GROUP B STREPTOCOCCI: COLONIZATION RATE AMONG PREGNANT WOMEN AND THEIR NEWBORN AND BURDEN OF NEONATAL DISEASE IN SELECTED HOSPITALS OF ETHIOPIA

MUSA MOHAMMED (BSc, MSc)

A Dissertation Submitted to the Department of Microbiology, Immunology and Parasitology in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Medical Microbiology

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ADDIS ABABA UNIVERSITY, COLLEGE OF HEALTH SCIENCES, SCHOOL OF MEDICINE, DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY

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ABBREVIATIONS/ACRONYMS

AAP…….....American Academy of Pediatrics

ACP…. ……....Alpha C protein

AD……...Atopic dermatitis

AHMC…….Adama Hospital Medical College

Alp……...Alpha like protein

AMPs……...Antimicrobial peptides

ACOG……American college of Obstetrician & Gynecologist

ATCC……..American type culture collection

BAP…….Blood agar plate

BBB…. ……...Blood–brain barrier

BD……..Becton Dickinson

BibA……..GBS immunogenic bacterial adhesin

BMECs…… …….Brain microvascular endothelial cells

BP……..Base pair

C3bp…….C3 binding protein

CAMP…… …….. ……Christie, Atkins, and Munch-Petersen

CBC…….. …....Complete blood count

CC…….. ……..Clonal complex

CD4…….....Cluster differentiation 4

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

CFU……...Colony forming unit

CGH……..Comparative genome hybridization

CIP…….....Complement interfering protein

CNS……..Central nervous system

CPS….. ……Capsular polysaccharide

CRISPR……..Clustered regularly interspaced short palindromic repeats/CRISPR-associated proteins

CRP……..C reactive protein

CSF…….. .....Cerebrospinal fluid

CspA…….Serine protease

DLVs…….....Double-locus variants

DNA……Deoxyribonucleic acid

DNA-DBH…….. ……DNA dot blot hybridization

dNTPs……...Deoxynucleotide triphosphate

DPPC…. ……..Dipalmotyl phosphatidylcholine

DT….. ……..Diphtheria toxin

ECM…. ……...Extracellular matrix

EOND……..Early Onset Neonatal Disease

EOND-GBS…….Early Onset Neonatal Disease due to GBS

EOND-GBS…….Early Onset Neonatal Disease due to GBS

FAT….. ……Fluorescent antibody test

FbsA.....Fibrinogen-binding protein A

FbsB…… ……Fibrinogen-binding protein B

GAG…….....Glycosaminoglycan

GAPDH ……...Glyceraldehyde-3- phosphate dehydrogenase

GAS….. ……Group A Streptococcus

GBS…….Group B Streptococcus

GM-CSF…….....Granulocyte Monocyte -colony stimulating factor

HA…….. …….Hyaluronan

HIV…….....Human immunodeficiency virus

HK……...Histidine kinase

HPTH…. ……Hitchens-Pike-Todd-Hewitt

HRH……Hawassa Referral Hospital

IagA……. ……...Invasion-associated gene

IAP……..Intrapartum Antibiotic prophylaxis

ICAM-1……...Intercellular adhesion molecule-1

ICEs…….Integrative and Conjugative Elements

Ig…….....Immunoglobulin

IL…….....Interleukin

IRB……Institutional Review Board

IV…….....Intravenous

LBW……Low birth weight

Lmb…… …….....Laminin-binding protein

LOND…….Late onset neonatal disease

LOND-GBS…….Late Onset Neonatal Disease due to GBS

LTA….. …….....Lipoteichoic acid

MALDI-TOF MS….. ……..Matrix-assisted laser desorption ionization–time of flight mass

MDG…. ……..Millennium Development Goal

MEE…….Multilocus Enzyme Electrophoresis

MIC……..Minimum inhibitory concentration

MLST……Multilocus sequence typing

mPCR……multiplex PCR

MtaR…… ……Methionine transport regulator

NAATs…… ……Nucleic Acid Amplificatin tests

NERC……..National Ethics and Research Committee

NO…….....Nitric oxide

NPV….. ……..Negative predictive value

NT……Non typable

NVU …….....Neurovascular unit

PBP1a…… …….Penicillin-binding protein 1a

PCR……Polymerase chain reaction

PFGE…….....Pulsed Field Gel Electrophoresis

PI……Pilus island

PI3K……..Phosphoinositide-3 kinase

PPV……Positive predictive value

PROM….. ……Prolonged rupture of the amniotic membranes

RDP…… ……..Restriction digest pattern

RFLP……Restriction fragment length polymorphism

RLB…….....Reverse line dot blot

RNA……..Ribonucleic acid

ROS….. …….Reactive oxygen species

ScpB……..C5a peptidase

SLVs……. ……...Single-locus variants

SNNRP…….Southern nation, nationalities, and people's region

SodA…….Super oxidase dismutase

Srr…….Serine-rich repeat proteins

ST……. ….....Sequence type

TASH……..Tikur Anbesa speiclized Hospital

TCS…….....Two-component regulatory systems

Tdap……..TT reduced diphtheria toxoid

TLR…. …….....Toll-like receptors

TNF-α…….....Tumor Necrosis factor -α

TSSs…….Transcription start sites

TT…….Tetanus toxoid

UK……United kingdom

UPGMA……..Unweighted pair group method with algorithm mean

USA……United States of America

VLBW…. …….....Very Low Birth Weight

VTR……Vertical transmission rate

WHO……...World Health Organization

β-H/C, CylE…….β-hemolysin/cytolysin

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OPERATIONAL DEFINITION

GBS colonization: Isolation of GBS from pregnant women (rectovaginal area) and their newborn (ear, nasal nares, umbilical cord, throat)

Infant: Babies whose age is less than 90 days.

Eearly Onset disease (EOD): Disease that occur among newborn whose age is less that seven dasys.

Late Onset disease (LOD): Disease that occurs among babies whose age is in between 7 and 90 days.

Vertial Transmission: Is passage of GBS from pregnant women to their newborn as confirmed by isolation of the same GBS serotype from pregnant women and their newborn.

ABSTRACT

Background: The newborn of today have a good chance of survival compared to several years back. The number of worldwide child deaths has decreased, from 12.7 million in 1990 to 5.9 million in 2015. Neonatal mortality reduction is still far behind MDG4 particularly in sub Saharan Africa, including Ethiopia. About 44% of world-wide mortality in children less than five years occurs in the first month of life. Several microorganisms are involved in neonatal ill health; among them Group B streptococcus (GBS), was identified relatively recently as the leading cause of neonatal disease in developed countries. Group B streptococcus is transmitted from colonized mother to newborn during birth. The primary risk factor for development of Early Onset Diseade due to GBS (EOD-GBS) is maternal colonization with GBS during late trimester.

Objective: The objectives of this study were to determine GBS colonization rate among pregnant women and their new born who were attending selected hospitals, vertical transmission rate, burden of neonatal disease due to GBS and phenotypic and genotypic characterization of GBS.

Materials and Methods: A cross-sectional and follow-up study was conducted in three different Hospitals starting from June 2014 to August 2015: Adama Hospital Medical College (AHMC), Hawassa Referral Hospital (HRH), and Tikur Anbessa Specialized Hospital (TASH). A total of 1873 participants were recruited in the present study. Among them, 840 of them were pregnant women and 857 of them were their new born from three study sites. All of them were screened for GBS using standard methods recommended by Center of Disease Control and Prevention (CDC). Out of the total study participants, 176 of them were infants suspected of early and late onset disease whose age was less than 90 months from Tikur Anbesa Specialized Hospital. Their blood and CSF were cultured to isolates GBS. The isolated GBS were serotyped by using serotype specific antisera. Selected GBS isolates were further characterized by multiplex PCR, antimicrobial susceptibility pattern and Multilocus Sequence typing. Structured questionnaire was used to collect Socio-demographic and clinical data of pregnant mother and newborn. Data was analyzed using SPSS version 20; P value <0.05 was considered statistically significant.

Result: The overall maternal, Newborn GBS colonization and vertical transmission rate were 17.4%, 9.2% and 54.1% respectively. The overall prevalent serotypes detected in the present study include; type II (30.3%), Ia (20.2%), V (17.9%), Ib (17.1%), III (10.1%), VII (0.9%), and non typeable (3.5%). The burden of neonatal disease due to GBS was 1.7%. No significant association between maternal and newborn GBS colonization rate and measured risk factors was found (P>0.05). All GBS isolates tested were sensitive to Penicillin, Vancomycin, Cefotaxime and Linezolid. Most of the GBS isolates were resistant to Tetracycline (91.2%), majority of them were sensitive to Levofloxacin (92.8%), Daptomycin (97.6%), Erythromycin (91.2%) and Clindamycin (98.4%). Among 123 GBS tested 89.4% of them contain antibiotic resistance genes. The antibiotic resistance genes detected include; *tet* (M-1) (88.6%), *tet* (L-1) (31.7%), *erm* (TR-1) (5.7%), *gyra* (GBS-1) (4.1%), *par C* (GBS-1) (4.1%), *erm* (B-1) (0.8%), and *tet* (O-1) (0.8%). MLST characterization grouped all GBS strains in to 17 STs, two clonal complexes (CC-2 and CC-249) and one singleton. ST-10, ST-19 and ST-23 are the most prevalent STs found in the study. ST-932, ST-933, ST-934, ST-935, and ST-936 were new sequence types that have been identified in this study.

Conclusion: This multi center study reveals the presence of primary risk factor, maternal GBS colonization, for early onset neonatal disease due to GBS in three Referral Hospitals of Ethiopia. The study also showed significant importance of invasive neonatal disease due to GBS from selected Hospital of Ethiopia for the first time. Epidemiology of GBS serotypes, antibiotic resistance pattern, MLST profile, and population structure of GBS collection from Ethiopia is partially different from other parts of the world. Therefore, it is important to measure neonatal disease due to GBS at large scale and consider prevention strategy such as Intrapartum prophylaxis and vaccine.

Keywords: Group B Streptococcus, Colonization rate, Vertical transmission rate, Antibiotic Susceptibility, Serotype, Sequence type, Ethiopia

CHAPTER I: INTRODUCTION

1.1. INTRODUCTION

Today newborns have a better chance of survival compared to early time newborn. The number of worldwide child deaths has decreased, from 12.7 million in 1990 to 5.9 million in 2015 (Liu *et al.,* 2015). However, there has been less progress in decreasing neonatal mortality rate, with 2.7 million neonatal deaths in 2015 (Blencowe *et al.,* 2016; Lawn *et al.,* 2014). Total child mortality rate has declined substantially; however, neonatal mortality reduction is still behind Millennium Development Goal (MDG4) particularly in sub Saharan Africa, including Ethiopia (Ann, 2014). About 44% of worldwide children mortality occurs in the first month of life (Starrs, 2014). Every year 2.9 million newborns die from preventable diseases (Udani and Richard, 2014). Contribution of infectious disease to neonatal morbidity is high; about 75% of infection related deaths occur in developing countries (Lawn *et al.,* 2014). Many microorganisms are involved in neonatal disease; among them Group B streptococcus (GBS) was identified recently as the leading cause of Early Onset Diseases (EODs) such as pneumonia and sepsis and Late Onset Neonatal Diseases (LODs) such as meningitis in developed and developing countries (Gray *et al*., 2007; Dangor *et al.,* 2015a).

Group B Streptococcus (GBS) has emerged in mid 1960s as the major cause of diseases among newborn and pregnant women (Schuchat, 1998). The first cases of neonatal disease due to GBS were reported from USA and Germany in between 1961 to 1965 (Da Cunha *et al*., 2014; Kexel and Schoenbohm 1965). According to Da cunha *et al.* (2014) the observed emergence of GBS infections in the 1960s was not due to past under diagnosis but was is due to modification of the human GBS population. Da cunha *et al.* (2014) reported that the human disease causing and carriage GBS population is dominated by few Tetracycline Resistant (TcR) clones that have spread all over the globe.

The primary risk factor for EOD due to GBS (EOD-GBS) is maternal genitourinary colonization with GBS. About 10-30% of pregnant women carry GBS in their rectovaginal compartment. GBS transmission from colonized mother to newborn occurs vertically during birth (Regan *et al.,* 1991; Nandyal, 2008). About 50-65% of infants who are born from

colonized mothers have positive GBS cultures. Approximately 1-2% of colonized infants develop invasive GBS infection such as sepsis and meningitis (Baker and Edwards 2001). Mortality rate of 20–25% and permanent neurologic sequelae in the majority of survivors of GBS infection will occur (Schuchat, 1999).

It has been about five decades since GBS emerged as an important agent in early onset neonatal infection in United States of America (USA) and other developed countries. It is in 1970s that GBS was recognized as the leading cause of early onset neonatal morbidity and mortality in USA with initial case fatality rate as high as 50% (Verani *et al.*, 2010). Several organizations were convened to develop a prevention strategy, Intrapartum Antibiotic Prophylaxis (IAP). After the release of the first prevention guideline in 1996 and its revised version in 2002, the incidence of EOD-GBS was substantially reduced in USA (Figure 1.1) and other countries (Verani *et al.*, 2010). In 2009, the prevention guidelines were also updated based on the data collected after the release of the 2002 guideline to further reduce the burden of EOD-GBS (Verani *et al.*, 2010).

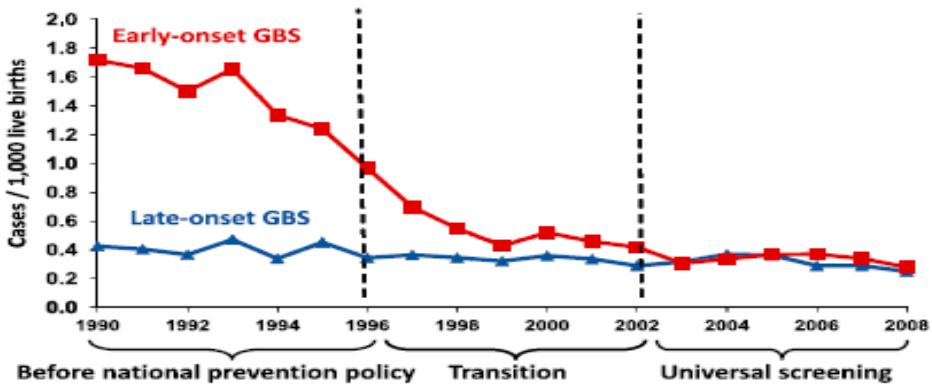


Figure 1.1 Rates of EOGBS and LOGBS, 1990–2008. Source: Active Bacterial Core surveillance/Emerging Infections Program (Puopolo, 2014).

Intrapartum Antibiotic Prophylaxis (IAP) recommends adminstraion of antibiotics before delivery based on either screening result or certaion risk factors. Risk factors include; gestaional age less than 37 weeks, prolonged ruprture of membrane and maternal intrapartum fever (Verani *et al.*, 2010). The current strategy, IAP, is not without limitations; LOD-GBS disease remains unchanged. Despite antibiotic prophylaxis during delivery, it is

estimated that about 3,600 neonates develop invasive GBS infections annually in USA (Schrag *et al.,* 2000). Research is going on to come up with vaccine to prevent EOD-GBS, LOD-GBS, GBS disease among adults and a strategy that does not cause emergence of antibiotic resistance bacteria (Kim *et al.,* 2014).

Even though the role of GBS in neonatal disease is not well known in developing countries, about 900,000 sepsis cases associated neonatal death in a year occurs in developing countries (Bryce *et al.,* 2005; Seale *et al.,* 2009). According to World Health Organization (WHO) estimates, 5 million children under one month of age die every year, and about 98% of them occur in developing countries (WHO, 1996). About 3.4 million neonatal deaths take place in the first week of life (Wessels and Kasper, 1994). Studies from different parts of Africa indicate similar colonization rate of GBS among pregnant women, risk factors for GBS disease and hence exposure of neonates to GBS is similar to those from developed countries (Wessels and Kasper, 1994; WHO, 1996). Moreover, meta-analysis estimated the burden of neonatal disease due to GBS in sub-Saharan Africa to be 2.05/1000 live birth which is high compared to other regions (Dagnew *et al.,* 2012; Edmond *et al.,* 2012).

So far there is no data about burden of neonatal disease due to GBS and there is scarce information concerning colonization of pregnant women with GBS, the primary risk factor, in Ethiopia (Mohammed *et al.,* 2012). As IAP is not standard of care in many developing countreis including Ethiopia, it can be estimated that several newborn could have been afflicted by GBS and it will continue until appropriate action is taken. More than 30 years experience from developed countries revealed that IAP significantly reduced the incidence of EOD-GBS. Therefore, to identify EOD-GBS and LOD-GBS as priority and in the future, to consider prevention strategy which best suit developing countries, data on disease burden due to GBS, risk factors and possible prevention strategies is required. MDG 4 and 5 identifies maternal and child health as a priority for international development, which is still unmet in sub-Saharan Africa (Seale *et al.,* 2009). GBS may be the leading cause of morbidity and mortality among newborns in Ethiopia, and to achieve the goal set by MDG-4, the epidemiology of invasive GBS and its risk factors among Ethiopian newborns needs to be investigated.

Therefore, the present study was undertaken to provide update information on maternal and infant colonization rate with GBS, vertical transmission, burden of neonatal disease due to GBS and to determine prevalent GBS serotypes, sequence types, including antimicrobial susceptibility pattern in three selected Hospitals in Ethiopia.

1.2. LITERATURE REVIEW

1.2.1. MICROBIOLOGY OF GROUP B STREPTOCOCCUS

a. General Characteristics

Streptococcus agalactiae (Group B streptococcus; GBS) is the species representation for *Streptococci* belonging to the Lancefield group B. It is facultative anaerobic gram positive cocci and form chains that can grow well on a blood agar plate (BAP). Colonies of GBS on BAP measures 1-3 mm in diameter, β-hemolytic and grayish-white mucoid in color as shown in Figure 1.2. About 1-2% of the GBS strains are non-hemolytic (Martins, 2007). They are catalase negative, hydrolyze hippurate, ferment ribose and trehalose, and produce acetoin (Kilian, 1998; Lachenauer *et al.*, 1999).

