CHROMagar *Strep*B base in powder form, CHROMagar *Strep*B-S1 (substrate 1) in liquid form and CHROMagar *Strep*B-S2 (substrate 2) in powder form and it was prepared following manufacturers instruction.

The two swabs were processed as one by direct inoculation on to CHROMagar^{TM} *Strep*B plate and incubated aerobically at 37°C for 24 hours. Suspected colonies of *S. agalactiae* i.e. mauve color (pink colonies) was confirmed as *S. agalactiae* by microscopic (gram stain) as gram positive cocci (dark purple) arranged in chains, catalase test negative, CAMP test positive and agglutination was identified by serological test. If *S. agalactiae* is not identified after 24h the media was re inoculated for additional 24h to identify the suspected organism.

CAMP testing was performed on sheep blood agar plate (SBAP) by streaking of *S.aureus* down the middle of SBAP and the test organism was then streaked perpendicular to the *Staphylococcal* streak. And the streaks did not touch. CAMP factor produced by *S. agalactiae* and β lysine produced by *S. aureus* act synergistically on SBAP to produce enhanced hemolysis. After incubation over night under candle jar atmospheres, the SBAP was examined for an arrowhead shaped zone of enhanced lysis at the junction of the two streaks as shown in Figure 3.

Figure 3: The CAMP test for identification of *S. agalactiae* (horizontal streak, GBS) demonstrates an arrowhead shaped area of enhanced hemolysis when grown near *S. aureus* (vertical streak, SA) (Picture from Yeh *et al*., 2009).

Pink colonies from CHROMagar™ *Strep*B was taken and sub cultured onto SBAP and incubated under candle jar atmospheres for 18 hours over night and colonies from SBAP was taken and serological test was done by latex agglutination method (PASTOREX™ Meningitis, France) according to the manufacturers instruction. Five colonies were suspended in test tube with 2ml of Todd Hewitt broth (Oxoid, England) and incubated in 37^0c for 2 hours then the test tube was centrifuged for 5 minutes at 3,000g after this one drop (40 to 50µl) of supernatant was placed in a circle on the disposable card and the latex reagent bottle was gently shaked; holding the bottle up right, one drop of this latex was placed at the periphery of the drop of supernatant. The latex and the sample were mixed using a rod then the card rotated (~120 rpm). The appearance of any clear agglutination was observed with in less than 1 minute and was reported as positive for *S. agalactiae*.

4.6.2 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out on all *S. agalactiae* isolates by using the disc diffusion technique on Mueller-Hinton agar (Oxoid, England) supplemented with 5% sheep blood. A suspension of the test organism was prepared by removing 3-5 colonies from

a pure culture plate by emulsifying in 3 ml of sterile physiological saline and incubated at 37^{o}C until the turbidity of the suspension become matched with turbidity standard equivalent to 0.5 McFarland. Using a sterile swab, the surface of Mueller-Hinton agar was completely covered by pressing and rotating the swab against the side of the tube above the level of suspension. After the plate dry(3-5 minutes), using sterile forceps the six discs was evenly distributed on the inoculated plate then was incubated at 37^{0}c in 5% co_2 for 20-24 hours according to the guideline recommendations of the CDC (CDC, 2010). The diameter of the zone of inhibition was measured using a ruler; interpret according to Clinical and Laboratory Standards Institute (CLSI) guide lines as 'Resistant', 'Intermediate/Moderately susceptible', 'Susceptible' (CLSI, 2010).

The following antimicrobial discs and concentrations (in brackets) were used: ampicillin (10µg); amoxicillin (2µg); erythromycin (15µg); clindamycin (2µg), Vancomycin (30 mg), and penicillin G (10 mg) (All of the antibiotics are product of Oxoid, England).

4.6.3 Quality control

Reference Strain

S. aureus (ATCC-25923) was used as a quality control throughout the study for culture, antimicrobial susceptibility testing and for CAMP test.

E. fecalis (ATCC -25212) was used as negative control for CAMP testing. The control strains were obtained from Ethiopian Health and Nutrition Research Institute (EHNRI).

Sample was collected and processed aseptically by using standard operating procedure (SOP).

4.7. Sample size: power calculation

The minimum sample size (n) was calculated by taking carriage rate of *S. agalactiae* in pregnant women done in Gondar university medical hospital, which is 9% carriage rate (Schmidt *et al.*, 1989). The expected margin of error (d) was 0.05 and the confidence interval (Zα/2) was 95%.

$$n = \frac{(Z\alpha/2)2 * (p*q)}{d^2} = \frac{(1.96)2 * (0.09*0.91)}{(0.05)^2} = 126$$

Where; n = sample size; z = confidence interval; a = level of significance;

d = tolerable error and p= proportion.

A total of 150 subjects were included in the study.

4.8. Statistical Analysis

Data entry and analysis was done using Statistical Package for Social Science (SPSS) version 16 software. Descriptive summarization of data consisted of frequency counts and percentages were used to compare certain data. A univariate and multivariate binary logistic regression model was used to ascertain the association between the frequencies of colonization in relation to the different variables. All statistical tests were two tailed, and a *p*-value of <0.05 was considered as indicative of a statistically significant association.