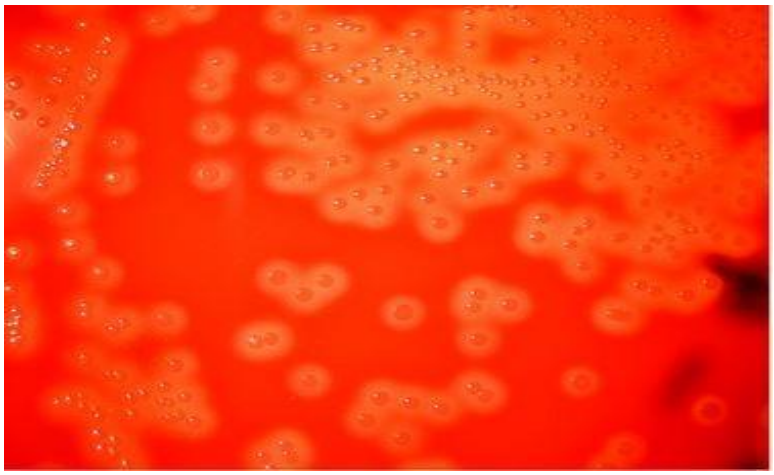


Figure 1.2. Beta Hemolytic properties of GBS (Adopted from Color Atlas of Diagnostic Microbiology).

b. Capsular Polysaccharides (CPS)

Group B Streptococcus **(**GBS) has two different polysaccharide antigens; the group B specific antigen which is common for all strains and the type specific capsular polysaccharides (CPS) which further divide GBS into 10 serotypes (Ia, Ib-IX). All the genes

responsible for the synthesis and cell wall of the GBS capsular polysaccharides (CPS) are clustered in the *cps* operon. This operon is composed of about 16–18 genes; the sequences of which differ among serotypes (Cieslewicz *et al.,* 2001). In many encapsulated bacteria, genes conserved across diverse capsular serotypes flank genes encoding enzymes unique to a specific capsular serotype (Yother, 1999). In GBS, a central group of genes for serotype-specific glycosyltransferases and polymerases is flanked on one side by genes encoding enzymes that synthesize and activate sialic acid (Haft *et al.,* 1996; Cieslewicz *et al.,* 2001; Suryanti *et al.,* 2003). Flanking the other side of the serotype-specific glycosyltransferase genes is a group of genes which are hypothesized to function in the export of the polysaccharide capsule.

The type specific polysaccharides are repeating units of five to seven monosaccharides: glucose, galactose, glucosamine and N-acetylneuraminic acid or sialic acid (Lancefield and Freimer, 1966; Slotved *et al.,* 2007). Glucose, galactose, and *N*-acetylneuraminic acid are found in all nine capsule serotypes. Except in serotypes VI and VIII, *N*-acetylglucosamine is also present in capsule of all serotypes. Rhamnose occurs only in the type VIII repeating unit (Cieslewicz *et al.,* 2005).

Analysis of the nine capsule serotypes revealed two basic motifs. The first motif a disaccharide backbone of β-D-Gal*p*- (1→4)-β-D-Glc*p* is found in eight serotypes. A second motif is a variable trisaccharide side chain, consists of α-D-Neu*p*NAc-(2→3)-β-D-Gal*p*-(1→4) [or (1→3)]-β-D-Glc*p*NAc (or β-D-Glc*p*). Serotype VIII is structurally unique in that it contains a variable trisaccharide that is interrupted by a β-L-Rha*p*. The linkage between the variable trisaccharide and the disaccharide can further distinguish the polysaccharide repeating units (PRUs). In five of the nine serotypes (Ia, Ib, II, III, and VI), the variable trisaccharide and the disaccharide have a 1→3 linkage. In serotypes IV, V, and VII, an additional α-D-Glc*p* is linked 1→4 to the β-D-Gal*p* of the disaccharide and the 1→3 linkage between the disaccharide and the variable trisaccharide is altered to a 1→6 linkage (Cieslewicz *et al.,* 2005).

Similar to other gram negative and gram positive bacteria, each repeating polysaccharide parts are assembled on a carrier lipid (Undecaprenyl phosphate) by sequential activities of

glycosyltransferase. Repeat units are transferred across the plasma membrane by flippase protein. Polymerization occurs at the periplasmic face of the plasma membrane and is catalyzed by polymerase enzyme (Berti *et al.,* 2014).

Definitive identification of GBS requires detection of the group B specific antigen by using hyperimmune group B specific antisera or monoclonal antibodies. The primary serologic method used for serotype determination of GBS is antigen extraction and precipitation reactions with adsorbed whole-cell antisera developed by Lancefield in 1933 (Lancefield, 1933; Paoletti *et al.,* 1999a). The fluorescent antibody test (FAT) described by Bevanger and Maeland (Bevanger and Maeland, 1977) is another alternative method of serotyping. A Latex agglutination test was also developed for serotyping of GBS (Slotved *et al.,* 2003). Almost all GBS isolates can be classified into respective serotypes, but 4 to 7% do not react with hyper immune sera and are classified as non-typable (NT) (Ferrieri *et al.,* 2004). In US, approximately 2.9% of colonizing and 1.4% of invasive isolates are NT (Ramaswamy *et al.,* 2006a). Up to 12% of GBS isolates from Mexico were found to be NT (Palacios *et al.,* 1997)

There are several explanation for non typeability of GBS such as; it may be due to non-encapsulated variant, uncharacterized polysaccharide, mutation in genes essential for capsule expression, reversible non-capsular phase variation or technical problem (Sellin *et al.,* 2000; Cieslewicz *et al.,* 2001; Slotved *et al.,* 2002). As part of this Rosini *et al.* (2015b) identified many distinct mutations in the *cps* operon which can lead to inactivation of capsule synthesis. The common genetic change detected were point mutations leading to stop codons in the *cps* genes and the main target was found to be *cpsE* encoding the portal glycosyl trasferase of capsule biosynthesis. To minimize non-typability of GBS, molecular typing of GBS based on detection of serotype-specific gene clusters of the capsular region has been developed by (Kong *et al.,* 2002a; Manning *et al.,* 2005). Non-typeable isolates produce very low levels of capsule or may have modified capsular structures do not react to antiserum obtained from any of the nine known serotypes. Additionally, a single strain which lacks the whole capsule locus was recently mentioned by Creti *et al,* (2012).

Moreover, vaccine based on capsular polysaccharide will not be effective in inducing optimal protection for infection with non typeable GBS strains.

Furthermore, it has been observed that closely and distantly related clones of GBS may share the genes encoding a specific CPS suggesting that capsule switching may occur in GBS (Davies *et al.*, 2004a). Dual and triple molecular serotypes could have originated from recombination of serotype-specific capsular genes, since the *cps* locus is highly conserved at the ends and contains a serotype-specific variable gene region in the middle (Cieslewicz *et al.*, 2005). Out of 10 known GBS serotypes, eight of them appear to be closely related both structurally and genetically; on the other hand, serotype VIII is more distantly related. This similarity in polysaccharide structure strongly suggests that the evolutionary pressure toward antigenic variation exerted by acquired immunity is counterbalanced by a survival advantage conferred by conserved structural motifs of the GBS polysaccharides (Cieslewicz *et al.*, 2005).

c. Surface Proteins

In addition to capsule, GBS also expresses several surface proteins. Majority of them are able to induce protective immunity in animal models and they can serve as potential source for vaccine development (Larsson *et al.*, 2006). The first surface protein identified in GBS was the C antigen (Wilkinson and Eagon, 1971). It is composed of the trypsin resistant α-protein and the trypsin sensitive β-protein (Bevanger, 1985). GBS can express either α-C protein, β-C protein, or both. In addition to α-C and β-C protein, the major surface-localized proteins include the R proteins (R1, R3 and R4) (Bevanger *et al.*, 1995; Smith *et al.*, 2004), the alpha like proteins, Alp2 and Alp3 which are variants of the R1 protein and the epsilon (ε) protein which has also been known as Alp1 (Maeland *et al.*, 2004).

The Rib, α-C, Alp2, Alp3 and the epsilon/Alp1 protein are characterized by their property of generating ladder-like patterns on western blots (Wastfelt *et al.*, 1996). The patterns are due to identical repeat units which may vary in number from one strain to other (Flores and Ferrieri, 1989; Michel *et al.*, 1992; Wastfelt *et al.*, 1996). The number of repeats inside the alpha-C proteins (ACP) can undergo internal deletions to avoid the host immune response (Madoff *et al.*, 1996).These proteins are encoded by stable mosaic genes. The *bca, ε/alp1,*

bac, rib, alp2 and *alp3* genes encode for α-C protein, epsilon/Alp1, β-C protein, Rib, Alp2 and Alp3 respectively (Creti *et al.,* 2004). The possibility that these genes are part of mobile elements cannot be excluded because a surface protein, R28, highly homologous to Alp3 is present in some *Streptococcus pyogenes* strains (Lindhal *et al.,* 2005) and a new Alp variant in a clinical *Streptococcus dysgalactiae* subsp. *equisimilis* isolate has been found previously (Creti *et al.,* 2007).

Associations between molecular serotypes and protein antigens may indicate specific evolutionary lineages and indicate immune selection has favored strains with specific combinations of protein antigens because it improves their fitness (Gupta *et al.,* 1996). Accordingly, relationships between surface protein and serotype were noted (Lachenauer *et al.,* 2000; Kong *et al.,* 2002b). The association of serotypes Ia, Ib and II with the alpha protein; serotype III with Rib and serotypes V and VIII with Alp3 was reported, (Ramaswamy *et al.,* 2006b) but these relationship does not exist all time. For instance, the alpha protein was present in most serotypes, Rib was detected in serotypes II, V, VIII and in non typeable strains and Alp3 was also detected in serotype VII (Creti *et al.*,2004). The Alp2 protein was detected for the first time in serotype V (Lachenauer *et al.,* 2000) and followed by serotypes Ia, III and III (Kong *et al.,* 2002a). Epsilon protein is evenly distributed over several GBS serotypes both from human and bovine origin (Creti *et al.,* 2004) (shown in Table 1.1).

Table 1.1. Surface variable of protein of GBS and encoding genes (Adapted from Bergseng, 2009).

Surface Protein	Alternate label	gene	Associated capsular polysaccharide
c protein			
alpha		*bca*	Ib
Alpha, epsilon variants	Alp 1	*epsilon/Alp1*	Ia
Beta protein		*bac*	Ib
R protein			
R1	Alp2	*alp2*	III
R1	Alp3, R28	*alp3*	V, VIII
R3	-	-	V
R4	Rib	*rib; r4*	III, V
R5	BPS	*sar5*	-

d. The Genome

The genome of GBS is circular chromosome of about 2,160,267 bp genome sequences which is predicted to contain about 2,175 genes as shown in Figure 1.3. The G+C content of the genome is about 35.71%. Untill now the complete genome sequences have been obtained for several GBS strains: NEM316 (Glaser *et al.*, 2002), 2603V/R (Tettelin *et al.*, 2002), A909 (Tettelin *et al.*, 2005), ED-NGS-1000 (Kropp *et al.*, 2014), CNCT 10/84 (Hooven *et al.*, 2014), GB00112 ST-17(Singh *et al.*, 2012). Moreover, complete genome sequencing of GBS serotype V strain 2 isolated from buccal cavity of canine (Harden *et al.*, 2016), GBS strain H002 type III (Wang *et al.*, 2015), type III, ST 283 strain SG-M1(Mehershahi *et al.*, 2015) were also reported.

Genomic sequence analyses have revealed a stable backbone and 11-14 interspersed islands. Comparison of the genome sequences of several GBS strains have identified a core genome of about 1800 genes shared by all GBS isolates, contributing for about 80% of any single

genome, plus a dispensable genome consisting of partially shared and strain-specific genes (Tettelin, *et al.*, 2005). In silico analyses, combined with comparative genome hybridization (CGH) experiments between the sequenced serotype V strain 2603 V/R and 19 GBS strains from several serotypes using whole-genome microarrays, revealed the existence of genetic heterogeneity among GBS strains (Tettelin *et al.*, 2002).

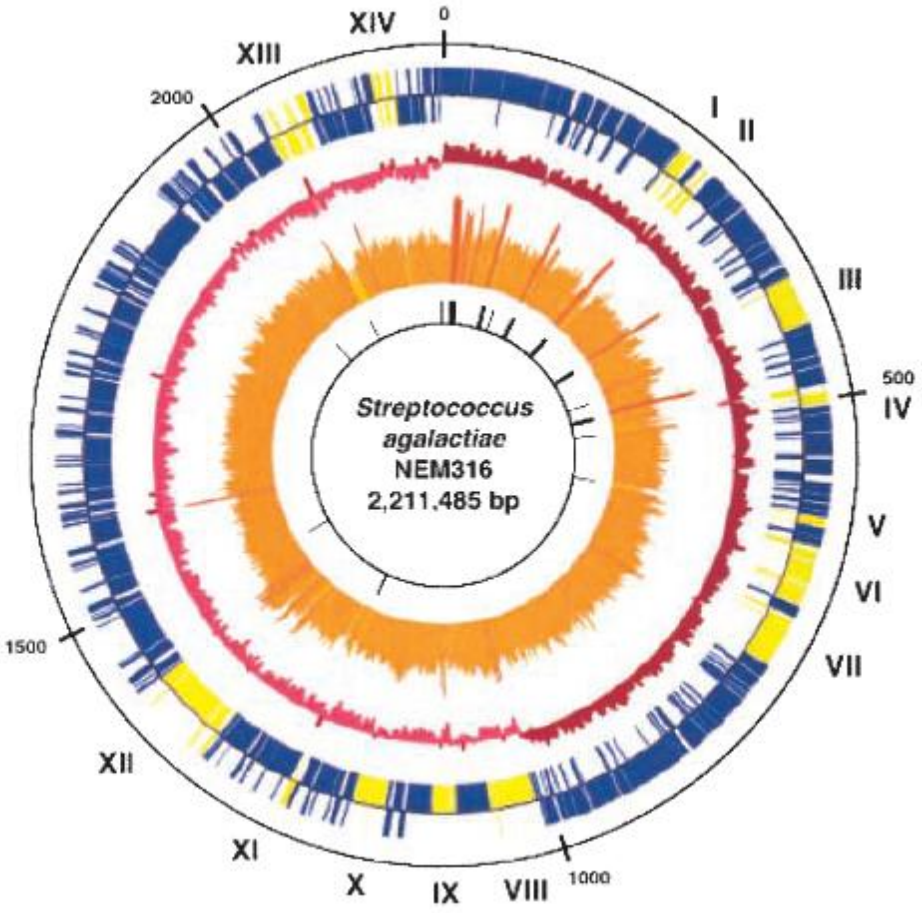


Figure 1.3. Circular genome map of *S. agalactiae* NEM316 showing the position and orientation of genes. From the outside: circle 1: protein coding genes on the + and - strands; genes in yellow belonging to the 14 islands numbered from I to XIV; circle 2: G/C bias (G + C/G -C); circle 3: G + C content with < 28.8% G + C in yellow, between 28.8% and 42.5% G + C in orange and with > 42.5% G + C in red; circle 4: stable RNA coding genes. The scale in kb is indicated on the outside of the genome with the predicted origin of replication being at position 0 (Adopted from Glaser *et al.*, 2002).

1.2.2. CHARACTERIZATION OF GROUP B STREPTOCOCCUS

To understand about the pathogenesis, epidemiology, transmission and possibility for vaccine development detail characterization of GBS is required. Several methods have been developed to characterize GBS at phenotypic and genotypic level. Phenotypic methods include capsular serotyping of GBS based on antisera and PCR and DNA based characterizations which include Pulsed field gel electrophoresis (PFGE), Multilocus Sequence Typing (MLST), Restriction Fragment Length Polymorphism (RFLP), Restriction Digest Pattern (RDP) analysis, Multilocus Enzyme Electrophoresis (MEE).

a. Capsular Polysaccharide Based Typing Using Antisera and PCR

Capsule production by GBS can be detected by using latex agglutination test (Slotved *et al.,* 2003), antigen extraction and precipitation reactions with adsorbed whole-cell antisera introduced by Lancefield in 1934 (Lancefield, 1933; Paoletti *et al.*, 1999a,b). It can also be detected by using FAT as described by Bevanger and Maeland (1977).

Genotypic methods complement phenotypic approaches and reduce problem of unreliable capsule expression, NT phenotypes and new antigenic variants. To identify capsular types of GBS, several researchers developed a protocol to detect gene coding for capsule. The *cps* cluster comprises genes *cpsA*-O, *cps*-R, *cps*-S *and cps*-Y (Chaffin *et al.,* 2000; Slotved *et al.,* 2003; Cieslewicz *et al.,* 2005), most of which are conserved across serotypes (Cieslewicz *et al.,* 2005). *Cps* G-K is highly variable in serotypes Ia, Ib, and II to VII, whereas *cps* E to K are variable in serotype VIII (Cieslewicz *et al.,* 2005) (Figure 1.4). Genetic analysis identified differences in *cps*-M, *cps*-O and *cps*-I gene sequences as responsible for the differentiation between the V, VII and IX capsular polysaccharide types, leading to hypothesis that type V emerged from a recombination event in a type IX background (Berti *et al.,* 2014).

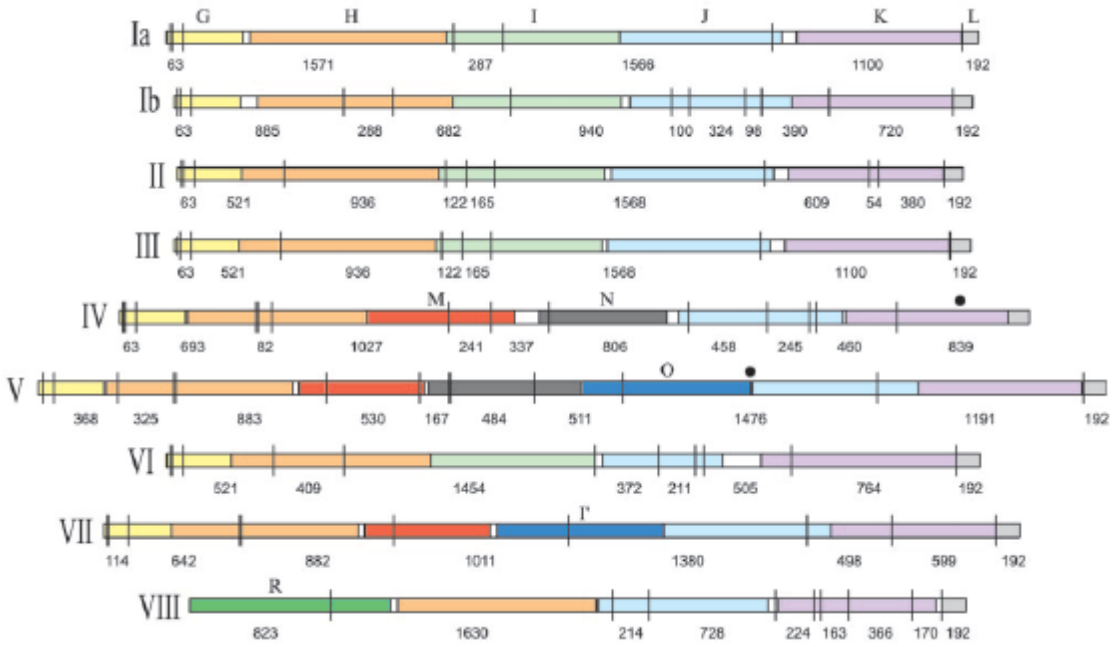


Figure 1.4. Diagram representing the serotype-specific variable region within the GBS *cps* gene cluster amplified by PCR. Homologous *cps* genes are illustrated by colors. The vertical lines and numbers represent the DdeI restriction sites and the expected restriction fragment sizes, respectively. Solid circles mark fragments affected in allelic variant (adopted from Manning *et al.*, 2005).

Several genotypic methods to characterize GBS have been described for capsular typing of GBS by molecular method. Most of the techniques are relatively easy to perform in the routine laboratory and all involve the combination of two different techniques sucha as; PCR plus sequencing, PCR plus hybridization and PCR plus enzymatic restriction (Table 1.2).

Table 1.2. CPS type-specific primers and prediction of PCR products by computer simulation (Poyart *et al.*, 2007).

Primer name	Sequence'5 to 3'	Gene target(s)	Amplicon size(s) (bp)	Gen Bank accession no. of targeted operon
Ia-F	GGTCAGACTGGATTAATGGTATGC	cps1aH	521and 1,826	AB028896
Ia-R	GTAGAAATAGCCTATATACGTTGAATGC			
Ib-F	TAAACGAGAATGGAATATCACAAACC	cps1bJ	770	AB050723
Ib-R	GAATTAACTTCAATCCCTAAACAATATCG	cpsIbK		
II-F	GCTTCAGTAAGTATTGTAAGACGATAG	*cps*2K	397	AY375362
II-R	TTCTCTAGGAAATCAAATAATTCTATAGGG			
III-F	TCCGTACTACAACAGACTCATCC	*cps*1a/2/3I	1,826	AF163833
III-R	AGTAACCGTCCATACATTCTATAAGC	*cps*1a/2/3J		
IV-F	GGTGGTAATCCTAAGAGTGAACTGT	*cps*4N	578	AF355776
IV-R	CCTCCCCAATTTCGTCCATAATGGT			
V-F	GAGGCCAATCAGTTGCACGTAA	*cps*5O	701	AF349539
V-R	AACCTTCTCCTTCACACTAATCCT			
VI-F	GGACTTGAGATGGCAGAAGGTGAA	*cps*6I	487	AF337958
VI-R	CTGTCGGACTATCCTGATGAATCTC			
VII-F	CCTGGAGAGAACAATGTCCAGAT	*cps*7M	371	AY376403
VII-R	GCTGGTCGTGATTTCTACACA			
VIII-F	AGGTCAACCACTATATAGCGA	*cps*8J	282	AY375363
VIII-R	TCTTCAAATTCCGCTGACTT			
dltS-F	AGGAATACCAGGCGATGAACCGAT	*dltS*	952	AL766853
dltS-R	TGCTCTAATTCTCCCCTTATGGC			

Molecular methods for serotyping such as PCR and sequencing of serotype-specific gene fragments within the *cps genes* (Kong *et al.,* 2002a; Cieslewicz *et al.,* 2005), DNA dot blot hybridization (DNA-DBH) (Borchardt *et al.,* 2004) and PCR-based RFLP analyses (Sellin *et al*, 2000), utilize genetic polymorphisms in the *cps* gene cluster to group GBS strains into the corresponding serotypes (Figure 1.4).

Manning *et al.* (2005) evaluated a new PCR-based method that utilizes RFLP of the *cps* cluster to detect DNA polymorphisms in the *cps* cluster. Kong *et al.* (2005) devised PCR/sequencing and Reverse line dot blot (RLB) assays which can be used to identify GBS serotypes. Borchardt *et al.* (2004) also developed typing methods that use DNA-DBH with probe generated by PCR from GBS capsular genes for serotype Ia, Ib, II-VII. Poyart *et al.* (2007) developed a method of capsular typing based on two set multiplex PCR and electrophoresis. Imperi *et al.* (2010) developed one round multiplex PCR electrophoresis for detection of 10 serotypes of GBS. Moreover, Creti *et al.* (2004) developed a multiplex PCR that allows determination of the following GBS surface protein genes directly by the analysis of the amplicon size: ACP, epsilon protein, Rib, Alp2/3, and Alp4.

The two set multiplex PCR developed by Poyart *et al.* (2007) (Primer is shown in Table 1.2) was published before the discovery of serotype IX (Slotved *et al.,* 2007) and serotype IX strains were found to yield a PCR product of the same size as those of serotype VII strains in this test (Yao *et al.,* 2013). In the one-set multiplex PCR developed by Imperi *et al.* (2010), the middle band in six and two strains of serotypes Ib and IV, respectively was very weak or not detectable and therefore, strains of these serotypes may be incorrectly typed as serotype Ia, which is characterized by the lack of the middle band (Figure 1.5).

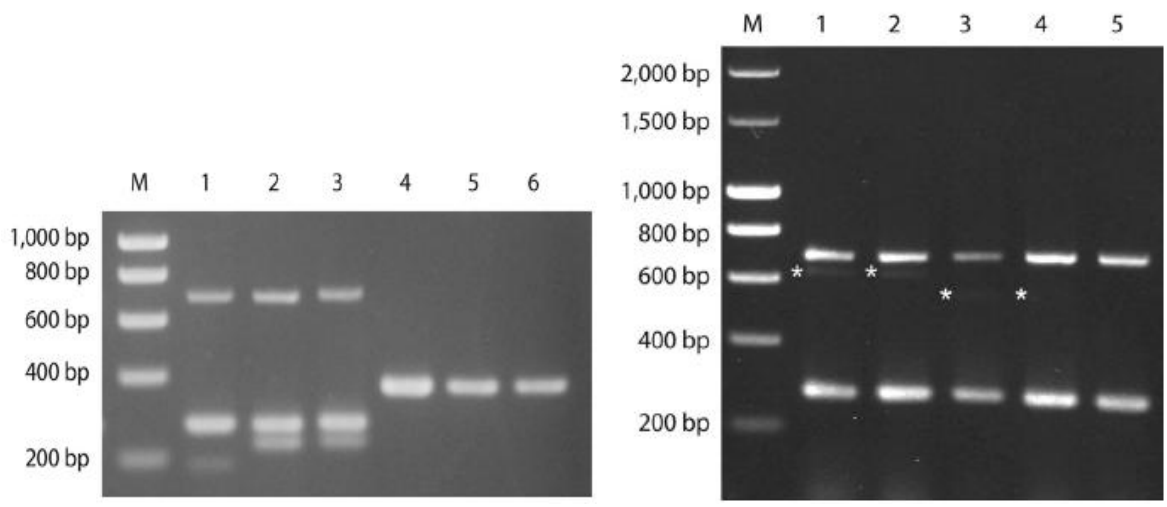


Figure 1.5. Right, serotype IX identified as VII in two set mPCR; Lane 1 to 3 amplicon from mPCR as described by Imperi *et al.* (2010); Lane 4 to 6 amplicon from mPCR 2 as described by Poyart *et al.*(2007); Lane 1 and 4 is control for strain VII; Lane 2 and 5 is control for IX. Left, week band in mPCR performed as described by Imperi *et al.* lane 1 is serotype Ib, lane 2 is serotype Ib, lane 3 is IV, lane 4 is IV, lane 5 is type Ia. (adopted from Yao *et al.*, 2013).

The serotype-specific PCRs described by Kong *et al.* (2002, 2005) which are used to confirm the results of the two multiplex PCRs described above do not include a PCR specific for serotypes II, VII, or VIII.

b. DNA Based Typing

Capsular serotyping along with protein profile has been commonly used to study the epidemiology of GBS. Diverse lineages of GBS serotypes can be distinguished with MLST (Jones *et al.*, 2003); MEE (Quentin *et al.*, 1995; Hauge *et al.*, 1996); PFGE (Rolland *et al.*, 1999) and RDP analysis (Quentin *et al.*, 1995). The lineages of GBS strains can vary in pathogenic potential with sequence type-17 (ST-17) being highly pathogenic type.

Pulsed Field Gel Electrophoresis (PFGE) is a gel-based analysis and dependent on digestion with smaI and visual interpretation. Unlike PFGE, MLST is an unambiguous genotyping method and sequence data are transferable between laboratories and can be used to build population structure of bacteria. MLST utilizes sequences of 450-500 base pairs from

fragments of seven housekeeping genes that encode central metabolic enzymes (Maiden *et al.,* 1998; Turner and Feil, 2007). For each housekeeping gene, the nucleotide sequences can vary. The variants of the genes are assigned as alleles and the combination of seven alleles at each of the seven loci represent an allelic profile which is defined as the sequence type (ST) of each isolate.

In MLST, the number of nucleotide differences between alleles is ignored and sequences are given different allele numbers whether they differ at a single nucleotide site or at many sites. Weighting according to the number of nucleotide differences between alleles would imply that the latter allele was more distantly related to the original allele than the former which would be only true if all nucleotide changes occurred by mutation but this is not true if the changes occurred by a recombinational replacement (Maiden *et al.,* 1998; Maiden, 2006). Nucleotide sequences of alleles and STs are available at http://pubmlst.org/sagalactiae and up to now 1169 unique STs are registered in the GBS online database.

There are many ways that can be used to analyze genetic relationship between isolates typed by MLST. The two commonly used are: the first method determines relationships on the basis of allele designations and STs (e.g. eBURST). The second approaches analyze nucleotide sequences directly (UPGMA and Neighbor joining method). UPGMA and Neighbor Joining methods are methods for the construction of phylogenetic trees by converting aligned sequences into a distance matrix of pairwise differences between the sequences. UPGMA constructs clusters by assuming that all sequences are equally distant from a root, which is unlikely. The Neighbor Joining method is based on a distance matrix similar to UPGMA but it calculates distances directly to internal nodes (Hall and Sinauer, 2004). The different STs at the ends of the branches are called external nodes. The lengths of the branches between the nodes illustrate the differences in nucleotide sequences between the STs. Thus, the tree illustrates phylogenetic relationships between the sequence types.

The eBURST analysis focuses on identifying groups of closely related isolates, clonal complexes (CC) within a bacterial population which is assumed to share a recent common ancestor and on exploring how these may have emerged and diversified (Feil *et al.,* 2004). eBURST divides a MLST data set of any size into groups of related isolates and clonal

complexes, predicts the genotype of each clonal complex and computes the bootstrap support for the assignment (Feil *et al.*, 2004). Bootstrap is a way of estimating the reliability of the model by finding the sampling distribution from one sample (Hall and Sinauer, 2004).

Isolates that have common alleles at six of seven loci and only differ from each other at one allele are designated single-locus variants (SLVs). Isolates with five out of seven shared alleles are called double-locus variants (DLVs). CCs are composed of a founder, which is defined as the ST that has the greatest number SLVs. If two STs have the same number of associated SLVs, the one with the greatest number of DLVs is selected as the founding ST (Hall and Sinaue, 2004).

Haguenoer *et al,* (2011) described a genotyping method based on multiple locus variable number of tandem repeat (VNTR) analysis (MLVA) applied to a population of GBS strains of various origins characterized by MLST and serotyping. The MLVA scheme generated clusters which corresponded well to the main clonal complexes obtained by MLST. It also provided a higher discriminatory power. The diversity index obtained with MLVA was 0.960 compared to 0.881 with MLST for a given population of GBS strains.

In addition, Nitschke *et al*. (2014) as part of better typing of GBS developed a microarray method targeting GBS virulence associated markers and resistance genes. They validated the method with reference strains and clinical isolates. For array-based typing, a set of 11 markers: *bac, alp, pil1 locus, pepS8, fbsB, capsule locus, hylB, abiG-I/-II plus Q8DZ34, pil2 locus, nss plus srr plus rogB2, and rgfC/A/D/B* was defined that provides a framework for splitting GBS strains that clustered according to MLST-defined clonal complexes. Microarray based typing of GBS along with MLST is expected to provide a better way of typing of GBS. Recently, Lier *et al.* (2015) also studied type II-A CRISPR-cas loci, which has been commonly used in *M. tuberculosis,* from human isolates of GBS belonging to different clonal complexes that represent the diversity of the species for the epidemiological typing of GBS. They found distinctive features according to the genetic lineage.

1.2.3. PATHOGENESIS OF GROUP B STREPTOCOCCUS

Group B Streptococcus (GBS) is recognized to be the leading cause of EOD and LOD worldwide, it is important to study the interaction between GBS and human host to come up with useful information about GBS pathogenesis and shed light on molecular targets for therapeutic intervention and vaccine development.

To cause disease, GBS has developed several mechanisms for successful colonization of the vaginal epithelium, penetration of placental or epithelial barriers, resistance to innate and adaptive immune response and the ability to breach the endothelial blood brain barrier (BBB). GBS are able to produce many different surface associated and secreted virulence factors which can be used for specific host-cell interactions and interfere with innate immune clearance mechanisms (Maisey *et al.,* 2008a,b).

a. Attachment to Epithelial Cells

Neonatal disease caused by GBS first begins with mucosal colonization of the maternal genito-urinary tract (Campbell *et al.,* 2000). GBS attach to vaginal epithelial cells at low pH with the low avidity interactions of cell wall associated lipoteichoic acid (LTA) and with high affinity interactions mediated by hydrophobic GBS surface proteins. The attachment of GBS to the host cell is facilitated by extracellular matrix (ECM) which includes fibronectin, fibrinogen and laminin (Nizet and Rubens, 2000).

A cell-surface protein of GBS, ScpB has ability to cleave C5a and fibronectin binding (Beckmann *et al.,* 2002; Cheng, 2002). GBS adherence to laminin is mediated by the adhesin, Lmb (Spellerberg *et al.,* 1999; Rajagopal, 2009) (Table 1.4); attachment to fibrinogen is mediated by repetitive motifs within the surface-anchored protein FbsA (Schubert *et al.,* 2004); and the serine-rich repeat domain protein (Srr-1) binds human keratin 4 (Samen *et al.,* 2007). In addition, GBS surface protein LrrG binds to epithelial cells, suggesting its role in facilitating attachment (Seepersaud *et al.,* 2005).

Group B Streptococcus (GBS) are revealed to express pili, which facilitate host-cell attachment and colonization (Lauer *et al.,* 2005). Among eight sequenced GBS genomes, two genetic loci encoding pili were found: PI-1 and PI-2. The second existing in one of two forms: PI-2a and PI-2b (Rosini *et al.,* 2006). GBS pilus island 2 includes the genes encoding

PilB, a LP(x)TG-motif-containing protein that polymerizes to form a pilus backbone and accessory pilus proteins PilA and PilC (Dramsi *et al.,* 2006; Maisey *et al.,* 2007). Epithelial cell adherence was reduced in isogenic GBS mutants lacking PilA or PilC but not those lacking PilB (Dramsi *et al.,* 2006; Krishnan *et al.,* 2007; Krishnan *et al.,* 2013) (Table 1.4). Component of GBS pili, PI-2a and PI-1 can attach to glycoprotein-340 assisting in oral colonization of GBS (Brittan and Nobbs, 2015; Rosini and Margarit, 2015a). Additionally, genes encoding putative surface proteins and in particular an antigen I/II have been identified on Integrative and Conjugative Elements (ICEs) found in GBS genome. Antigens I/II are multimodal adhesins promoting colonization of the oral cavity by *Streptococci* such as *Streptococcus gordonii* and *Streptococcus mutan* (Chuzeville *et al.,* 2015).

Adhesion factors also promote invasion either by disruption of the epithelial cell layer or by modulation of the epithelial cytoskeleton and the junctional protein assembly, which then allows for paracellular translocation (Landwehr-Kenzel and Henneke, 2014).

After attachment to cells, GBS can use secreted toxins or cell-surface virulence factors to facilitate bacterial entry to the host cells. They can also facilitate invasion by using the ECM and host cellular signal transduction pathways mechanisms. Entry of GBS into epithelial cells provides GBS with an intracellular niche, result in breakdown of host tissue integrity and induce inflammation (Nizet and Rubens, 2000). Infection of the placenta can promote ascending infection, whereas invasion of pulmonary epithelium and endothelium promote systemic dissemination (Melin, 2011).

The surface-anchored GBS–epithelial-cell adhesin, FbsB binds fibrinogen via its N-terminal domain (Schubert *et al.,* 2004), Lmb mediates ECM adherence (Spellerberg *et al.,* 1999; Tenenbaum *et al..,* 2007) and ScpB interacts with fibronectin (Cheng *et al.,* 2002). In addition to attachment, the above factors are able to promote efficient epithelial or endothelial cell invasion. Another GBS surface protein, Spb1 play a specific role in serotype III GBS invasion of epithelial cells (Adderson *et al.,* 2003). In addition, the surface-anchored ACP is known to mediate GBS invasion of human cervical epithelial cells (Li *et al.,* 1997; Bolduc *et al.,* 2002). ACP specifically interacts with host cell glycosaminoglycan

(GAG) on the epithelial cell surface to promote bacterial internalization (Baron *et al.,* 2004; Baron *et al.,* 2007; Bolduc and Madoff, 2007).

Group B Streptococcus (GBS) secrete hyaluronate lyase, which can degrade ECM component that is abundant in placental tissues (Lin *et al.,* 1994). In response to tissue injury, hyaluronan (HA) polymers are cleaved by host hyaluronidases, generating small fragments that ligate Toll-like receptors (TLRs) to elicit inflammatory responses. GBS secrete hyaluronidases as a mechanism for tissue invasion, but it is not known how this activity relates to immune detection of HA. Kolar *et al.* (2015) reported that bacterial hyaluronidases secreted by GBS degrade pro-inflammatory HA fragments to their component disaccharides. In addition, HA disaccharides block TLR2/4 signaling elicited by both host-derived HA fragments and other TLR2/4 ligands, including lipopolysaccharide.