4.9. Ethical considerations

The M.Sc research project was ethically approved by the Department of Microbiology, Immunology and Parasitology Ethical Review Committee (DERC). Written informed consent was obtained from all of the study participants **(Annex I)**. Permission to conduct a study in Adigrat Zonal Hospital and Adigrat Health Center and to process the sample at APF was also obtained from responsible body. In the event of a positive culture for S. agalactiae the result was communicated with the gynecologist for appropriate management.

5 Results

5.1. Socio-demographic characteristics of study participants

A total of 825 women attended ANC follow up at Adigrat Zonal Hospital and Adigrat Health Center between December 1, 2011 and April 02, 2012. Out of these 150 pregnant women who have fulfilled our inclusion criteria and willing to participate were included in this study. A socio-demographic characteristic of study participants was summarized in Table 1. The mean age of the women in this study was 26.14±4.9 year (ranging from 14 to 40 years and its median was 25 years). Many of the pregnant women, 109(72.7%) were in 21-30 years age group followed by greater than or equal to 31 years 24(16%) and less than or equal to 20 years 17(11.3%). The mean gestational age (± SD) at examination was 35.7± 0.77weeks (median 35.5 weeks, range 35–37 weeks). Half of the study participants (50%) were in 35 weeks of gestational age, 47(31.3%) of them were in 36 weeks of gestational age and 28(18.7%) were in 37 weeks of gestational age. One hundred forty six (97.3%) of the study participants had Tigray ethnicity, 3(2%) had Amhara and 1(0.7%) had Sidama ethnicity. Out of the pregnant women evaluated 3(2%) of them were with HIV disease. Almost half of the study participants, 65(43.3%) were primary school educated, 20(13.3%) were uneducated, 52(34.6%) were secondary school educated and 13(8.7%) were tertiary level.

Table 1: Socio-demographic characteristics of study participants investigated for *S. agalactiae* colonization at Adigrat Zonal Hospital and Adigrat Health Center, Tigray, Ethiopia from [December 2011-March2012].

Variables	**No Study participants (N=150)**	**% of Study participants**
Age, yrs		
≤20	17	11.3
21-30	109	72.7
≥31	24	16
Address		
Town	134	89.3
Outside of the town	16	10.7
Marital status		
Married	146	97.3
Single	3	2
Cohabitant	1	0.7
Educational status		
Uneducated	20	13.3
Primary	65	43.3
Secondary	52	34.6
Tertiary	13	8.7
Gravida		
Primi gravida ≤3	110	73.3
Multi gravida >3	40	26.7
Gestational age in wks		
35	75	50
36	47	31.3
37	28	18.7
Ethnicity		
Tigray	146	97.3
Amhara	3	2
Sidama	1	0.7
HIV		
Yes	3	2
No	147	98

5.2. Carriage rate of *S. agalactiae*

As shown in Table 2 among the 150 pregnant women evaluated 93(62%) of them were from health center and 57(38%) were from hospital. Seventeen (11.3%) of the pregnant women were colonized by *S. agalactiae*. Four (7%) and 13(14%) of the pregnant women were those who have attended ANC follow up at Adigrat Zonal Hospital and Adigrat Health Center, respectively. Thirteen (76.5%) of the isolates were from health center and 4(23.5%) were from hospital. The study revealed a higher colonization rate among the age group 21 to 30 years (76.5%) but one pregnant woman with *S. agalactiae* was identified (5.9%) in women aged lesser than or equal to 20 years.

Table 2: Age distribution of *S. agalactiae* colonized pregnant women at Adigrat Zonal Hospital and Adigrat Health Center, Tigray, Ethiopia from [December 2011-March 2012].

Age in years	Hospital (n=57) No. (%)	Health Center (n=93) No. (%)	Total *S. agalactiae* isolated (n=17) No. (%)
≤ 20	0(0)	1(1.07)	1(5.9)
21-30	3(5.26)	10(10.75)	13(76.5)
≥ 31	1(1.75)	2(2.15)	3(17.6)
Total	**4(7.01)**	**13(13.97)**	**17(100)**

5.3. Risk factors associated with colonization of *S. agalactiae*

After analyzing the carriage rate of *S. agalactiae*, next we assessed the risk factors associated with colonization of *S. agalactiae* in the pregnant women. Among the several factors assessed on univariate analysis (initial analysis), there was statistically significant association in the isolation frequency of *S. agalactiae* with Preterm labor (OR = 0.115, 95% CI = 0.015-0.873, P-value = 0.037) and PROM (OR=0.154, 95% CI= 0.38 - 0.615, P-value= 0.008). However none of the other risk factors such as age, early neonatal death, gestational age and other history of the study participants had not any significant association in the isolation frequency of *S. agalactiae* ($p > 0.05$) as provided in Table 3.