Studies by electron microcopy demonstrated that host cytoskeletal changes are triggered by GBS, which lead to endocytotic uptake of the bacteria in to the cell (Nizet *et al.,* 1997; Valentin-Weigand *et al.,* 1997).The intracellular uptake of GBS involves activation of cytoskeletal rearrangements in the target cell. Rho family GTPases are known to be modified by pathogenic bacteria at the cell surface to trigger downstream regulation of actin polymerization and rearrangement (Dumenil and Nassif, 2005). GBS infection of epithelial cells increases activated levels of Rho family members RhoA, Rac1 and Cdc42. Penetration of GBS in to the cell can be inhibited by dominant-negative expression of these proteins and by Rho family GTPase inhibitors (Burnham *et al.,* 2007a).

Another host signal transduction pathway involved in GBS uptake involves phosphoinositide-3 kinase (PI3K)/Akt. It is a lipid kinase that catalyses the recruitment, phosphorylation and activation of the intracellular effector Akt, which then triggers downstream signalling to modulate cytoskeletal activities. Akt phosphorylation is demonstrated in the epithelial cell response to GBS infection and chemical inhibition of PI3K or Akt and genetic inactivation of PI3K results in reduced GBS invasion (Burnham *et al.,* 2007b). Moreover, Pooja *et al.* (2015) data suggest that H9C2 cells internalized GBS through energy–dependent endocytic processes and the LTA of GBS play important role in host cell internalization and cytotoxicity induction.

Even though cellular invasion has principal role in bloodstream penetration for LOD-GBS, extensive lung epithelial and endothelial destruction may be evident in severe EOD-GBS**.** Damage to cell is caused by the actions of the GBS β-haemolysin/cytolysin, a pore-forming toxin that lyses lung epithelial and endothelial cells and compromises their barrier function (Nizet *et al.,* 1996; Gibson *et al.,* 1999). GBS β-haemolysin/cytolysin promotes lung epithelial cell invasion and triggers release of interleukin-8 (IL-8), a principal neutrophil chemoattractant (Doran *et al.,* 2002; Hensler *et al.,* 2005). The cytolytic, pro-invasive and pro-inflammatory effects of the GBS β-haemolysin/cytolysin are neutralized by dipalmotyl phosphatidylcholine (DPPC), surfactant-deficient in neonates (Nizet *et al.,* 1996). On the other hand, Six *et al*. (2016) studied non hemolytic and non pigmented GBS strain originated from invasive infection and colonization. They found a mutation localized predominantly in the *cyl* operon, encoding the β-haemolysin/cytolysin pigment biosynthetic pathway and, in the *abx1* gene, encoding a CovSR regulator partner. This may indicate β-haemolysin/cytolysin hemolysin and pigment production is not absolutely required to cause human invasive infections by GBS.

The glycolytic enzyme glyceraldehyde-3- phosphate dehydrogenase (GAPDH) has been implicated as virulence for a number of bacterial pathogens including group A Streptococcus (GAS), through binding and activation of host plasminogen (Terao *et al.,* 2006). GAS acquisition of surface plasmin activity promotes host invasion and systemic spread (Cole *et al.,* 2006). GBS GAPDH shares homology with GAS GAPDH and it is expressed on the cell surface. GBS can bind lysine residues of host plasminogen via GAPDH, activate the bound pro-enzyme to plasmin, and gain the ability to degrade host matrix proteins such as fibronectin (Seifert *et al.,* 2003; Magalhaes *et al.,* 2007).

In addition to penetration of host cell barriers by intracellular invasion or direct damage to cells and extracellular matrix, GBS can also cross cell monolayers through paracellular route. GBS have been shown to associate with junctional protein complexes in electron microscopic studies. The GBS strain expressing a GAG-binding-deficient ACP variant could not invade cervical epithelial cells, but could still accomplish transcytosis (Baron *et al.,* 2007).

Table 1.3. GBS virulence factors and their role in transition from colonization to invasive disease (Adapted from Landwehr-kenzel and Henneke, 2014).

Virulence factors	Colonization	Adhesion	Invasion	Immune evasion	Neurotropism
FbsA	+	+			
FbsB			+		
Lmb			+		+
BsaB	+	+	(+)		
ACP	+	+	+	+	
Srr	+	+	+		
Pili	+	+	+	+	+
HvgA	+	+	+	(+)	+
ß-H/C	+	+	+	+	+
CPS				+	
ScpB				+	
BibA				+	
Factor H				+	
IgA-binding β-antigen				+	
D-alanylation				+	
SodA				+	

FbsA: fibrinogen-binding protein A; FbsB: fibrinogen-binding protein B; Lmb: Laminin-binding proteins; BsaB: GBS surface adhesion; ACP: Alpha C proteins; Srr: Serine rich repeat proteins; HvgA: Hypervirulent GBS adhesiin; β-H/C: ß-hemolysin/cytolysin; CPS: Capsular polysaccharides; ScpB: Streptococcal C5a peptidase of GBS; BibA: GBS immunogenic bacteria adhesion; SodA: Superoxide dismutase

In a recent study which involved total of 194 GBS strains, 55 isolates from bovines and 139 from humans the *scpB, hly, bca* and *bac* virulence genes were only found among human isolates (Emaneini *et al.*, 2016). Moreover, GBS strains differ in their abilities to attach to distinct host cell types and express key virulence genes that are relevant to the disease process (Korir *et al.*, 2014).

b. Evasion of Innate Immune Response

After penetration of the lung tissue or bloodstream of the newborn by GBS immunological response is initiated. Phagocytosis of GBS by neutrophils and macrophages requires opsonization of the GBS by specific antibodies or complement (Maruvada *et al.,* 2008). Newborn are susceptible to GBS disease because of the deficiencies in phagocytic cell function, specific antibody and complement. In addition to deficiency in immunity, GBS has several virulence factors that help to overcome or evade the key components of host immune system (Campbell *et al.,* 1991; Marques *et al.,* 1992).

The CPS of GBS is important for limiting the effectiveness of host complement defense. Capsule can allow the organism to survive within the host by covering antigenic determinants associated with the bacterial surface, by mimicking host antigens, or by interfering with complement mediated killing (Cieslewicz *et al.,* 2005). The serotype-specific epitopes of ten known GBS are created by different arrangements of four monosaccharides into unique repeating units, all of which contain a terminal sialic acid bound to galactose in an α2-3 linkage (Jennings *et al.,* 1983; Wessels *et al.,* 1987; Wessels *et al.,* 1989; Kogan *et al.,* 1995; Kogan *et al.,*1996; Slotved *et al.,* 2007).

The terminally attached sialic acid molecule provides antiphagocytic protection by limiting surface deposition of opsonically active complement C3 on the bacterial surface (Campbell *et al.,* 1991; Marques *et al.,* 1992). Lewis *et al.* (2004) found that some of the Neu5Ac residues of the GBS type III capsule are O-acetylated at carbon position 7, 8, or 9, a major modification evidently missed in previous studies. O-acetylation often generates immunogenic epitopes on bacterial capsular polysaccharides and can modulate human alternate pathway complement activation. The evolution by a pathogen of antigenically distinct capsular types is thought to be driven by selective pressure imposed by host immunity (Lipsitch, 1999). However, selective pressure to evolve new capsular types may be counterbalanced by a specific virulence benefit conferred by the conservation of a particular polysaccharide structure. However, the finding of more than one genetic lineage among serotype III GBS strains, ST-17 and ST-19 is indicative that the type III capsule of GBS is not associated with pathogenicity, which requires other factors (Jones *et al.,* 2006b).

Additionally, bacterial persistence, immune evasion and pathogenesis often involve biofilm formation which is enhanced in the presence of human plasma (Di Xia *et al.,* 2015). Analysis of mutants impaired for various surface components revealed that the GBS capsule is a key component in biofilm formation (Di Xia *et al.,* 2015). Di Xia *et al.* (2015) demonstrated GBS's ability to from biofilm, which is mediated by the PI-2a pilus, in medium supplemented with glucose and cell culture medium supplemented with human plasma.

Another cell-surface GBS immunogenic bacterial adhesin (BibA) was described by Santi *et al.* (2007). BibA binds human C3bp, a component of the classical complement pathway, promotes resistance to phagocytic killing, mediates adherence to epithelial cells and contributes to virulence in a mouse model of infection. GBS β-protein was shown to prevent opsonophagocytosis by binding factor H and enabling the unbound active region to block C3b deposition on the bacterial cell surface (Jarva *et al.,* 2004). GBS interference with C3b deposition is hypothesized to result from recruitment of endogenous factor H by Neu5Ac residues. The β antigen of C protein binds human IgA antibody and IgA deposited nonspecifically on the bacterial surface inhibits interactions with complement (Jerlstrom *et al.,* 1991).

Cell-surface protease, CspA, targets host fibrinogen and produce adherent fibrin-like cleavage products that coat the bacterial surface and interfere with complement-mediated opsonophagocytic clearance (Harris *et al.,* 2003). GAPDH dependent induction of IL-10 and β-protein binding to the inhibitory phagocyte receptors sialic acid binding immunoglobulin like lectin 5 and 14 down regulate the immune response. On the host side, sensing of GBS nucleic acids and lipopeptides by both Toll-like receptors and the inflammasome appears to be critical for host resistance against GBS (Landwehr-Kenzel and Henneke, 2014).

Once inside the cell, GBS in the phagosome will encounter the rapid release of toxic reactive oxygen species (ROS) produced in the oxidative burst. GBS is able to protect itself from ROS and survive inside macrophage phagolysosomes (Wilson and Weaver, 1985; Cornacchione *et al.,* 1998; Teixeira *et al*., 2001). Moreover, GBS has oxygen-metabolite scavenger glutathione (Wilson and Weaver, 1985) and the GBS super oxidase dismutase

(SodA) enzyme can neutralize superoxide anions (Poyart *et al.*, 2001b). GBS also produce an orange carotenoid pigment (Spellerberg *et al.,* 2000), which can neutralize hydrogen peroxide, superoxide, hypochlorite and singlet oxygen, and as a result provide protection against several elements of phagocytic ROS killing (Liu *et al.,* 2004).

Group B Streptococcus (GBS) can incorporate positively charged D-alanine to their cell-wall teichoic acids to increase resistance to Antimicrobial peptides (AMPs) (Poyart *et al.*, 2001a). A surface-anchored penicillin-binding protein, PBP1a, enhances GBS resistance to AMPs by reducing GBS susceptibility to killing by alveolar macrophages and neutrophils (Hamilton *et al.,* 2006). Expression of the pilus backbone protein PilB makes GBS more resistant to killing by AMPs and is associated with increased phagocyte resistance and systemic virulence (Maisey *et al.,* 2008a) (Table 1.4).

On the other hand, GBS can also induce phagocytic apoptosis to avoid phagocytic clearance. Unlike some cell-death ligands, macrophage apoptosis triggered by GBS requires caspase-3 activation and utilizes unique changes in regulation and localization of Bcl-2 family members (Ulett *et al.,* 2005). GBS-induced macrophage apoptosis can also progress independently of caspases (Carlin *et al.,* 2007; Fettucciari *et al.,* 2015). Costa *et al.* (2016) reported oxidative stress is one of the cellular events that lead to apoptosis during GBS human invasive infections.

Recently GBS was found to secret a novel protein named as complement interfering protein (CIP) (Pietrocola *et al.,* 2016). This protein down regulates complement activation via the classical and lectin pathways but not the alternative pathway. The CIP protein showed high affinity toward C4b and inhibited its interaction with C2, presumably preventing the formation of the C4bC2a convertase (Pietrocola *et al.,* 2016).

c. Activation of Inflammation

One of the main factors for GBS to cause septicemia is inability of the host immune system to control the bacteria. IL-1 is an early produced cytokine which plays a key role in the deleterious cytokine cascade of septic shock (Vallette *et al.,* 1995). On the other hand, IL-12, which is produced lately, has an important role in regulating the systemic response to GBS infection (Mancuso *et al.,* 1997; Lione *et al.,* 2014).

Peptidoglycan of GBS is more effective than lipoteichoic acid and capsular polysaccharide as a stimulator of cytokine release from macrophages (Vallejo *et al.,* 1996). Cell wall peptidoglycan-induced activation of p38 and NF-kB depends upon the cytoplasmic TLR adaptor protein MyD88, but does not precede through the pattern recognition receptors TLR2 or TLR4 (Mancuso *et al.,* 2004). GBS activation of TLR2 was shown to depend on surface expression of lipoproteins, which also has a significant role in the development of GBS sepsis (Henneke *et al.,* 2008).

The nitric oxide (NO) pathway has also been implicated in the overproduction of proinflammatory cytokines and initiation of cellular injury during GBS infection of lung tissue (Raykova *et al.,* 2003). The proinflammatory effects of the GBS β-haemolysin/cytolysin also contribute to sepsis pathophysiology. The toxin acts to stimulate iNOS and NO release in macrophages (Ring *et al.,* 2002; Puliti *et al.,* 2000; Gendrin *et al.,* 2015; Costa *et al.,* 2016).

d. Penetration of Blood and Brain Barrier

So as to cause meningitis, GBS should able to pass Blood Brain Barrier (BBB). The pathogenesis of GBS meningitis varies according to age of onset. In EOD-GBS, there is little evidence of leptomeningeal inflammation inspite of abundant bacteria, vascular thrombosis and parenchymal haemorrhage. Where as in infants with LOD-GBS, usually there is diffuse purulent arachnoiditis with involvement of the base of the brain (Simonsen *et al.,* 2014). The difference in histopathological observation in the above two cases may indicate underdevelopment of the host immunological response in the immediate neonatal period.

The BBB is comprised of specialized brain microvascular endothelial cells (BMECs) and together with astrocytes, pericytes, neurons and extracellular matrix constitute the neurovascular unit (NVU). The functions of BBB is to protect the brain from circulating microbial infection and toxins by maintaining tight intercellular junctions that comprise gap, adherens, and desmosomal junctions that link cells together and prevent pinocytosis. Penetration of the BBB by GBS may involve a complex interaction between the GBS cell surface factors and the endothelial cells of the BBB; however, the mechanism by which

GBS crosses the BBB and engages the NVU are not clearly revealed (Berman and Banker, 1966).

Analysis of GBS transposon mutant library for reduced BMEC invasion, hypo-invasive mutant was found to possess a disruption of a *iagA* gene which encodes an enzyme for biosynthesis of diglucosyldiacylglycerol, a membrane glycolipid that functions as an anchor for lipoteichoic acid (Doran *et al.,* 2005). In other study, GBS mutants lacking the GBS FbsA, Lmb, or PilB also showed decreased attachement or invasion of BMECs *in vitro* (Tenenbaum *et al.,* 2005; Maisey *et al.,* 2007; Tenenbaum *et al.,* 2007). A study conducted by Mu *et al.* (2014) suggested that GBS SfbA plays an important role in bacterial interaction with BBB endothelium and the pathogenesis of streptococcal meningitis.

Human BMEC invasion by GBS is accompanied by evidence of β-haemolysin/cytolysin-induced cellular injury (Nizet *et al.,* 1997; Doran *et al.,* 2003). GBS invasion of human BMECs can be blocked by inhibition of actin polymerization, suggesting that GBS trigger rearrangement of the host cytoskeleton and induce their own uptake (Nizet *et al.,* 1997). Moreover, GBS induction of host transcriptional repressor, Snail1, contributes to BBB disruption (Kim *et al.,* 2015). Tight junction components ZO-1, claudin 5, and occludin were decreased at both the transcript and protein levels in hBMECs following GBS infection, and this repression was dependent on Snail1induction. GBS induction of Snail1 expression was dependent on the ERK1/2/MAPK signaling cascade and bacterial cell wall components.

Hypervirulent ST-17 GBS clone, which possess Srr2, is strongly associated with invasive neonatal meningitis. Non-ST-17 isolates express Srr1 (Da Cunha *et al.,* 2014). Srr2, a cell wall-anchored protein, it can bind to plasminogen, plasmin, fibrinogen, and increase bacterial survival to phagocytic killing (Six *et al.,* 2015). By doing this, Srr2 hijack ligands of the host coagulation system, it contributes to bacterial dissemination and invasiveness and ultimately to meningitis. Tazi *et al.* (2010) showed important determinant of the pathophysiology of ST-17 associated LOD-GBS, the CC-17-specific surface-expressed protein HvgA, which is critical for GBS intestinal colonization and translocation across the BBB during the onset of meningitis (Tazi *et al.,* 2010). HvgA was exploited to develop real time PCR based assay for rapid detection of CC17 strain (Lamy *et al.,* 2006; Tazi *et al.,*

2010). Recently, HvgA encoding gene was detected among 3 GBS type IV indicating the shift of type of III to IV (Bellais *et al.,* 2012). Another protein, PbsP, is conserved among the main GBS lineages and is a major plasminogen adhesin in non-CC17 GBS strains (Buscetta *et al.,* 2016).

Human astrocytes encircle BMEC with their pseudopodia and maintain direct contact with cerebrospinal capillaries and play a unique role in GBS infection and contribute to the development of meningitis. Stoner *et al.* (2015) used a well characterized human fetal astrocyte cell line, SVG-A, and examined GBS infection *in vitro*. They observed that all GBS strains of representative clinically dominant serotypes (Ia, Ib, III, and V) were able to adhere to and invade astrocytes. Cellular invasion was dependent on host actin cytoskeleton rearrangements and was specific to GBS as *Streptococcus gordonii* failed to enter astrocytes.

The host inflammatory response to GBS contributes significantly to the pathogenesis of meningitis and CNS injury. The initiation of the inflammatory response is triggered by BBB endothelium, which activates a specific pattern of gene transcription for neutrophil recruitment, including production of chemokines, endothelial receptors and neutrophil activators (Doran *et al.*, 2003; Kim, 1995). GBS β-haemolysin/cytolysin induces IL-8 and ICAM-1, promoting neutrophil migration across polar BMEC monolayers, suggesting that the toxin is crucial to this particular manifestation of GBS CNS disease (Doran *et al.,* 2003). TNF-α production by astrocytes, microglia and infiltrating leukocytes also contribute to apoptosis of hippocampal neurons and further increases in BBB permeability (Bogdan *et al.,* 1997; Kim *et al.,* 1997). Moreover, GBS signal through TLR2 to activate and stimulate NO production by microglia cells, resulting in neuronal destruction (Lehnardt *et al.,* 2006).