Table 3: Univariate analysis of respondents' characteristics related with *S. agalactiae* colonization at Adigrat Zonal Hospital and Adigrat Health center, Tigray, Ethiopia from [December 2011-March2012].

Variable	Total	Yes	No	95% CI	OR	P-value
Age						
≤20	17	1	16		1	
21-30	109	13	96	0.056-3.775	0.462	0.471
≥31	24	3	21	0.042-4.609	0.438	0.491
Preterm labor						
Yes	4	2	2	0.015-0.873	0.115	0.037
No	146	15	131		1	
Parity						
Primi parity(≤3)	130	14	116	0.178-2.630	0.684	0.580
Multi parity(>3)	20	3	17		1	
PROM						
Yes	10	4	6	0.38-0.615	0.154	0.008
No	140	13	127		1	
Gestational age						
35	75	8	67		1	
36	47	5	42	0.385-5.059	1.396	0.612
37	28	4	24	0.343-5.718	1.400	0.639
Early neonatal death						
Yes	5	2	3	0.27-1.120	0.173	0.66
No	145	15	130		1	
HIV disease						
Yes	3	1	2	0.351-4.72	4.094	0.261
No	147	16	131		1	

CI-Confidence Interval; OR-Odds Ratio; 1-Reference Group

PROM-Prolonged Rupture of Membrane

Finally, after univariate analysis was done we did multivariate logistic analysis, in order to ascertain association by adjusting for confounders. For the 2 risk factors (Preterm labor and PROM) in which statistically significant association was obtained by univariate analysis, stepwise logistical regression identified PROM only had significant association with *S. agalactiae* colonization (OR=5.864, 95% CI=1.395 – 24.643, P-value=0.016). Prolonged rupture of membrane independently contributes 5.86 times for colonization of *S. agalactiae* as provided in Table 4.

Table 4: Multivariate analysis of respondents' characteristics related with *S. agalactiae* colonization at Adigrat Zonal Hospital and Adigrat Health center, Tigray, Ethiopia from [December 2011-March2012].

Variable	95 % CI	OR	P=value
Preterm labor			
Yes		1	
No	0.822-61.438	7.105	0.075
PROM			
Yes	1.395-24.643	5.864	0.016
No		1	

CI-Confidence Interval; OR-Odds Ratio; 1-Reference Group

5.4. Antibiotic susceptibility pattern of isolates

As shown in Table 5 all the 17 isolates were susceptible to ampiciillin (100%), penicillin G (100%), vancomycin (100%), and amoxacillin (100%). However, 2 isolates were resistant to erythromycin (11.8%) and three isolates to clindamycin (17.6%). Common resistant to erythromycin and clindamycin was found in two isolates. Furthermore, intermediate susceptibility was also detected against erythromycin and clindamycin in 2 isolates (11.8%) for each.

Table 5: Antibiotic susceptibility profiles of *S. agalactiae* isolated from pregnant women in Adigrat Zonal Hospital and Adigrat Health Center, Tigray, Ethiopia from [December 2011-March2012].

Antibiotic	**No. (%) of the isolates with indicated Antibiotic response**		
	Susceptible	Intermediate	Resistant
Penicillin G[a]	17(100)	-----	-----
Amoxacillin[a]	17(100)	-----	-----
Ampicillin[a]	17(100)	-----	-----
Clindamycin[a]	12(70.6)	2(11.8)	3(17.6)
Erythromycin[a]	13(76.5)	2(11.8)	2(11.8)
Vancomycin[a]	17(100)	-----	-----

[a=]Clinical and Laboratory Standards Institute (CLSI) breakpoint for *Streptococcus* spp β- haemolytic group (CLSI, 2010).

6. Discussion

While the rates of serious *S. agalactiae* infections are much higher among newborns than among any other age group, serious *S. agalactiae* infections occur in other age groups in both men and women. In developed countries, these organisms are the leading cause of neonatal sepsis and meningitis (Heath *et al.*, 2004; CDC, 1996; Mayor-Lynn et al., 2005). CDC recommends to screen pregnant women routinely for colonization of *S. agalactiae* and to treat those colonized pregnant in order to prevent transmission to neonate (CDC, 2010). Unfortunately, there are no local guidelines for screening and prophylaxis of *S. agalactiae* colonization at the study area. This study, the first to be carried out at Adigrat Zonal Hospital and Adigrat Health Center, was aimed at addressing the question whether *S. agalactiae* is a threat necessitating the development of prevention strategies.

This study showed that 11.3% of pregnant women who attended ANC follow up in the study area had rectovaginal colonization with *S. agalactiae*. A comparable rate of colonization with the current study was reported in the previous study done in Gondar, Ethiopia (9%) (Schmidt *et al*., 1989) and in Saudi Arabia (9.2%) (Uduman *et al.*, 1985).