1.2.4. GROUP B STREPTOCOCCUS DISEASE

1.2.4.1. GBS in Pregnant Women

The incidence of pregnancy associated GBS disease in USA before IAP was 0.29/1000 live births but it has declined to 0.11- 0.14/1000 after the introduction of IAP strategies (Lin *et al.,* 2004). Recently the incidence was reported to be 0.02/1000 among non pregnant women; 0.49/1000 in post-partum women (Deutscher *et al.,* 2011). Most of the GBS cases are associated with bacteremia, endometritis, chorioamnionitis, pneumonia and puerperal

sepsis as result of infection of the endometrium, placenta or amniotic sac (Nazer, 1981; Ipe *et al.,* 2016).

Colonization with GBS during pregnancy also increases the risk of spontaneous abortion, premature rupture of the fetal membranes, preterm labor and low neonatal birth weight (LBW) (El Beitune *et al.,* 2005; Petersen *et al.,* 2014). The serotypes causing maternal GBS disease are similar to those that cause EOD-GBS (Kunze *et al.,* 2011; Turner *et al.,* 2012; Kalin *et al.,* 2015).

1.2.4.2. GBS in Non-Pregnant Adults

Group B Streptococcus (GBS) has also been associated with high rates of invasive diseases such as skin and soft tissue infection among adults, especially in patients >65 years of age, black race and those with underlying medical conditions such as diabetes, cancer, cirrhosis and Human Immunodeficiency virus (HIV) infection (Nuccitelli *et al.*, 2015; Ranz, 1940). In addition, GBS is also indicated in severe infective endocarditis in a healthy adult (Fujita *et al.,* 2015), brachial plexus neuritis (Seo *et al.,* 2014), supraglottitis (Nagaraja *et al.,* 2015), large intra-abdominal abscess (Crum-Cianflone, 2015) and septicemia in patients with diabetes and hepatic cirrhosis (Batistaa and Ferreira, 2015). Mortality from GBS bacteremia among non pregnant adults remains high (Larppanichpoonphol and Watanakunakorn, 2001). Mortality rate for elderly adults is about 15% (CDC, 1999, 2002, 2004). Adults >65 years of age are at greater risk of dying from GBS disease compared with adolescents and adults 15–64 years of age (Schrag *et al.,* 2000).

Nursing home residents had a greater incidence of GBS infection than community-dwelling residents for all age groups (Henning *et al.,* 2001). The age-adjusted annual incidence of GBS infection was 72.3 cases per 100,000 population for nursing home residents and 17.5 cases per 100,000 populations for community dwelling residents (Edwards, 2005).

1.2.4.3. GBS in infants less than 90 days

The organisms most frequently involved in EOD of term and preterm infants are GBS and *Escherichia coli* which accounts for approximately 70% of infections combined (Simonsen *et al.*, 2014). Other infectious agents which are being reported from neonatal disease are; Viridians group streptococci and *Streptococcus pneumoniae* (Hoffman *et al.,* 2003),

Staphylococcus aureus, *Enterococcus* species, Gram-negative enteric bacilli such as *Enterobacter* species, non-typeable *Haemophilus* species and *Listeria monocytogenes* (Stoll *et al.,* 1996; Bizzarro *et al.,* 2005;Weston *et al.,* 2011). Gram negative rods are the most common etiologies of EOD among preterm and Very Low Birth Weight (VLBW) infants (Westen *et al.,* 2011).

Fungal pathogens such as *Candida* species are also indicated in neonatal disease (Klinger *et al.,* 2009; Bizzarro *et al.,* 2015). Among viruses, herpes simplex virus, enteroviruses and parechoviruses are some of them to be implicated in EOD (Simonsen *et al.,* 2014).

Historically GBS emerged as an important pathogen around 1960s and replaced *S. aureus* as the most common cause of neonatal sepsis (Hood *et al.,* 1961; Eickhoff *et al.,* 1964). Current epidemiological trends in developed countries are showing a decrease in the frequency of EOD-GBS related directly to prenatal screening and treatment with IAP (Puopolo and Eichenwald, 2010; Mischler *et al.,* 2015).

Group B Streptococcus (GBS) disease in infants is classified based on the time of onset of infection into early onset disease and late onset disease. EOD-GBS occurs within the first 7 days of life. Most affected infants with EOD-GBS develop signs and symptoms within the first day of life. More than 75% of GBS cases are early onset (Nandyal, 2008).

Early Onset Disease due to GBS (EOD-GBS) can be confirmed by serotyping GBS isolates from colonized mothers and their new born. GBS is transmitted vertically from colonized pregnant women to new born. Intrauterine infection of the fetus results from ascending spread of GBS from the vagina of a colonized woman. Aspiration of infected amniotic fluid by the fetus can lead to neonatal pneumonia, sepsis, and stillbirth. Infants can also become infected during passage through the birth canal, although the majority of infants who are exposed to the organism through this route become colonized on skin or mucous membranes but remain healthy (Schrag *et al.,* 2002).

The initial presentation of EOD-GBS is respiratory distress in more than 80 % of new born. Pneumonia and septicemia are the most common manifestations and 5 to 10 % new born will also have meningitis. The incidence of EOD-GBS is about 10 times higher in premature

than in term neonates (Schuchat, 1998; Shah *et al.,* 2001). The case-fatality ratio of EOD-GBS was as high as 50% in 1970s in USA and recently declined down to 4% – 6%, this is largely due to advancement in neonatal care. Mortality is higher among preterm infants, with case-fatality rates of approximately 20% even higher among those ≤33 weeks' of gestation compared with full-term infants (Verani *et al.,* 2010).

Late Onset Neonatal Disease due to GBS (LOD-GBS) develops in infants after 7 days and up to 3 months of age. Transmission can be either horizontal or vertical (Shet and Ferrier, 2004). Cases of GBS transmission via human milk were also reported (Shah *et al.,* 2001; Nandyal, 2008). The pathogenesis of LOD-GBS including its risk factors is not well understood. In some occasions acquisition of GBS during passage through the birth canal can occur. About 50% of mothers of infants with LOD were found to carry the same GBS serotype as that causing infection in their infants (Schuchat, 1998). Term and preterm infants are equally vulnerable to LOD-GBS. The role of maternal obstetric complications for LOD-GBS is rare. LOD-GBS usually presents with bacteremia and meningitis. Other clinical manifestations include osteomyelitis, septic arthritis, and cellulitis (Nandyal, 2008). Over 20% of survivors of GBS meningitis have permanent neurological sequelae (Dangor *et al.,* 2015a). Even though experience from USA revealed substantial reduction in incidence of EOD-GBS by administration of IAP the incidence of LOD-GBS remains the same (Nandyal, 2008). Cases occurring among infants 90 days after birth are also described, but rare and account for only 0.37–0.73 per 100,000 in USA (Phares *et al.,* 2008).

Between 15 and 35% of pregnant women are colonized by GBS in the vagina and/or rectum, yet the incidence of neonatal GBS disease is 1 to 2 infants per 1,000 births and there should be other factors which increase the chances that an infant will develop EOD-GBS. These factors include; previous delivery of infants with invasive GBS infection, GBS bacteruria, GBS colonization late in pregnancy, onset of labor or rupture of membrane in less than 37 weeks of gestation, prolonged rupture of membrane greater than or equal 18 hours and Intrapartum fever (Schuchat, 1998; Schrag *et al.,* 2002; Verani *et al.,* 2010).

1.2.5. RISK FACTORS FOR EOD-GBS

a. Maternal GBS Colonization

The primary risk factor of EOD-GBS is maternal colonization with GBS. Even though it is necessary, all maternal GBS colonization does not lead to EOD-GBS. There are factors which may facilitate vertical transmission of GBS from mother to newborn. Neonatal disease due to GBS has also been associated with transmission of GBS via infected breast milk (Le Doare and Kampmann, 2014). GBS is a common and normal inhabitant of the gastrointestinal and genitourinary tracts of child bearing women. Local hygiene or sexual practices can increase the risk for vaginal colonization. Vertical transmission of GBS from mother to fetus mostly occurs after the onset of labor or membrane rupture (Figure1.6). However, colonization early in pregnancy is not predictive of EOD-GBS as the colonization rate is variable during pregnancy (Shet and Ferrieri, 2004). About 50% of vaginally delivered infants from GBS culture positive mother will become colonized and from those colonized infants about 1% to 2% of them may develop GBS invasive diseases such as sepsis and meningitis (Maniatis *et al.*, 1996; Nandyal, 2008).

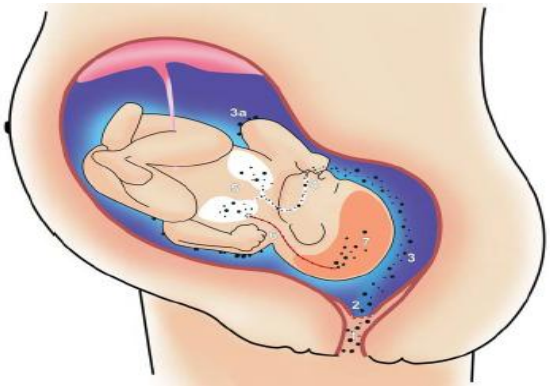


Figure 1.6. Hypothesized pathogenesis of GBS-EOD. Designed by Vincent Khouw (VMK designs) (adopted from Muller *et al.*, 2010) Numbers in this figure indicate ascending infection of GBS (1, 2 & 3) number 4, 5, 6 & 7 indicates infection of the fetus's lung, blood and CNS.

Maternal GBS colonization can be transient, intermittent or persistent; little is known about the host and bacterial factors controlling GBS persistence (Kwatra *et al.*, 2014). Studies

have identified an association between high maternal serotype-specific anti CPS antibody concentrations with reduced risk of recto-vaginal colonization and reduced risk of newborns developing EOD-GBS (Baker *et al.,* 1981). Patras *et al.* (2015a) used human cervical and vaginal epithelial cells and mouse model of GBS vaginal colonization to characterize key host factors that respond during GBS colonization. They identified GBS strain that persisted beyond a month in the murine vagina, whereas other strains are more readily cleared. They have detected differential cytokine production in human cell lines after challenge with the persistent strain vs. non persistent GBS strains. They have also demonstrated that the persistent strain more readily invades cervical cells compared with vaginal cells, suggesting that GBS may potentially use the cervix as a reservoir to establish long-term colonization.

Culture screening of both the vagina and rectum for GBS late in gestation during prenatal care can detect women who are likely to be colonized with GBS at the time of delivery and are thus at higher risk of perinatal transmission of the organism to the newborn (Regan *et al.,* 1991). Colonization with GBS varies throughout pregnancy, this creates problematic situation from a culture based screening perspective with results obtained at 35−37 weeks' of gestation correlating best with the culture status at birth (Kurz and Davis, 2015). The sensitivity and specificity in predicting colonization status at term is 87% and 96% respectively (CDC, 1996). Specimens collected from the anorectal region and vagina increased GBS isolation rate from 5% to 27% over vaginal cultures alone. The use of selective media increases the yield by as much as 50% (Kvam *et al.,* 2011). Studies conducted in the 1980 indicated that women with GBS colonization late in pregnancy were >25 times more likely than pregnant women with negative cultures to deliver infants with EOD-GBS (Baker and Kasper, 1976).

The source GBS in human is the gastrointestinal tract with the genitourinary tract being the major additional sites of colonization in women. GBS colonizes the genitourinary tract of 10-40% of all pregnant women. Colonization rates may vary among ethnic groups, geographic locations, age of pregnant women and sexual habits. Studies from other countries indicate significant geographic variation in the prevalence of GBS colonization among pregnant women (Shah *et al.,* 2001); higher colonization rate is reported from

Norway, 34.8% (Bergseng *et al.,* 2007) and lower colonization rate is reported from India, 2.5% (Kulkarni *et al.,* 2001) (Table 1.4). Specimen collection and microbiologic methods play an important role in identification of colonized women and contribute to observed colonization rates differences in several countries (Shah *et al,* 2001; Shet and Ferrier, 2004; Kvam *et al.,* 2011). The higher colonization rate may explain the higher risk of both EOD-GBS and LOD-GBS among African Americans, but whether socioeconomic factors and health care system also influence the risk of GBS disease is not clear. On the other hand, high colonization rates observed among Scandinavian women may challenge the hypothesis of more GBS disease in populations with high colonization rates (Regan *et al.,* 1991; Bergseng *et al.,* 2007; Hakansson *et al.,* 2008).

Although some women are colonized with GBS during pregnancy, they will have a chance to be colonized during their subsequent pregnancies where as a significant proportion will not be colonized (Cheng *et al.,* 2008; Turrentine and Ramirez, 2008). However, study by Colicchia *et al*. (2014) indicated GBS colonization in a prior pregnancy is an indication to be colonized in a subsequent pregnancy. Moreover, heavy colonization of pregnant women with GBS during delivery is associated with high risk for EOD-GBS (Regan *et al.,* 1996). There is scarce information regarding vaginal and rectal colonization with multiple serotypes of GBS, which may influence prevention strategy; in this regard Ferrieri *et al.* (2004) reported about 21.6% of pregnant women to be colonized with multiple GBS serotypes.

Kwatra *et al*. (2014) studied the association of GBS serotype-specific CPS antibody on new acquisition and clearance of rectovaginal GBS colonization in pregnant women from 20 weeks until 37 to 40 weeks' gestation. Higher serotype III CPS antibody concentration was associated with lower risk of rectovaginal acquisition of serotype III during pregnancy. They also found serotype-specific antibody titers to Ia and III were higher in women who remained free of GBS colonization throughout the study compared to those who acquired the homotypic serotype.

Table 1.4. Colonization rates of GBS among pregnant women in different countries.

Country	Colonization rate (%)	References
Sweden	25.4	Hakansson *et al.*, 2008
Norway	34.8	Bergseng *et al.*, 2009
Germany	16	Brimil *et al.*, 2006
UK	13.59, 21.3	Colbourn *et al.*, 2007; Jones *et al.* 2006a
The Netherland	21	Valkenburg-van *et al.*, 2006
Iceland	24.3	Bjarnadottir *et al.*, 2003
Italy	11.3	Busetti *et al*., 2007
USA	20-30	Park and Vizet, 2018
New Zealand	20	Grimwood *et al.*, 2002
Australia	20-24	Hiller *et al.*, 2005; Dyke *et al.*, 2009
Brazil	24.3	Soares *et al.*, 2013
India	2.5	Kulkarni *et al.* 2001
Greater Accra	26.8	Banni, 2014
Congo	20	Mitima *et al.*, 2014
Malawi	21.7	Gray *et al.*, 2011
Ethiopia	20.7	Mohammed *et al.*, 2012

b. Other Risk Factors

In addition to maternal colonization with GBS, other factors that can increase the risk for EOD-GBS includes gestational age of <37 completed weeks, prolonged rupture of the amniotic membranes (PROM) more than >18 hours, intrapartum fever, chorioamnionitis, young maternal age and black race (Dangnor *et al.*, 2016).

Low maternal levels of GBS specific anticapsular antibody and kinetics of antibody transfer from mother to newborn can be another risk factor of invasive GBS disease among newborn (Baker *et al.*, 1981; Boyer *et al.*, 1983; Schuchat *et al.*, 1990; Schuchat *et al.*, 1994; Schuchat *et al.*, 2000; Zaleznik *et al.*, 2000; Oddie and Embleton, 2002). Newborn susceptibility to GBS is increased when the level of anticapsular antibodies to the infecting serotype is low. This is when the maternal antibody level is low, when there is problem of

antibody transfer and also when newborn are born before 34 weeks of gestation (Baker, 1976; Baker *et al.,* 1981). In a low-middle income setting with a high burden of invasive disease, Dangor *et al*. (2015a) have demonstrated a sero-correlate of protection for GBS serotypes Ia and III which could facilitate vaccine licensure.

History of previous delivery of an newborn with invasive GBS disease was also counted as a risk factor for EOD-GBS. Women may remain colonized with the same strain of GBS for prolonged periods and may fail to develop protective levels of type-specific serum antibodies despite long-term colonization (Dykes *et al.,* 1985). It is therefore likely that the risk in subsequent pregnancies is higher for women who have had a child with EOD-GBS disease.

Newborn from women with GBS bacteriuria during pregnancy are at high risk for invasive GBS disease. Moreover, mothers with GBS bacteriuria and GBS colonization demonstrate a higher incidence of adverse obstetric outcomes such as habitual abortion, intrauterine growth restriction, still birth, preterm labor, chorioamnionitis and premature rupture of membranes (Carstensen *et al.,* 1988; Faxelius *et al.,* 1988; Kessous *et al.,* 2012; Nan *et al.,* 2015).

Preterm and LBW infants have an increased risk of EOD-GBS with a progressive increase in risk for neonatal sepsis with decreasing gestational age and birth weight (Schuchat *et al.,* 1994; Yancey *et al.,* 1996; Benitz *et al.,* 1999). Hakansson *et al*. (2008) showed that even infants born at 37 weeks of gestational age had a threefold increased risk of EOD-GBS compared with infants born at 40 weeks.

Gestational diabetes, diabetes mellitus (Hakansson and Kallen, 2006) and frequent vaginal examination (Schuchat *et al.,* 2000) are reported to increase the risk of having a newborn with EOD-GBS (El Beitune *et al.,* 2005; CDC, 1996; Ramos *et al.,* 1997; Schrag *et al.,* 2002; Lin *et al.,* 2004; Boyer and Gotoff, 1985). Few observational studies have reported an association between EOD-GBS and some obstetric procedures, such as the use of internal fetal monitoring devices (Adams *et al.,* 1993; Adair *et al.,* 2003). More than five or six digital vaginal examinations after onset of labor or rupture of membranes can increase the risk (Adams *et al.,* 1993; Schuchat *et al.,* 2000; Gibbs *et al.,* 2004). Concern has been raised

on performing other obstetric procedures such as membrane stripping and mechanical or pharmacologic cervical ripening on GBS-colonized women (Boulvain *et al.,* 2005; Heinemann *et al.,* 2008; Verani *et al.,* 2010). Women who had been in one of the above risks factors but who have negative prenatal screening cultures are at relatively low risk for EOD-GBS (Verani *et al.,* 2010).

The high prevalence of maternal HIV infection is contributing to the high incidence of EOD-GBS disease in South Africa; however, maternal HIV infection was not associated with increased risk of maternal bacterial colonization and vertical transmission (Cutland *et al.,* 2015; Dangor *et al.,* 2015c). The increased risk is due to low transfer IgG antibody from HIV infected mother to newborn (Cutland *et al.,* 2012; Dangor *et al.,* 2015c). Higher burden of EOD-GBS among newborn exposed to HIV was also reported from a Belgium (Epalza *et al.,* 2010). Moreover, maternal HIV infection was associated with lower anti-GBS surface binding antibody concentration and antibody-mediated C3b/iC3b deposition onto GBS bacteria of serotypes Ia, Ib, II, III and V (Le Doare *et al.,* 2015a). This may make these infants more susceptible to EOD-GBS and LOD-GBS.