Different rates of maternal carriage rate were reported from various countries; reports of low carriage rates of *S. agalactiae* were in Iran (5.2%) (Nahaei *et al.*, 2007), in Greek (6.6%) (Tsolia *et al.*, 2003), in Peru (6%) (Collins *et al*., 1998). The high carriage rate in the present study might be due to the difference in culture media used. Sheep blood agar was used in the above studies where as in our study selective and differential media chromogenic agar was used. Similarly, lower carriage rate were reported in Israel (6.5%) (Naor *et al.*, 1982) and in Japan (2.9%) (Yamane *et al.*, 1983). This may be explained by sample site collection difference as vaginal swab was taken in the above studies where as in our study vaginal and rectal swab was taken which have significantly higher detection rate than vaginal samples (Bergseng *et al*., 2007).

Higher carriage rates than the present study were found in different countries like: in Gambia (22%) (Suara *et al.*, 1994), in Egypt (26%) (Abdelmoaty *et al.*, 2009) and (17.89%) (Elbaradie *et al.*, 2009), in British (28.6%) (Knox, 1979), in Norway (34.3%) (Bergseng *et*

al., 2007), in Thammasat (16%) (Tor-Udom *et al.*, 2006), in Singapore (14.1%) (Chua *et al*., 1995), in Canada (18%) (Church *et al.*, 2008), in Trindad (32.9%) (Orrett, 2003), in Brazil (20.4%) (Costa *et al.*, 2008). This difference could be attributed to differences in the study design, specimen collection and culture techniques as well as patient characteristics, geographical and socio-economic factors (Stoll and Schuchat, 1998). This highlights the importance of individualizing preventive strategies according to local colonization rates.

Most of the *S. agalactiae* isolates (76.5%) were identified from the health center and 23.5% were from the hospital. This higher colonization in the health center might be due to hygienic difference of the study participants.

Data collected from questionnaires showed *S. agalactiae* colonization among pregnant women was significantly correlated with the PROM (OR=5.864, 95% CI= 1.395 – 24.643, P-value= 0.016). This was in agreement with a recent study in India (Dechen *et al*., 2010) and in Iran (Namavar *et al*., 2008). But this is in contrast with other study in Tanzania (Joachim *et al*., 2009). However no significant relationship was found between maternal *S. agalactiae* carriage rate and number of parity and abortion history ($p>0.05$). This might be probably due to smaller sample size, geographical or socio economic characteristics of the study participants. This was in agreement with previous findings in Iran (Nahaei *et al.*, 2007). But other colleagues get association; pregnant women with previous spontaneous abortion were more frequently colonized by *S. agalactiae* (Sharmila *et al*., 2011). In the current study colonization was not significantly greater in multigravid (>3) than in primigravid (≤3) women and this is in agreement with other collogues in India (Dechen *et al*., 2010), in Trindad (Orrett and Olagundoye, 1994) and in Peru (Collins *et al.*, 1998). But this is in contrast with a study in south India (Sharmila *et al*., 2011).

Young maternal age was associated with risk of *S. agalactiae* disease (Schuchat *et al*., 1994). However old age has been proposed by some investigators as a risk factor for maternal colonization (Orrett, 2003). This controversy of maternal age with *S. agalactiae* colonization could be due to subjective classification of age in to young and old. In the present study more *S. agalactiae* were isolated from women >21 years than those younger

than 21 years. However, our finding has shown that subjects' age had no influence on the rate of *S. agalactiae* colonization (age group had no significance difference on *S. agalactiae* colonization). And this is in agreement with study in India (Dechen *et al*., 2010), in Greek (Tsolia *et al*., 2003), in Peru (Collins *et al.,* 1998) and in Brazil (Costa *et al.,* 2008).

Some authors found a higher frequency of colonization by *S. agalactiae* in pregnant women with complicated pregnancy (Strus *et al*., 2009). In the present study there was not significant association (OR=4.094, CI= 0.351-4.72, P=0.261) of *S. agalactiae* colonization with HIV positive women. However, these results cannot be considered definitive, due to the small number of pregnant women with HIV disease in which HIV positive women evaluated were 2%. From the 3 HIV positive women only one (1/3, 33.3%) was colonized by *S. agalactiae*. This is in agreement with a study in San Francisco (Shah *et al*., 2011), in Tanzania (Joachim *et al*., 2009) and in Zimbabwa (Mavenyengwa *et al*., 2011).

In our study, all *S. agalactiae* isolates were uniformly sensitive to penicillin, which is in agreement with previous reports (De Mouy *et al*., 2001; Castellano-Filho *et al.*, 2010; Tor-Udom *et al*., 2006; Abdelmoaty *et al.,* 2009, Quiroga *et al*., 2008; Motlova *et al*., 2004; Andrews *et al.*, 2000). 100% of isolates were sensitive to vancomycin and ampiciillin which is in agreement with a study in Brazil (Castellano-Filho *et al*., 2010). All isolates were uniformly sensitive to amoxacillin which is in agreement with a study in France (De Mouy *et al*., 2001).

Since the introduction of protocols for the prevention of the newborn *S. agalactiae* diseases, the use of erythromycin and clindamycin has increased, especially in patients allergic to penicillin. At the same time, there was an increase in rates of *S. agalactiae* resistance to these antibiotics, according to the results observed in studies performed in different countries (Abdelmoaty *et al.*, 2009; Joachim *et al*., 2009; Simoes *et al*., 2004; Castellano-Filho *et al.*, 2010). Similarly, in Adigrat it is common to prescribe erythromycin by health professionals to patients with complication that didn't improve with the treatment of ampicillin or a patient with allergic to penicillin. However, it isn't common to prescribe clindamycin by health professionals routinely. But it is a common practice in Adigrat similar

to other cities that antibiotics can be purchased without prescription, which leads to misuse of antibiotics by the public thus contributing to the emergence and spread of antimicrobial resistance.

In the current study, the prevalence of resistance to clindamycin was 17.6%. This was in agreement with finding observed in Egypt (17.9%) (Abdelmoaty *et al.,* 2009), Tanzania (17.6%) (Joachim *et al*., 2009) and France (17.5) (De Mouy *et al*., 2001), but the rate was higher than that of other reports from Czech Republic (3.2%) (Motlova *et al*., 2004), Thammasat (3%) (Tor-Udom *et al*., 2006), Argentina (3.2%) (Quiroga *et al*., 2008), East Lansing (6.4%) (Manning *et al*., 2008). This may be indicate that frequent utilization of clindamycin that obtained outside of recognized treatment centers, and taken without medical authorization or supervision. This leads to the inappropriate use of antimicrobials and their being taken at sub-optimal dosages and for an insufficient length of time. Often the high cost of an antibiotic, results in an incomplete course being purchased, sufficient only to alleviate symptoms (Cheesbrough, 2006). This may contribute to the emergence and spread of clindamycin resistance in Adigrat and it needs further investigation whether this resistance is related to erythromycin resistance due to the presence of *erm* gens or the rare lincosomaid and streptogramin A (LSA) phenotype resistance. In contrast it was lower than a study in Brazil (19%) (Simoes *et al*., 2004). This lower finding in this study might be explained due to method variation. As disk diffusion and micro dilution method was used in the above study where as in the current study disk diffusion method only was used.

In addition, the resistance to erythromycin was 11.8% this was in agreement with resistance observed in Argentina (9.7%) (Quiroga *et al*., 2008) and in Canada (14%) (Andrews *et al*., 2000). However lower than our study resistance was observed in Czech Republic (3.8%) (Motlova *et al*., 2004), in Thammasat (1.5%) (Tor-Udom *et al*., 2006), in Trindad (8%) (Orrett, 2003). This may be indicate that frequent utilization of erythromycin for treatment and prophylaxis of other infectious diseases in the study area due to infection control difference, poor controlling condition that led to multiplication of resistance strains including poor adherence of patients, provision of poor drug quality and regimens, administration of incorrect drug combination and utilization for treating other bacterial

infection (CDC, 2006). But the resistance was lower than in East Lansing (19.1%) (Manning *et al*., 2008) and in France (21.4%) (De Mouy *et al*., 2001). This lower finding in our study may be due to method variation. Disk diffusion and Etest strips method was used in East Lansing and agar dilution method was used in France. However, disk diffusion method was used in our study.

The implementation of intrapartum antibiotic prophylaxis in the United States was followed by a reduction in early-onset neonatal *S. agalactiae* infection (Schrag *et al*., 2000). Similarly, in Australia the incidence of early-onset *S. agalactiae* sepsis fell from 2.0 per 1000 live births in 1991 to 1993, to 1.3 in 1993 to 1995, to 0.5 in 1995 to 1997. The incidence decrease from 1991 until 1997 this was because, in 1991, 3 of 9 study hospitals had a formal policy on intraparatum antibiotic use, whereas in 1997 all 11 hospitals had a formal policy (Isaacs and Royle, 1999).

Perinatal *S. agalactiae* disease is both an expensive and serious condition that can be effectively prevented by relatively low-cost and fast screening strategies during gestation. The carriage rate of *S. agalactiae* in the present study was similar with industrialized countries carriage rate and prevention method of *S. agalactiae* is necessary in Adigrat. Maternal and child health are high priorities for international development. Currently, Ethiopia is on the way of development and works to decrease maternal and neonatal, mortality and morbidity. In the current study we administered to the colonized pregnant women penicillin G according to the recommendation by CDC (CDC, 2010) to reduce the transmission of the bacteria from mother to the neonate and we expect our study may contribute its own merit to achieve millennium developmental goal of Ethiopia.

7. Conclusion and Recommendations

7.1. Conclusion

The carriage rate of *S. agalactiae* in the study area was 11.3%. High *S. agalactiae* isolates were detected from Adigrat Health Center. Prolonged rupture of membrane was strongly associated with the colonization of *S. agalactia*e. Based on the finding, penicillin G was the best antibiotic for the treatment of *S. agalactia*e. Two (11.8%) isolates were resistance to erythromycin and three isolates were resistance to clindamycin (17.6%). Common resistance to erythromycin and clindamycin was seen in two isolates.