1.2.6. EPIDEMIOLOGY OF EOD AND LOD-GBS

Eickhoff *et al.* (1964) published the first report on GBS infections among neonates and adults. 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 EOD-GBS and LOD-GBS was reduced to 4% because of advances in neonatal care (Verani *et al.,* 2010).

Several clinical trials in the middle of 1980s demonstrated that IAP administered during labor to mothers colonized with GBS was effective in preventing disease in newborns (Schrag *et al.,* 2000). In 1996 consensus guidelines for the prevention of perinatal GBS disease were issued by the AAP (American Academy of Pediatrics Committee on Infectious Diseases and Committee on Fetus and Newborn) (AAP, 1997), the AOG (American College of Obstetricians and Gynecologists Committee on Obstetric Practice (ACOG) 1996), and the CDC (Centers for Disease Control and Prevention (CDC, 1996). IAP strategy greatly influenced the epidemiology of EOD-GBS in developed countries.

The introduction of consensus guidelines has been followed by substantial decline in the incidence of EOD-GBS in USA and other countries in similar manner (Table 1.6). However the burden of EOD-GBS is not well known in most of the countries around the world particularly in Africa and the prevention strategy is largely being used in most developed countries (Schrag and Schuchat, 2004; Luthander *et al.*, 2015; Darlow *et al.*, 2016).

Table 1.5. Incidence of EOD-GBS in different countries.

Country	Incidence of EOD before IAP	Incidence after IAP	References
United States	1-3/1000 LB*	0.6/1000LB	Verani et al., 2010
Europe	0.5-2/1000LB	-	Trijbels-Smeulders *et al*., 2004
Australia	2.0/1000 LB	0.5/1000 LB	Garland *et al.*, 2011
UK & Wales	0.74/1000 LB	No data	Weisner *et al.*, 2004
Taiwan	0.11/1000 LB	No data	Ho *et al.*, 1999
Netherlands	1.9/100 LB	No data	Trijbels-Smeulders *et al.*, 2007
Brazil	0.39/1000 LB	No data	Vaciloto *et al.*, 2002
Singapore	0.2/1000 LB	No data	Niduvaje *et al.*, 2006
Malawi	0.92/1000 LB	No data	Gray *et al.*, 2007
South Africa	2.06-1000/LB	No data	Dangor *et al.*, 2015a
Check Republic	0.7-.0/1000 LB	No data	Strakova and Motlova, 2004

*LB-live birth

*IAP- Intrapartum Antibiotic Prophylaxis (IAP)

According to Edmond *et al.* (2012) although substantial heterogeneity exists, the overall global incidence of neonatal GBS disease is estimated to be 0.53 per 1000 live births. Incidence was highest in Africa (1.21/1000) followed by the Americas (0.67/1000) and Europe (0.57/1000). Southeast Asia had the lowest incidence (0.02/1000) (Edmond *et al*., 2012). A low incidence of neonatal invasive diseases is also reported from Japan (Matsubara *et al.*, 2013).

The most common serotypes among GBS infective isolates are Ia, Ib, II, III, and V, although there are some geographic and historical variations (Le Doare and Heath, 2013). The

frequency of serotype V has increased in the last few years, starting from 1990, whereas type IV has recently emerged as a cause of adult and neonatal infection in USA and Canada (Ferrieri *et al,* 2013; Teatero *et al.,* 2015).

Studies from many countries indicated that several serotypes of GBS to be associated with new born EOD-GBS and LOD-GBS. According to report from USA, United Kingdom, and Finland (Kalliola *et al.,* 1999) serotypes Ia, III, are V are the most isolated serotype from EOD –GBS and LOD-GBS (Table 1.6). However study from Japan reported high proportion of serotype VIII and VI from pregnant women (Lachenauer *et al.,* 1999). The serotype IX, which was identified in 2007, can also cause neonatal infections (Lamagni *et al.,* 2013; Melin and Efstratiou, 2013).

Studies of GBS serotype distribution in other countries showed that different serotypes to be associated witht invasive disease; GBS type IV in Brazil (Dutra *et al.,* 2014), Ireland (Kiely *et al.,* 2011) and Iran (Jannati *et al.,* 2012); type VI in Egypt (Shabayek *et al.,* 2014) and in Japan (Lachenauer *et al.,* 1999); type VII in Kuwait (Boswihi *et al.,* 2012) and type VIII Japan (Lachenauer *et al.,* 1999). The emergence of type IV colonization among American women has been recently noted (Diedrick *et al.,* 2010). Generally, Epidemiological data collected worldwide revealed that the distribution of CPS types was remarkably similar to the WHO region over the past years (Edmond *et al.,* 2012).

Table 1.6: Distribution GBS serotypes in EOD and LOD across various countries.

		Ia	Ib	II	III	IV	V	VI	VII	NT
German (Fluegge *et al.,* 2005)	EOD-GBS	17%	5%	8%	58%	1%	9%	-	-	-
	LOD-GBS	11%	4%	2%	77%	-	6%	-	-	-
USA (Shet and and Ferrier 2004)	EOD-GBS	32.8%	9.2%	9.2%	28.2%	1%	19%	-	-	0.5%
	LOD-GBS	27.3%	5.1%	3%	50.5%	-	14.1 %	1%	-	-
South Africa (Madhi *et al.,* 2003)	EOD-GBS	31%	7%	7%	49.2%	-	5.6%	-	-	2
	LOD-GBS	24.3%	-	-	75.7%	-	-	-	-	-
Malawi (Gray *et al.,* 2007)	EOD-GBS	26%	7%	11%	52%	-	4%	-	-	-
	LOD-GBS	20%	4%	4%	72%	-	-	-	-	-
Czech Republic (Strakova and Motlova, 2004)	EOD-GBS	13%	8%	14%	42%	3%	13%	1%	-	-
	LOD-GBS	26%	4%	20%	22%		11%	4%	2%	2%

Data on serotype distribution in particular geographic location has valuable input for the development of multivalent GBS vaccines tailored to specific geographic location, which should include those serotypes most commonly found. Moreover, serotype distribution may change with time and may vary between countries, which emphasize the importance of epidemiological surveillance of serotype distribution in the population for effective vaccine development (Wessels and Kasper, 1994).

1.2.7. LABORATORY METHODS FOR GBS DETECTION

To identify candidates among pregnant women for IAP to prevent EOD-GBS laboratory method with high sensitivity and specificity is required. There are several methods to detect GBS from genital tract of pregnant women such as; culture, Immunological assay and nucleic acid testing (Picard and Bergeron, 2004).

Consensus prevention guidelines of 1996/1997 from the CDC, AOG, and AAP recommended that GBS culture should be performed at 35–37 weeks 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) (Lim broth) or with gentamicin (10 μg/ml) and nalidixic acid (15 μg/ml) (TransVag broth). Selective enrichment broth can be made to contain chromogenic substrates that can change in color in the presence of beta-hemolytic GBS. Such broths can facilitate the identification of beta-hemolytic GBS; however, non-hemolytic isolates will not be detected by these broths alone (Picard and Bergeron, 2004).

The inoculated selective medium is incubated for 18–24 hours and then subcultured onto sheep blood agar. 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. Recently, chromogenic agars that undergo color change in the presence of beta-hemolytic colonies of GBS have become available but these agars cannot detect non hemolytic GBS colonies (Picard and Bergeron, 2004). 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 (Davies *et al.,* 2001; Picard and Bergeron, 2004).

A β-hemolytic *Enterococcus faecalis* strain agglutinating Lancefield group A, B, C, D, F, and G antisera was observed from rectovaginal swab during antenatal screening for GBS. This multi-Lancefield antisera-agglutinating isolate may mimic GBS isolation and leading to false positive reporting and mislead receipt of intrapartum antibiotics prophylaxis (Savini *et al.,* 2015).

To further improve culture method, Joubrel *et al*. (2014) compared the performances and the cost-effectiveness of 5 selective media, two were Granada-based media: Granada and BD GBS differential agar and 3 were chromogenic media: Brilliance GBS, StrepBSelect, and ChromID Strepto B for GBS screening in vaginal samples from pregnant women. They recommended the usefulness of these media for GBS screening; the choice will depend on the laboratory organization and the availability of a matrix-assisted laser desorption

ionization–time of flight mass spectrometry (MALDI-TOF MS). Xie *et al*. (2016) also evaluated different culture methods for GBS detection and found the sensitivity of SCB to be significantly higher than that of the SBA medium.

de Melo *et al.* (2015) demonstrated that Hitchens-Pike-Todd-Hewitt (HPTH) medium and SBA were more sensitive than Todd-Hewitt enrichment broth for GBS screening in pregnant women and good GBS recovery in culture, indicating that the two media should be used together for vaginal and anorectal specimens (de Melo *et al.*, 2015). Binguha *et al*. (2014) reported the high performance of chromogenic medium chromID Strepto B agar (STRB) compared to BAP due to its higher sensitivity. Furthermore, MALDI-TOF MS could reliably differentiate the putative GBS isolates on STRB.

Group B Streptococcus (GBS) strains are identified reliably by the production of group B Lancefield antigen. Consequently, many latex agglutination tests and immunoassays that detect this antigen for GBS identification have been developed. However, the overall sensitivity of these commercially available immunological assays is low. When compared with the results of selective broth culture, the sensitivity of popular rapid immunoassays to detect GBS colonization in pregnant women directly from rectovaginal 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 (Picard and Bergeron, 2004).

Faro *et al.* (2013) developed accelerated method in which sample is processed by an immunoblot-based test, sample is placed over an antibody-coated nitrocellulose membrane, and after a six-hour culture, bound GBS is detected with a secondary antibody. It demonstrated a sensitivity of 97.1% and a specificity of 88.4%. GBS test provides a high level of validity for the detection of GBS colonization in antepartum patients within 6.5 hours and demonstrates a substantial agreement between observers.

The sensitivity of the standard culture method for the detection of GBS colonization is only about 54-87% (Davies *et al.*, 2004b). It has long turnaround time, and requires up to 36-72 hour before the results can be reported (Davies *et al.*, 2004b). It also requires an experienced technician to identify non-beta-hemolytic GBS.

To overcome culture problems, more rapid and sensitive techniques have been developed, including DNA probe and nucleic acid amplification tests (NAATs). Ke *et al.,* (2000) developed an RT-PCR method based on amplification of a *cfb* gene fragment that is present in virtually every strain of GBS. While PCR continues to show promise for use in intrapartum testing, current CDC guidelines allow for PCR to be performed in an antepartum setting, but the guidelines caution against the use of PCR alone when susceptibility testing is required and recommend that in situations when anaphylaxis is a concern in a penicillin-allergic patient, culture be performed as well (Verani *et al.*, 2010).

There are several NAATs which can be used for screening of mothers colonized with GBS. Studies on the performance of commercially available NAAT on non enriched samples have demonstrated varying sensitivities (range: 62.5%–98.5%) and specificities (range: 64.5%–99.6%) compared with the gold standard method. There are many commercially available assays for detection of GBS using PCR to mention some of them; IDI-Strep B, Gene Xpert, and BD GeneOhm, Smart GBS assay (Verani *et al.,* 2010; Park *et al.,* 2013; Buchan *et al.,* 2015).

The IDI-Strep B is a qualitative in vitro diagnostic test for rapid detection of GBS in vaginal/anal specimens from maternity patients. The test uses a PCR assay to amplify GBS-specific DNA and a fluorogenic probe to detect the amplified GBS target. The test can be performed on the Smart Cycler (Cepheid) automated analyzer. In a single-site investigational trial, the selected primers and probe yielded a sensitivity of 97.0%, a specificity of 100%, a negative predictive value (NPV) of 98.8%, and a positive predictive value (PPV) of 100% for detecting GBS compared with culture results (Davies *et al.*, 2004b).

Study from Canada (Davies *et al.,* 2001) indicated that IDI-Strep B test to be highly sensitive and specific for detecting GBS from combined vaginal and anal specimens obtained from pregnant women in labor. The availability of such a test during labor may lead to a further reduction in the rates of neonatal GBS disease. Morozumi *et al.* (2015) also used *dltS* gene (encoding a histidine kinase specific to GBS) for detection of GBS using PCR. The sensitivity of PCR is 10 CFU/well for a 33-35 threshold cycle.

Buchan *et al.* (2015) reported the sensitivity and specificity of the Xpert GBS LB assay to be 99.0% and 92.4%, respectively, compared to those for the gold standard culture and the for Smart GBS molecular test they demonstrated sensitivity and specificity of 96.8% and 95.5%, respectively. The sensitivities of the two broth-enriched molecular methods were superior to those for direct testing of specimens using the Xpert GBS assay, which demonstrated sensitivity and specificity of 85.7% and 96.2%, respectively. Park *et al.*, (2013) reported that the sensitivity and specificity of intrapartum Xpert GBS assay compared to intrapartum culture were 95.8-98.5% and 64.5-99.6%, respectively.

1.2.8. TREATMENT OF EOD AND LOD-GBS

The 2002 prevention of perinatal GBS disease guidelines provided recommendations for the management of infants born to mothers who have received IAP (Schrag *et al.*, 2002). If a woman receives intrapartum antibiotics for the treatment of suspected chorioamnionitis, her newborn should have a full diagnostic evaluation, and empiric antibiotic therapy (ampicillin and gentamicin) pending culture results, regardless of clinical condition at birth, duration of maternal antibiotic therapy before delivery, or gestational age at delivery (Tumbaga and Philip, 2006).

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 white blood cell count, blood culture, chest radiograph with respiratory signs and spinal tap. Blood culture can be sterile in 15% to 38% of infants with culture proven meningitis (Tumbaga and Philip, 2006). For any symptomatic infants, 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).

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 (Tumbaga and Philip, 2006). Limited evaluation includes CBC with differential and blood culture. They need close observation; including frequent vital signs, frequent assessment of the infant. 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 requires immediate notification to the physician team and reassessment (Tumbaga and Philip, 2006).

Full evaluation including spinal tap and empiric antibiotic therapy is required, 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).

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 (Nandyal, 2008). 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 (Nandyal, 2008). Serial CRP measurements 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 OF GBS DISEASE

a. Intrapartum Antibiotic Prophylaxis (IAP)

By the late 1980s, once GBS is recognized as the leading cause of neonatal disease, a number of efforts have been made to prevent and treat GBS disease. The intravenous antimicrobial prophylaxis, administered to women who are GBS colonized during labor and delivery, was found to be the most practical and effective method to prevent vertical transmission of GBS from the mother to the infant and reduce the risk of perinatal sepsis (Boyer and Gotoff, 1986).

Two preventative approaches are used; a culture screening-based and a risk-based approach to administer IAP. The first approach involves universal screening for GBS colonization of

all pregnant women between 35 and 37 weeks of gestation using vaginal and rectal swab cultures to detect for GBS colonization. Properly obtained and processed antenatal cultures correctly identified most women colonized at the time of labor (Schrag *et al.,* 2002).

The risk-based approach involves administration of antibiotics based on the presence of antenatal or intrapartum risk factors. Maternal risk factors for EOD-GBS are as follows; preterm labor or premature rupture of membranes (< 37 weeks' gestation); prolonged rupture of membranes (≥18 hours); intrapartum fever ≥ 38.0°C; history of a previous newborn with GBS disease and GBS bacteruria during pregnancy (Schrag *et al.,* 2002).

The IAP is indicated for all GBS carriers except for those in whom cesarean delivery is planned in the absence of or membrane rupture. Penicillin *G* remains the drug of choice for prophylaxis with ampicillin as the alternative medication. Anaphylaxis caused by penicillin can range from 4/10000 to 4/100000. In addition; as many as 10% of the adult populations have less severe allergic reactions to penicillin (Nandyal, 2008). Recent report by May *et al.* (2016) intrapartum antibiotic exposure does not alter the risk of penicillin allergy. A study by Wohl *et al*. (2015) indicated exposure to antibiotics for <24 hours during a vaginal delivery does not increase the risk of atopic dermatitis among children.

For patients who are allergic to penicillin but without a history of anaphylaxis, Cefazolin is the preferred antibiotic (Table 1.8). 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 Erythromycin or Clindamycin, either of them is recommended for IAP. But, their efficacy in the prevention of EOD-GBS was not evaluated. In the presence of maternal chorioamnionitis, early treatment of the mother with broad-spectrum antibiotics is required (Table1.7). Criteria for the diagnosis of maternal chorioamnionitis include; maternal fever, uterine tenderness, fetal tachycardia, foul smelling amniotic fluid, prolonged rupture of membranes of equal to or more than 18 hours, or maternal leukocytosis (Nandyal, 2008).

A population-based comparison of strategies to prevent EOD-GBS in new born indicated routine screening for GBS during pregnancy prevents more cases of early-onset disease than the risk-based approach (Schrag *et al.,* 2002). Based on this information a new guideline

which states a superiority of screening of pregnant women for GBS than risk factor was issued in 2002 by CDC. Based on data collected after the release of 2002 prevention guidelines, the upgrading of the existing prevention guidelines was made in 2009 and published by CDC (Verani *et al.*, 2010).

A retrospective cohort study in a population of over 600,000 live born infants compared the effectiveness of the screening and risk-based approaches in preventing EOD-GBS. Data showed that the screening approach was more effective than the risk-based approach at preventing perinatal group B streptococcal disease (Schrag *et al.*, 2002). In the USA, after the widespread implementation of preventive strategies, the incidence decreased dramatically from 1.7 cases per 1000 live births in 1990 to <0.4 cases per 1000 live births in 2010 (Nuccitelli *et al.* 2015). However, no prevention strategy is currently totally effective in the eradication of EOD-GBS and, most important IAP has had no impact on late-onset infection where the burden is still substantial.

Table 1.7: Recommended regimen for intra partum antimicrobial prophylaxis for perinatal GBS disease prevention (Verani *et al.*, 2010).