7.2. Recommendations

Based on the findings of the present study the following recommendations are made:-

- The rates, risk factors of maternal *S. agalactia*e colonization may vary in different communities and need to be thoroughly evaluated in each country or region to allow the most appropriate preventive strategy to be selected.
- Empirical antibiotic treatment for *S. agalactia*e infection must be taken into consideration. Especially the increasing rates of erythromycin and clindamycin resistance that is now prevalent.
- Penicillin G must be administered to treat specific *S. agalactia*e colonized pregnant women rather than giving ampicillin.
- The role of *S. agalactia*e in neonatal infection should be investigated.
- The serotype distribution of *S. agalactia*e must be investigated.
- Coexisting bacteria like *E. coli* that can cause similar effect like *S. agalactia*e in pregnant women must be investigated.
- Routine *S. agalactia*e bacteria surveillance by culture and the study of its resistance patterns must be an essential component of pregnant women and neonatal care.

If culture and susceptibility test is not possible:

- The hospital and health center must have local guidelines for screening and prophylaxis for *S. agalactia*e by risk assessment according to CDC recommendations.
- Vancomycin should be reserved for penicillin-allergic women at high risk of anaphylaxis due to unknown susceptibility of a prenatal isolate to clindamycin and erythromycin.

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9. Annexes

Annex 1. Patient information & Consent form

Information for Pregnant/ candidates

Purpose: *S. agalactia*e infections are major public health problems in human worldwide. It is associated with adverse outcomes in pregnant women like UTI, sepsis, chorioamnionitis, endometritis and septic abortion and in your neonate this bacteria cause sepsis, pneumonia and meningitis. The aim of this study is to determine the colonization of pregnant women and anti microbial susceptibility pattern of *S. agalactia*e.

Procedure: To determine the colonization of pregnant women and anti microbial susceptibility pattern of *S. agalactia*e infection we invite you to take part in this study. If you are willing to participate in this project, you will be examined for your carriage status: rectal and vaginal swab samples will be collected from you.

Risk and Discomfort: there is no risk for you if you participate in the study.

Benefits: If you participate in this research, you will get a clinical assessment of your health condition. You will be informed your result if positive for further treatment.

Incentives: You will not be provided any incentives to take part in this research.

Confidentiality: The information that we collect from this research project will be kept confidential. Information about you that will be collected from the study will be stored in a file, which will not have your name on it, but a code number assigned to it. Which number belongs to which name will be kept under lock and key, and it will not be revealed to anyone.

Sharing of results: The finding of this research will be disseminated to responsible body and will be published. But it will not be known the result is being yours.

Right to refuse or withdraw: You have full right to refuse from participating in this research if you do not wish to do so.

Whom to contact: If you have any further question you can contact at any time through
Tel: 0913513513 or E-mail: kahsaytsega@yahoo.com

Consent form

Pregnant consent form (To be translated in to the pregnant's language)

Serial no…….

Card no…..……....

The objective of this study is to determine the colonization of pregnant women and pattern of antimicrobial susceptibility of *S. agalactiae*. Because isolation of *S. agalactiae* infection and determining pattern of antimicrobial susceptibility of *S. agalactiae* in pregnant women is good to decrease morbidity and mortality of neonate, the results of this study are believed to be important to treat pregnant women appropriately. Therefore, we are requesting you to participate in the study, which would require your response to an interview, physical examination and to provide sample rectal and vaginal swab for laboratory examination. Results will be reported to the requesting Gynecologist for appropriate treatment and management.

I_____ hereby give my consent for giving of the requested information and specimens as the doctors find best for me.

I have been informed verbally and in writing about this study that plans to determine the *S. agalactiae* infection. I understand what is involved and I have been requested to give rectal and vaginal swab for laboratory investigation. I also know whom to contact if I need more information. I understand that confidentiality will be preserved. Moreover, I also understand that I have a right to withdraw from participating in this study at any time and my actions will have no impact on the overall management of my conditions. The investigators have briefed me there are no risks associated with the swab sample collection. I have been given enough time to think over before I signed this informed consent. It is therefore, with full understanding of the situation that I gave my consent and cooperate at my will to participate fully in the study.

My signature below indicates that I agree to participate in this study.

Subject's signature …… Date of signature……..

Signature of Person Obtaining Consent …….

Date of signature……

<u>የነፍሰጡር እናቶች መረጃ መስጫና ፍቃደኝነት መጠይቅ</u>

<u>ትርጉም (በኣማርኛ)</u>

<u>ለነፍሰጡር እናቶች የሚሰጥ መረጃ</u>

<u>ጠቀሜታ፡</u> የዚህ ጥናት ኣላማ በነፍሰጡር እናቶች ሰትሪፕቶኮከስ አጋላክሽያ ታህዋስ መኖሩን ማረጋገጥና የመድሃኒት መለማመድ መኖሩን ማወቅ ነው።ጥናቶች እንደሚያመለክቱት ይህ ተህዋስ በኣለም ዙርያ የህዝብ ችግር ሁኖ ቆይተዋል።ይህ ተህዋስ በእናት ነፍሰጥሮች የኩላሊት፣ሰፕሲስ፣ጥንስ መቋረጥ እና በጨቅላው ደግሞ ሰፕሲስ እና መንንጃይተስ ጋር የተያያዘ ነው።