Recommended	Penicillin G, 5 million units IV initial dose, then 2.5 million units IV every 4 hours until delivery
Alternatives	Ampicillin 2 gm IV initial dose, then 1 gm IV every 4 hours until delivery
If penicillin allergic and at low risk for anaphylaxis	Cefazolin, 2 gm IV initial doze and then 1 gm IV every 8 hours until delivery
If penicillin allergic and at high risk of for anaphylaxis	Clindamycin, 900 mg IV every 8 hours until delivery or Erythromycin, 500 mg IV every 6 hours until delivery (if GBS is susceptible)
If GBS is resistant to erythromycin or clindamycin or unknown	Vancomycin, 1 gm every 12 hours until delivery

National consensus guidelines for the prevention of GBS perinatal disease recommending use of IAP have been issued by public health authorities in a number of developed countries.

The screening-based approach is recommended in the USA, Japan and a number of European countries (Belgium, France, Germany, Italy, Poland, Spain and Switzerland). A risk-based approach is recommended in guidelines from Australia, Denmark, the Netherlands, New Zealand and the United Kingdom (Melin and Efstratiou, 2013).

As an alternative search for prevention strategy, *Lactobacillus reuteri* CRL1324 was found to possess a preventive effect on GBS vaginal colonization in an experimental mouse model. It can be considered as a biological agent to reduce infections caused by GBS (Gregorio *et al.,* 2014). *Lactobacillus reuteri* CRL1324 has a protective effect against GBS colonization that can be mediated by the modulation of the immune response; decrease the number of neutrophils activated by the pathogen, increase the activate macrophage population. (De Gregorio *et al.,* 2016). On the other hand, Patras *et al.* (2015b) reported that *S. salivarius* K12 may also have potential as a preventative therapy to control GBS vaginal colonization and thereby prevent its transmission to the neonate during pregnancy.

b. Vaccine

Evidence from developed countries indicated that IAP substantially reduced EOD-GBS but it is not without limitation. IAP strategy did not reduce LOD-GBS, it may not be feasible for developing countries, and above all it is not 100% effective in preventing EOD-GBS. Missed opportunities for prevention were also identified among women delivering preterm and among those showing adverse antibiotic events, such as anaphylaxis. Moreover, the widespread use of β-lactam antimicrobials for the IAP raises concerns about the selection of antibiotic resistance among GBS isolates or other newborn pathogens. Recently, a small number of clinical isolates have been characterized as having decreased penicillin susceptibility (Kimura *et al.,* 2008). However, resistance to β-lactams remains rare and it is not increasing, whereas resistance to alternative drugs such as erythromycin and clindamycin is increasing (Castor *et al.,* 2008). GBS resistance to clindamycin and erythromycin has already affected IAP options for penicillin allergic women. As a result the best option in the future is searching for effective vaccine.

For the first time, the possibility of vaccine for GBS was demonstrated by the work of Rebecca Lancefield and she demonstrated the protective ability of CPS-specific antibodies

in animal model (Lancefield and Hare, 1935). Later on, Baker and Kasper (1976) provided proof of concept that maternal vaccination can prevent EOD-GBS. They indicated that low maternal antibodies level against type III CPS were strongly associated with neonatal susceptibility to EOD-GBS. Maternal CPS-specific antibodies transferred from the mother to the newborn via transplacental route were able to protect newborn against GBS infections. The association between maternal antibody levels against serotype Ia and III and infant susceptibility was also confirmed by subsequent multicentre epidemiological studies (Lin *et al.*, 2001a; Baker *et al.*, 2014).

The first human clinical trials were conducted in the 1980s with purified native GBS serotype Ia, II or III polysaccharides injected in healthy adult volunteers, including pregnant women. Phase I trials demonstrated that these vaccines were safe and well tolerated, but it was poorly immunogenic. Among 10 GBS serotype, serotype II CPS was found to be the most immunogenic (Baker and Kasper, 1985; Baker *et al.*, 1988). Unconjugated polysaccharide vaccines induce T cell independent B-cell activation without B-cell memory response. Boosting vaccine will not result in an enhanced immune response to the vaccine antigen, and there was high variability among the responders, especially among GBS-naive individuals (Baker and Kasper, 1985). To increase the immunogenicity of CPS-based vaccines the next GBS vaccines was based on the generation of glycoconjugates, attaching CPS with protein. The conjugation of immunogenic protein to CPS antigens produced strong and durable immune response against the polysaccharide (Avci and Kasper, 2010).

Conjugate vaccines prepared with GBS type-specific CPS (Ia, Ib, II, III, IV, V, VI, VII and VIII) attached to tetanus toxoid (TT) showed better immunogenicity when tested in animal models (Wessels *et al.*, 1990; Paoletti *et al.*, 1992; Paoletti *et al.*, 1994; Paoletti *et al.*, 1999; Paoletti and Madoff, 2002a; Paoletti and Kasper, 2002b). The first GBS conjugate vaccine for phase I clinical trials was obtained from purified type III CPS coupled by reductive amination to monomeric TT (Kasper *et al.*, 1996). The study was conducted in healthy non-pregnant women and compared three dosages of III–TT, unconjugated type III CPS, and saline placebo. The vaccine was safe, the immunogenicity of III–TT was dose-dependent

and women who received the unconjugated type III CPS had significantly lower immune responses (Kasper *et al.,* 1996).

Next trial comparing III–TT with unconjugated type III CPS in pregnant women showed that, after vaccination, titers of protective IgG to type III CPS were elevated in cord blood, persisted for at least 2 months in the neonates and correlated with levels of type III CPS-specific antibody in maternal serum (Baker and and Edwards, 2003; Baker *et al.,* 2003). In one trial reported that women immunized with III–TT had decreased levels of vaginal and rectal colonization with GBS (Hillier *et al.,* 2009). Monovalent conjugate vaccines representing the most frequent disease-causing serotypes (Ia, Ib, II, III and V) have been prepared coupled to TT and tested in phase I and II clinical trials in healthy women. For each vaccine, an improved immunogenicity was demonstrated over the isotypic unconjugated polysaccharide, which was dose-dependent and more consistent with a memory response. In addition, glycoconjugate vaccines were able to induce functionally active serotype specific IgG (Baker *et al.,* 1999; Baker *et al.,* 2000; Baker *et al.,* 2004; Baker *et al.,* 2007).

Vaccine may have different effect among HIV infected pregnant women. Le Doare *et al.* (2015b) reported that anti-GBS IgG2 placental transfer is not affected by HIV infection. This is important for functional antibody against the capsular polysaccharide of GBS and provides confidence that maternal GBS vaccination may result in functional activity in HIV-infected and uninfected women. Maternal HIV infection was associated with lower anti-GBS surface binding antibody concentration and antibody-mediated C3b/iC3b deposition onto GBS bacteria of serotypes Ia, Ib, II, III and V (Le Doare *et al.,* 2015a). This can make infants more susceptible to EOD-GBS and LOD-GBS.

In other studies, HIV-1 is associated with lower GBS capsular and surface-protein IgG antibody levels and reduced transplacental antibody transfer in pregnant women (Dangor *et al.,* 2015c). The vaccine was less immunogenic in women infected with HIV than it was in those not infected, irrespective of CD4 cell count, resulting in lower levels of serotype-specific maternal antibody transferred to infants, which could reduce vaccine protection

against invasive GBS disease. A validated assay and correlate of protection is needed to understand the potential protective value of this vaccine (Heyderman *et al.,* 2016).

Although the immunogenicity in humans of GBS type-specific CPS antigens was successfully increased through conjugation to TT, the immune responses were serotype-specific. Thus, to develop a broadly effective vaccine against the most common disease-causing strains circulating worldwide, conjugate formulations should be multivalent (Phares *et al.,* 2008; Le Doare and Heath, 2013). A combination of four TT-conjugated serotypes (Ia, Ib, II and III) was successfully tested in a mouse infection model (Paoletti *et al.,* 1994), and subsequently the safety and immunogenicity of this tetravalent vaccine formulation was evaluated in healthy adults (Kotloff *et al.,* 1996). The vaccination was well tolerated and higher IgG titres were observed in those subjects with pre-existing anticapsular antibody to GBS**.** A bivalent vaccine containing II–TT and III–TT glycoconjugates was tested in humans in terms of safety and immunogenicity (Baker *et al.,* 2003). The safety and immunogenicity of a trivalent GBS vaccine based on type Ia, Ib and III CPS, conjugated to CRM197 has been evaluated in a phase I study in healthy nonpregnant women.

A trivalent GBS polysaccharide-protein conjugate vaccine for vaccination of pregnant women is under development to protect their newborns against invasive GBS disease. Establishing sero-correlates of protection against invasive GBS disease in infants could accelerate the licensure pathway of polysaccharide-protein conjugate vaccine. To complement this Dangor *et al*. (2015b) indicated maternal capsular antibody concentrations to be associated with the risk of invasive GBS disease in infants from low-middle income setting and demonstrated a sero-correlate of protection for GBS serotypes Ia and III which could facilitate vaccine licensure. A systematic review also described the association between low capsular antibodies levels in invasive GBS cases compared to controls. However, different assay methods and the lack of standardized reference ranges for serotype-specific antibody levels makes it difficult to select an antibody level that may be used as a reliable sero-correlate of protection (Dangor *et al.,* 2016).

Multivalent capsular conjugate vaccines are in advanced stage of development; however, it has some limitations: it is serotype-specific**,** lack of coverage against NT isolates and the

problems of serotype replacement/switching as happened among *S. pnemoniae* (Brueggemann *et al.,* 2007). CPS-based vaccination may exert the selective pressure for virulent genotype to switch capsules and escape vaccine coverage. As a result capsular switch from vaccine to non-vaccine type may compromise the success of vaccination program. Analysis of GBS strain comparative genome hybridization (CGH) (Brochet *et al.,* 2006, 2009), MLST (Luan *et al.,* 2005), PFGE (Martins *et al.,* 2010) indicated capsular switch occurred among clinical isolates.

To overcome this limitation efforts have been also directed to the identification of highly protective antigens for the development of a broad coverage GBS protein based vaccine. Protein candidate for vaccine should be surface-exposed, conserved and expressed in a wide panel of strains, strongly immunogenic, and able to confer protection from disease. Different to the CPS antigens, the proteins are able to induce protective T-cell dependent antibody responses and long-lasting immunity (Rinaudo *et al.,* 2009; Rappuoli, 2011).

Until the publication of the first complete genome sequences of two GBS clinical isolates (Glaser *et al.,* 2002; Tettelin *et al.,* 2002) only a limited number of proteins involved in GBS pathogenesis were identified also as potential vaccine candidates for their ability to elicit protective immunity in animal models and induce complement dependent opsonophagocytosis killing of bacteria *in vitro* (Lindahl *et al.,* 2005). Some of these proteins there are the tandem repeat-containing α and β components of the C protein complex (Michel *et al.,* 1991), Rib protein (Stalhammar-Carlemalm *et al.,* 1993), the surface-immunogenic protein (Sip) (Brodeur *et al.,* 2000), and the serine-protease C5a peptidase that interferes with host defenses by inactivating human complement component C5a (Cheng *et al.,* 2001).

Maione and co-workers (2005) have identified 4 potential vaccine antigens among 312 putative surface/secreted proteins that were produced as recombinant proteins in *E. coli* and tested for their capacity to elicit protection in infant mice in an active maternal/ neonatal challenge model. Only one of these antigens, the Sip protein, belonged to the core genome (Brodeur *et al.,* 2000). The other three proteins were never described and were part of the dispensable portion of GBS pan-genome, not present in all analyzed strains. Maione and co-

worker (2005) demonstrated that the combination of these proteins, each effective against overlapping populations of isolates, in a multivalent vaccine formulation can confer broad serotype-independent protection.

Further work revealed that the three dispensable genome antigens were structural components of the pilus-like structures (Lauer *et al.,* 2005). Following the identification in GBS of three pilus variants whose genes are present in three different PIs (named PI-1, PI-2a and PI-2b) (Rosini *et al.,* 2006) and the evidence that each pilus contains two structural subunits eliciting protective immunity in mice (Maione *et al.,* 2005; Rosini *et al.,* 2006; Margarit *et al.,* 2009). Margarit *et al.* (2009) demonstrate extensive study of pili distribution and conservation in a large collection of clinical isolates, that all GBS isolates carried at least one or a combination of two PIs and that a pilus-based vaccine exclusively constituted by three pilus components, one from each pilus type, can be effective in preventing GBS infections and capable of providing a broad protection (Margarit *et al.,* 2009). Moreover, GBS isolates from China carried at least one pilus island. The most frequently detected pilus island was PI-2a alone (Lu *et al.,* 2015).

This evidence provides a baseline for the development of a universal pilus-based vaccine that is potentially capable of preventing disease by all GBS serotypes. However, not all the protective pilin subunits could be included in the vaccine due to their antigenic variation (Margarit *et al.,* 2009). Dressing pilus proteins with surface glycan antigens could be an attractive approach to extend vaccine coverage. Nilo *et al.* (2015) have recently developed an efficient method for tyrosine-directed ligation of large glycans to proteins via copper-free azide–alkyne [3 + 2] cycloaddition. They demonstrated that polysaccharide-conjugated GBS80 pilus protein functions as a carrier comparably to $CRM_{197,}$ while maintaining its properties of protective protein antigen. Alves *et al.* (2015) demonstrated that the GAPDH-based vaccine is safe and stable and protects susceptible and diabetic adult mice against GBS infections.

In addition, mechanistic insights on how the vaccine works should be considered. In previous model of glycoconjugate vaccines, B-cell receptors specific to a vaccine's carbohydrate components recognize and phagocytose the glycoconjugate. The

glycoconjugate is then degraded in B-cell endosomes, and the peptide (but not the carbohydrate) components are presented on major histocompatibility complex (MHC) II molecules. The MHC II–peptide complexes signal through T-cell receptors and thus induce T-cell maturation through IL-2 and IL-2 receptor production; this causes T cells to produce IL-4, which induces B-cell maturation, class switching, and memory. Thus the B cell can continue to produce antibodies specific to the carbohydrate components of these glycoconjugates, while the T cells recognize the peptide components (Chena *et al.*, 2013).

Avci *et al.* (2011) proposed that endosomal degradation of glycoconjugates yields processed glycans conjugated to peptides (glycanp–peptides). It is glycanp–peptides not only peptide components that are presented on MHC II molecules. The peptide component binds to the MHC II molecule; on the other hand the covalently attached glycan component is recognized by the T-cell receptor.

Avci *et al.* (2011) further designed a prototype new-generation glycoconjugate vaccine by conjugating polysaccharide with peptides to achieve an optimal number of glycan–MHCII-binding peptide bonds per glycoconjugate. Compared with a conventional glycoconjugate vaccine, this new-generation vaccine was several times more immunogenic and greatly enhanced the survival of challenged mouse pups in a maternal immunization model.

This type of vaccine design benefit newborn with late-onset neonatal GBS disease, which typically occurs in premature infants who have low levels of transplacentally transferred antibody (Leineweber *et al.*, 2004). Increasing maternal antibody levels with better vaccine design will result in higher neonatal antibody levels. While the maternal antibody levels necessary to protect against early-onset GBS disease have been estimated (Lin *et al.*, 2001a,b), it remains to be determined what maternal/fetal antibody level is required for prevention of late-onset GBS disease. With maternal–fetal transfer compromised as it is in premature infants, it will be critical to design as immunogenic a vaccine as possible in order to maximize the chances of successful disease prevention.

1.2.10. SIGNIFICANCE OF THE STUDY

In 1970 GBS emerged as a leading infectious cause of neonatal morbidity and mortality in the USA and several other industrialized countries (Schrag *et al.*, 2000). 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 EOD-GBS and LOD-GBS was reduced to 4% because of recognition of the cases and application of advanced neonatal care (ACOG, 1996).

As early onset neonatal infection is due to vertical transmission of GBS from colonized mother to their newborn it can be prevented by administration of IAP based on Universal screening of pregnant women or those with risk factors. With the release of a new guideline in 2002 further reduction in EOD-GBS was observed in USA indicating the superiority of universal screening approach over risk based approach (American College of Obstetricians and Gynecologists Committee on Obstetric Practice (ACOG, 1996).

In USA as a result of prevention efforts based on the recommendation issued in 1996 and 2002, the incidence of EOD-GBS has declined dramatically over the past 15 years, from 1.7 cases per 1,000 live births in the early 1990s to 0.34–0.37 cases per 1,000 live births in recent years (Verani *et al.,* 2010) In Canada the incidence is reduced from 1.21/1000 live births to 0.77/1,000 live births as a result of adoption of prevention strategy (Goldenberg *et al.,* 1998). In Australia the incidence reduced from 2.0/1000 live births in 1991-93 to 0.5/1000 live births in 1995-97. This decline was associated with wide spread use of intrapartum chemoprophylaxis (Isaacs *et al.,* 1999).

In developing countries about 900, 000 sepsis associated neonatal death occur in a year (Bryce *et al.,* 2005; Seale *et al.,* 2009). According to WHO estimates, 5 million children under one month of age die every year, and about 98% of these occur in developing countries (WHO, 1996). About 3.4 million neonatal deaths take place in the first week of life (Wessels and Kasper, 1994).

The objective of MDG-4 is to reduce the toll of childhood mortality by 2/3 between 1990 and 2015; from 95/1000 LB in 1990 to 31/1000 LB in 2015 which is far away from objective set by MDG-4 in sub-Saharan Africa (Seale *et al.,* 2009). A huge reduction in neonatal deaths will be required to achieve MDG-4. Therefore, activities which reduce neonatal deaths should become a major public-health priority. Some of the reasons why the health of newborn babies has been neglected in developing countries despite the huge

number of deaths include; neonatal deaths are not visible to the public and not documented, neonatal deaths also have low visibility on the global agenda.

In Ethiopia, according to one study, GBS colonization rate among pregnant women was found to be comparable to other countries (Mohammed *et al.,* 2012). The study conducted at Hawassa, Ethiopia that involved screening of 139 pregnant women for GBS at their 35-37 weeks of gestation found a prevalence of 20.86% (Mohammed *et al.,* 2012). This indicates that exposure of newborns of Ethiopia to GBS during birth is similar to those in other countries indicating that the risk of EOD-GBS for new born do exist even though it was not properly investigated.

In Ethiopia there is no published data either from a hospital based or multicenter study on burden of early onset neonatal disease due to GBS which is currently recognized as the leading cause of perinatal infection in the world. There is no data how many new born have been lost so far due to readily preventable GBS disease in Ethiopia. Therefore, the present study was undertaken: 1) to provide valuable data about maternal and their newborn colonization with GBS 2) to provide information about the burden of neonatal disease due to GBS, and serotypes distribution of GBS isolated from mothers and their newborns. The data generated from this study will highlight the importance of routine screening of pregnant women for GBS and will also help to implement prevention strategies such as intrapartum antibiotic prophylaxis (IAP) to protect the new born from EOD-GBS and LOD-GBS.