<u>ኣሰራር፡</u> በነፍሰጥሮች ሰትሪፕቶኮከስ አጋላክሽያ ታህዋስ ላይ ጥናት ለማካሄድ ፍቃደኛ ከሆናችሁ ከመቀመጫችሁ እና ከብልታችሁ ጫፍ ፈሳሻ ለመውሰድ እንድትተባበሩን እንጠይቃለን።

<u>ኣደገኝነትና ሙቸት መንሳት፡</u> በጥናቱ ላይ ብትሳተፉ በጤናዎ ላይ ምንም ኣይነት ኣደጋ አያስከትልብዎም።

<u>ጥቅም፡</u> በጥናቱ ላይ ተሳታፊ ቢሆኑ ሰትሪፕቶኮከስ አጋላክሽያ ጋር በተያያዘ ስለ ጤናዎ ሁኔታ ታይቶ ህክምና ይደረግለዎታል።

<u>ማበረታቻ፡</u> ከጥናቱ ጋር በተያየዘ የገንዘብ ወይም የእቃ ስጦታ ኣይኖርም።

<u>ሚስጥራዊነት፡</u> ከዚ ጥናት የሚገኝ መረጃ ሚስጥራዊነቱ የተጠበቀ ነው። በጥናቱ ፋይል ስማችሁ ሳይሆን ሚስጢራዊ ኮድ ነው የሚፃፈው።

<u>ውጤት ማሰራጨት፡</u> የጥናቱ ውጤቶች ለሚመለከታቸው ኣካላት ይሰጣሉ።

<u>ጥናቱ ላይ ለመሳተፍ ኣለመገደድ፡</u> በዚህ ጥናት የመሳተፍ ወይም ያለመሳተፍ መብትዎ የተጠበቀ ነው።

ተጨማሪ መረጃ ማግኘት ከፈለጉ

በ 0913513513 ወይም ኢመይል <u>kahsaytsega@yahoo.com</u>

ንነፍሰፁራት ሓበሬታ መውሃብን ድልየት መሕተቲን

ትርጉም(ብትግርኛ)

ናይዚ ዕንዓት እዚ ዕላማ ኣብ ነፍሰፁር እኖታት ናይ ሰትሪፕቶኮስ ኣጋላክሽያ ባክተርያ ምህላው ምርግጋፅን ናይ መድሓኒት ምልምማድ ምህላው ምፍላጥን እዩ። ዕንዓታት ካም ዝሕብርዎ እዚ ባክተርያ ብዓለም ደረጃ ዓብይ ችግር ኮይኑ ፀኒሑ እዩ። እዚ ባክተርያ ኣብ ነፍሰፁር እኖታት ናይ ኩላሊት፣ሰፕሲስ፣ጥንሲምካድ፣ ዕሸል ኣብ ዘይመዓልቱ ምውላድ፤ ኢሉ ውን ኣብቲ ማማይ ድማ ምስ ሰፕሲስ ፣ናይ ሳንባ ምቹን መንንጃይተስን ዝተተሓሓዘ እዩ። ናይ እዚ ባክተርያ ምህላዉ ነረጋገፀሉ ካብ መቀመጫኣንን ብልዕተንን ጫፍ ፈሳሲ ብምውሳድ ኣንትኽውን ኣብዚ ዕንዓት እንተተሳቲፈን ምንም ዓይነት ሓደጋ ይኩን ናይ ዘይምምችቻው ኩነታት ኣየጋጥመንን። ሰትሪፕቶኮስ ኣጋላክሽያ ባክተርያ እንተድኣ ተረኺቡ ኣብእዋን ሕርሲ ግዜ መድሓኒት ክወሃበን እዩ። ይኩን እምበር ናይ ገንዘብ ይኩን ናይ ኣቅሓ ዝረክበኡ ውህብቶ የለን። ካብዚ ዕንዓት ዝርከብ መረዳእታ ምስጥሩ ዝተሓለወ እዩ። ኣብዚ ዕንዓት ንምስታፍ እንተዘይደልየን ብዝኮነ ይኩን መንገዲ ኣይግደዳን።

ንተወሳኪ ሓበሬታ በዚ ኣድራሻ ምርካብ ይከኣል። ስ.ቁ 0913513513 (kahsaytsega@yahoo.com)

ታ.ቁ ---------- ካርድ ቁ ----------

ካብዚ ብምብጋስ ንሰን ኣብዚ ዕንዓት ንክሳተፉ እናሓተትና ካብኣን ዝድለ ንሕቶታት መልሲ ምሃብ፣ብሓኪም ምርኣይ ካብኡ ድማ ንላባራቶሪ ዝከውን ናሙና ካብ መቀመጢኣንን ብልዕተንን ፈሳሲ ምሃብ እዩ።ውዕኢቱ ምስ ተወደአ ተደላይ መድሓኒት ንክረክባ ክንገብር ኢና።

ኣነ----------ስመይ ኣብዚ ዝተጠቀሰ ሓኪሙ ንዓይ ዝሓሸ ኮይኑ እንተረኪብዎ ብድልየተይ ዝተሓተትክዎ ሕቶ መልሲን ናሙናን ንምሃብ ዝግጁ እየ።ናይቲ ዕንዓት ዕላማ ብዕሑፍን ብቃልን ተነጊሩኒ ዝተረደኣኒ እንትኸውን ካብ መቀመጥየይን ብልዕተይን ናሙና ንምሃብ እናፈቀድኩ፣ተወሳኪ ሓበሬታ እንተደልየ ንመን ካነጋግር ካም ዘለኒ ፈሊጠ ኣለኩ። እዚ ብመረዳእታ ዝተደገፈ ድልተይ ዘረጋግፅ ካብ ምፍራመይ ኣቀዲሙ እኩል ዝኾነ ስዓት ተዋሂቡኒ ሓሲበሉ እየ።ፈርማይ ኣብዚ ታሕቲ ዘሎ ኣብዚ ዕንዓት ንምስታፍ ምስምዕማዐይ መረጋገፂ እዩ።

ናይ ተሳታፊ ፈርማ ---------- ዕለት ----------

ፍቃድ ናይ ዝተቀበለ ሰብ ስም---------- ፈርማ---------- ዕለት.....

Annex 2. Clinical Examination & Questionnaire Form

Questioner

Serial No……

Patient name……Card No……

Telephone home…….Mobile……

I. Demographic Data

1. Age in years ……....

2. Educational level I. Uneducated II. Primary school III. Secondary IV. Tertiary

3. Address; 1. With in the Town …… 2.Out side of the town …….

4. Marital status 1. Married 2.divorced 3.single 4.cohabitant 5.other

5. Religion 1.Orthodox 2. Catholic 3.muslim 4.other

6. Ethinic group 1. Tigray 2.Amhara 3.other

7. Reproductive hx 1. Gravida……....2.Para……..3. No of Abortions……

II. Clinical Data (obstetric and medical hx)

1. Gestational age in weeks …….

2. Previous history of Early neonatal death…… 1.Yes 2.No

If yes, cause of death 1. Infection 2. Asphyxia 3.prematurity 4.unknown

3. Previous history of preterm labor……1.Yes 2.No

4. Previous history of Preterm PROM ……..1.Yes 2.No

5. Previous History of *S. agalactiae* infections……1.Yes 2.No 3. I don't know

If yes, type of infection: UTI/ sepsis/ chorioamnionitis/ endometritis

6. Current History of chronic medical illness ……1.Yes 2.No

If yes which one of the following is /are present (more than one answer is possible)

1. HIV 2.Diabetes mellitus 3.Cancer 4. Cardiac diseases

5. Renal diseases 6.Chronic bronchitis 7.other, specify ……

7. If resistant to any antibiotic ……1.Yes 2.No

If yes, mention antibiotic/s……

III. Laboratory Form

1. Date and time of specimen collection: date__/___/2004 E.C, Time __:__PM/AM
2. Serial No…… ……3. Card No……
4. Fever at the time of sample collection……. 1.Yes 2.No
5. Type of specimen: Rectal and vaginal swab
6. *S. agalactiae* organism isolated 1) Yes 2) No
7. Antimicrobial susceptibility testing S (mm) I (mm) R (mm)
   - Penicillin G …… …….. …….
   - Ampicillin …… …… …….
   - Amoxicillin …… …… …….
   - Erythromycin …… …… …….
   - Clindamycin …… …… …….
   - Vancomycin …… …… …….
8. Comment _____

Name of investigator_____

Signature _____

Date __/__/04.

Annex 3. Work flow chart: Specimens

Rectal and vaginal Swab was collected from pregnant women whose gestation period is

35-37 week by attending physician

Transportation of swab in Amies Transport medium to APF

Inoculation directly to CHROM^{TM} *strep* agar incubated aerobically at 37^0c for 18– 24h

Gram stain

Bio chemical test (Catalase test, CAMP test)

Confirmed by latex agglutination Antisera for *S. agalactiae*

Anti microbial Susceptibility on Muller Hinton Agar supplemented with 5% sheep

Blood, was incubated at 37^0c for 20-24 hours

Interpreted according to Clinical and Laboratory Standards Institute (CLSI) guide lines as 'Resistant', 'Intermediate/Moderately susceptible', 'Susceptible'.

DECLARATION

I, the under signed, declare that this M.Sc thesis is my original work, has not been presented for a degree in any other university and that all sources of materials used for this thesis have been duly acknowledged.

M.Sc candidate: **Tsega Kahsay (B. Sc)**

Signature _____

Date _____

Advisor: Tamrat Abebe (B. Sc, M. Sc, PhD Candidate)

Signature _____

Date _____

Advisor: Adane Mihret (DVM, M. Sc, PhD Candidate)

Signature _____

Date _____

Place of submission: Addis Ababa, Ethiopia