1.2.11. HYPOTHESIS

The colonization rate of GBS among pregnant women and their new born in selected hospitals could be similar with colonization rate previously reported from Ethiopia and other countries.

The burden of Early and Late onset neonatal disease caused by GBS in Tikur Anbesa specialized Hospital could be similar with burden of Early and Late onset neonatal disease caused by GBS reported from other countries.

1.3. OBJECTIVE OF THE STUDY

1.3.1. GENERAL OBJECTIVES

- To determine group B Streptococcus (GBS) colonization rate among pregnant women and their new born attending selected Hospitals in Ethiopia
- To determine the burden of early and late onset neonatal disease due to GBS in a selected hospital in Ethiopia.
- To determine risk factors associated with colonization rate and vertical transmission of GBS
- To characterize GBS collected by using various molecular techniques

1.3.2. SPECIFIC OBJECTIVES

- To isolate and serotype GBS from pregnant women and their newborn
- To isolate and serotype GBS from newborn suspected of early and late onset neonatal disease.
- To determine the susceptibility pattern of GBS isolates to the common and new antimicrobial agents used for intrapartum antibiotic prophylaxis and treatment of GBS disease in newborns.
- To determine the vertical transmission of GBS from pregnant women to their new born.
- To identify the risk factors associated with colonization rate and transmission of GBS from pregnant women to their new born.
- To characterizes GBS isolates from mother and their new born using molecular techniques.

CHAPTER II: MATERIALS AND METHODS

2.1. STUDY DESIGN AND DURATION

Phase I:

A cross-sectional and follow up study was conducted to determine GBS colonization rate among pregnant women and their new born at: 1) Adama Hospital Medical College (AHMC), Adama from June 2014 to October 2014; 2) Hawassa Referral Hospital (HRH), Hawassa from November 2014 to March 2015; 3) Tikur Anbessa Specialized Hospital (TASH), Addis Ababa from March 2015 to August 2015.

Phase II:

A cross sectional study was conducted from March 2015 to September 2015 at Tikur Anbessa Specialized Hospital (TASH), Addis Ababa, Ethiopia to determine the burden of EOD and LOD- GBS in newborn.

2.2. STUDY AREAS

Tikur Anbessa Specialized Hospital (TASH), Hawassa Referral Hospital (HRH) and Adama Hospital Medical College Hospital (AHMC) were selected for the present study. These Hospitals are among referral hospitals found in Ethiopia and serve large population in their surroundings. These selected sites will also be considered as a surveillance center for GBS neonatal disease in Ethiopia after completion of the project.

Tikur Anbessa Specialized Hospital (TASH) is located in Addis Ababa the capital city of Ethiopia with a population of 3,384,569. Addis Ababa lies at an altitude of 2,300 meters and located at $9^{o}1'48"N\ 38^{o}44'24"E$. The city lies at the foot of Mount Entoto. From its lowest point around Bole International Airport, at 2,326 meters above sea level in the southern periphery, the city rises to over 3000 meters in the Entoto Mountains to the north. The city has a complex mix of highland climate zones, with temperature differences of up to $10^{o}C$ depending on elevation and prevailing wind patterns.

Tikur Anbessa Specialized Hospital (TASH) is the largest tertiary referral Hospital in Ethiopia and provides care for 24 hours including emergency services. TASH has 800 beds and provides care for approximately 370,000−400,000 patients per year. TASH is also the centre of excellence for training of undergraduate and postgraduate students in different

disciplines.

Hawassa Referral Hospital (HRH) is located at Hawassa town, the capital city of SNNRP 275 km to the south of Addis Ababa. The altitude of the town is 1697 km above sea level with mean annual temperature and rainfall of 20.9^{o}C and 997.6 mm respectively. The total population of Hawassa town is 235,000 with one to one male to female ratio. The hospital was inaugurated in November 2005 and has 850 beds and serves about 12 million people.

Adama Hospital Medical College (AHMC) is located at Adama town, Oromiya region 100 km to the east of Addis Ababa. The town has a total population of 220,212. It is located at 8^{o}33'N39^{o}16'E/8.55^{o}N39.27^{o}E at an elevation of 1712 meters. AMHC has 213 beds and provides services to one million inhabitants in Adama and the surrounding areas.

2.3. STUDY POPULATION

Phase I

a. Pregnant Woman and Their New Born

For phase I a total 840 pregnant women at time of delivery and their newborn were screened for GBS colonization in three selected hospitals: Adama Hospital Medical college (n=280) from June 2014 to October 2014; Hawassa Referral Hospital (n=280) from November 2014 to March 2015 and Tikur Anbessa Specialized Hospital (n=280) from March 2015 to August 2015. Neonates born to mothers with GBS and/or with GBS colonization was followed for EOD-GBS through telephone for 7 days (Table 2.1).

Sample size

Margin of error = 0.05, Confidence interval = 95%, prevalence from previous study conducted in Ethiopia, 20.86% (Mohammed *et al.*, 2012).

$$n = \frac{(Z\alpha/2)^2 * P(1-P)}{d^2}, \quad n = \frac{(1.96)^2 * 0.21(1-0.21)}{(0.05)^2} = 255 + 10\ \% = 280$$

Table 2.1 Distribution of sample size in three referral hospitals.

Hospitals	Sample size		
	Pregnant women	**Their New born**	**Total**
Tikur Anbessa specilized Hospital	280	280+	560
Hawassa Referral Hospital	280	280+	560
Adama Hospital Medical College	280	280+	560
Total	840	840+	**1680**

+ The number can be greter than this if there are tiwins

Phase II

b. New born with suspected EOD-GBS and LOD-GBS

For phase II, all new born admitted to TASH from March to September 2015 (n=176) for suspected Early onset neonatal disease (new born whose age is less than 7 days with suspected meningitis and sepsis) and Late onset neonatal disease (new born whose age is between 7 to 90 days with suspected sepsis and meningitis) were investigated for GBS.

2.4. INCLUSION AND EXCLUSION CRITERA

Inclusion criteria

- Pregnant women with age 18-45 able to understand and comply with planned study and provide informed consent were included in the study.
- Ifnats less than 90 days who are suspected of sepsis and mengitis.

Exclusioncriteria

- Pregnant women with age 18-45 unwilling to consent, having Cesarean Section as mode of delivery and unwilling to obtain swab from their new born were excluded from the study.
- Intans whome paretns refused to give consent.
- Pregnant women with planned ceseral section.
- Pretnant women who are on antibiotics during delivery.

2.5. STUDY VARIABLES

Dependent variables: GBS colonization, GBS prevalence, Antimicrobial susceptibility, Serotype, Sequence type, Antimicrobial resistance genes.

Independent variables

Pregnant women: Maternal age, Gestational age, Gravida, Previous mode of delivery, Duration of rupture of membrane, Premature rupture of membrane, Meconious stained amniotic fluid, other illness.

Their Newborn: Week of birth, APGAR score, Status of newborn during birth, EOD, Weight, other disease.

Newborn with disease: Week of birth, APGAR score, Diarrhea, Reduced movement, Reduced suckling, Seizure, Slow heart rate, Swollen belly, vomiting, Yellwish sking and eye.

2.5 SAMPING TECHNIQUE

To recruit study partipants for phase I and phase II of the present study convenient sampling technique was used.

2.6. SAMPLE COLLECTION, HANDLING AND TRANSPORT

2.6.1. Pregnant Women

Swabs from lower vagina and the rectal region were collected from informed and consented pregnant women at time of delivery by attending midwifes according to the CDC (Schrag *et al.,* 2002) and American College of Obstetricians and Gynecologists Committee on Obstetric Practice (ACOG, 1996) guidelines. The swabs were placed in Stuart's liquid media (BD Diagnostics, USA) and transported to the Microbiology Laboratory of three selected hospitals within 4 hours.

2.6.2. Their Newborn

Swabs from external ear, nasal area, throat and umbilical area were collected in parallel from new born immediately after delivery by attending midwifery. All the swabs were

placed in Stuart's liquid media (BD Diagnostics, USA) and transported to the Microbiology Laboratory of three selected hospitals within 4 hours.

2.6.3. Neonates with suspected EOD-GBS and LOD-GBS

Blood and cerebrospinal fluid were collected from newborn born admitted to TASH with suspected early and late onset neonatal diseases such as sepsis and meningitis, respectively by attending pediatrician.

I. Blood Specimen

Using aseptic technique by applying Povidone iodine and 70% alcohol at the site of vein puncture, 1 ml venous blood was drawn from the antecubital or femoral vein using sterile 23 gauge/20-25mm needle. Then the blood was inoculated into Tryptone Soy Broth (BD Diagnostics, USA) and transported within one hour to the Microbiology Laboratory of DMIP Addis Ababa University.

II. Cerebrospinal Fluid CSF)

Using aseptic technique by applying Povidone iodine and 70% alcohol to the site, 1-2ml CSF sample was collected using sterile 23 gauge/64mm wide-bore needle which was inserted between the fourth and fifth lumbar vertebrae. Then the CSF was transferred into sterile test tube and transported without delay to the Microbiology Laboratory of DMIP Addis Ababa University.

2.7. CULTURE AND IDENTIFICATION OF GBS

2.7.1. Vaginal, Anorectal, External Ear, Nasal Area and Umbilical Swabs

Vaginal and anorectal swabs from pregnant women during delivery and swabs from external ear, nasal, throat and umbilical area of their new born were placed into Lim broth (BD Diagnostics, USA) supplemented with colistin (10µg/ml) and nalidixic acid (15µg/ml). The inoculated selective medium was incubated for 18–24 hours at 37^{o}C in CO2 enriched atmosphere and then subculture was performed onto sheep blood agar plates (BAP) (BD Diagnostics, USA) and incubated in candle jar at 37^{o}C for 18−24 hours. If there was no growth, blood agar plate was re-incubated and examined at 48 hours (Annext VII) (Verani *et al*., 2010)

2.7.2. Blood and CSF

One ml blood was inoculated into Tryptone Soy Broth (BD Diagnostics, USA) for culture. All blood culture were incubated aerobically at 37°C and inspected daily for 7 days for presence of visible microbial growth by observing any of one of the following: turbidity, hemolysis, gas production and coagulation of broth. Blood cultures that showed signs of microbial growth sub-culture was made onto blood agar (BD Diagnostics, USA). The blood agar plate was incubated aerobically using a candle jar at 37°C for 24−48 hrs.

Cerebro Spinal Fluid (CSF) was gram stained and assessed for presence of bacteria and inflammatory cells. Then the CSF was inoculated on blood and chocolate agar (BD Diagnostics, USA) and incubated at 37^{o}C for 18-24 hours in carbon dioxide rich atmosphere.

All suspected GBS colonies on BAP from swabs, blood and CSF were characterized according CDC guideline (Verani *et al.,* 2010). Hemolytic reaction on BAP (beta-hemolytic or non-hemolytic), gram stain (gram positive cocci short chain), negative catalase test and positive CAMP test were used to confirm GBS. Ambiguous CAMP test results were re-tested using a Strep B Grouping Latex (Remel, USA). The confirmed GBS isolates were stored in Skim milk with glycerol (BD Diagnostics, USA) at -70°C for further characterizations such as serotyping using mPCR, antimicrobial susceptibility testing using Microdilution Method (MDM), MLST,whole genome sequencing. All characterizations were performed at Streptococcus laboratory of CDC, Atlanta, USA from January 2015 to March 2016 (Annex VII).

2.8. ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility was performed on stored GBS isolates at CDC, Atlanta, Georgia, USA using micro dilution method according CLSI guidelines (CLSI, 2014). The following nine antimicrobial agents obtained from BD diagnostics, USA were tested: Penicillin (PEN), Levofloxacin (LEVO), Tetracycline (TET), Erythromycin (ERY), Linezolid (LZD), Vancomycin (VAN)**,** Daptomycin (DAP), Clindamycin (CL), and Cefotaxime (FOT). In brief, from a pure GBS culture 3-5 selected colonies of bacteria was taken and transferred to a tube containing 5 ml sterile Muller Hinton broth (BD Diagnostics,

USA) and vortexed to a homogenous suspension (turbidity 0.09-0.16) and 1µl was transferred to lyzed sheep blood (BD Diagnostics, USA) and mixed gently then transferred to 96 well plate containing antibiotics using dispenser machine and incubated at 37^{o}C overnight. The result was read using magnifier and the isolates was classified as sensitive, intermediate, and resistant according to the standardized table supplied by the CLSI (CLSI, 2014).

2.9. TYPING OF GBS STRAINS

2.9.1. Capsular Serotyping

Capsular serotyping was done on all GBS isolates using slide agglutination tests using type specific 10 antisera for serotypes Ia, Ib, II, III, IV, V, VI, VII, VIII and IX (Statens Serum Institute, Denmark) as previously described by Slotved *et al.* (2003). Briefly, GBS strains were cultured on blood agar plates. A heavy suspension of the test organism harvested from the blood agar plate was prepared in 250 µl phosphate-buffered saline (PBS), pH 7.4. For each of the 10 serotypes, a 20µl aliquot of the bacterial suspension was applied to a disposable reaction card and mixed with 1 µl of latex suspension (reagents Ia, Ib, and II to IX; Strep-B-Latex kit; Statens Serum Institute, Copenhagen, Denmark). The reaction card was rotated slowly and observed for agglutination, and a positive reaction was scored when clear-cut agglutination appeared within 30s.

2.9.2. Molecular Characterizations

A total of 125 GBS strains were further characterized by Antimicrobial susceptibility testing and 123 by multiplex PCR (Imperi *et al.,* 2010). A total of 123 GBS strains were also further characterized by MLST and Antibiotic resistance gene detection (Jones *et al.,* 2003). Twenty eight GBS strains were collected at Adama Hospital Medical College; 35 GBS strains were collected at Tikur Anbessa Specialized Hospital and 60 GBS strains were collected at Hawassa Referral Hospital. Procedure for DNA extraction, multiplex PCR, MLST and whole genome sequencing is described briefly as follows (Annex VII).

I. DNA Extraction for mPCR and MLST

Group B streptococcus (GBS) isolates were cultured on Trypticase soy agar supplemented with 5% sheep blood and incubated overnight at 37°C. DNA of GBS isolates was extracted

by using chelex based boiling according (Imperi *et al.,* 2010). Briefly the isolates were mixed with chelex reagent in vials and incubated at 56^{o}c for 20 minutes, then vortexed in the middle followed by incubation at 95^{o}c for additional 20 minutes; the extract (pure DNA) was stored at -4^{o}c until used.

II. Multiplex PCR reaction system (mPCR)

The purpose of mPCR was to further reconfirm prevalent serotypes of GBS. Multiplex PCR was performed according to Imperi *et al,* (2010). Briefly, 19-primer multiplex PCR mixture (Master mix) was prepared as follows (Table 2.1): 0.25 µl each of all forward (50 pmol µl^{-1}) and reverse (50 pmol µl $^{-1}$) primers, 1.25 µl dNTPs (2.5 mM of each dNTP), 2.5 µl X PCR buffer, 3 µl 25mM MgCl2 (4.5 mM final), 0.1 µl Qiagen hotstart Taq polymerase (5 units µl $^{-1}$) and water was added to vials (total volume= 25µl). 23 µl of the preparation was loaded on to the PCR plate, and then 2 µl DNA was added, and placed in PCR cycler. The product was run on electrophoresis gel and the result was interpreted according to the publication of Imperi *et al.,* (2010). Primer for mPCR is listed in Table 2.2.

Table 2.2. List of primers used to amplify the *cps* genes of GBS using mPCR (Imperi *et al*., 2010).

SN^{o}	Primer Name	Sequence 5' to 3'
1.	Cpsl-Ia-6-7-F	GAATTGATAACTTTTGTGGATTGCGATGA
2.	Cpsl-6-R	CAATTCTGTCGGACTATCCTGATG
3.	Cpsl-7-R	TGTCGCTTCCACACTGAGTGTTGA
4.	CpsL-F	CAATCCTAAGTATTTTCGGTTCATT
5.	CapsL-R	TAGGAACATGTTCATTAACATAGC
6.	CpsG-F	ACATGAACAGCAGTTCAACCGT
7.	CpsG-R	ATGCTCTCCAAACTGTTCTTGT
8.	CpsG-2-3-6-R	TCCATCTACATCTTCAATCCAAGC
9.	CpsN-5-F	ATGCAACCAAGTGATTATCATGTA
10.	Cps-N-5-R	CTCTTCACTCTTTAGTGTAGGTAT
11.	CpsJ-8-F	TATTTGGGAGGTAATCAAGAGACA
12.	CpsJ-8-R	GTTTGGAGCATTCAAGATAACTCT
13.	CpsJ-2-4-F	CATTTATTGATTCAGACGATTACATTGA
14.	CpsJ-2-R	CCTCTTTCTCTAAAATATTCCAACC
15.	CpsJ-4-R	CCTCAGGATATTTACGAATTCTGTA
16.	CpsI-7-9-F	CTGTAATTGGAGGAATGTGGATCG
17.	CpsI-9-R	AATCATCTTCATAATTATCTCCCATT
18.	CpsJ-Ib-F	GCAATTCTTAACAGAATATTCAGTTG
19.	CpsJ-Ib-R	GCGTTTCTTTATCACATACTCTTG

Table 2.3. Size (in base pairs, bp) and type of the PCR amplification products expected for each serotype (Ia to IX). The names in parenthesis indicate the GBS capsular gene from which the primers have been designed (Adopted from Imperi *et al*, 2010).

Ia (2 band)	Ib (3 band)	II (3 band)	III (2 band)	IV (3 band)	V (2 band)	VI (3 band)	VII (3 band)	VIII (2 band)	IX (3 band)
688 bp (cpsL)	688 bp (cpsL)	688 bp (cpsL)	688 bp (cpsL)	688 bp (cpsL)	688 bp (cpsL)	688 bp (cpsL)	688 bp (cpsL)	688 bp (cpsL)	688 bp (cpsL)
	621 bp (cpsJ)								
					582 bp (cpsN)				
				538 bp (cpsJ)					
						470 bp (cpsI)			
		465 bp (cpsJ)							
								438 bp (cpsJ)	
			352 bp (cpsG)			352 bp (cpsG)			
272 bp (cpsG)	272 bp (cpsG)	272 bp (cpsG)		272 bp (cpsG)			272 bp (cpsG)		272 bp (cpsG)
									229 bp (cpsI)
							179 bp (cpsI)		

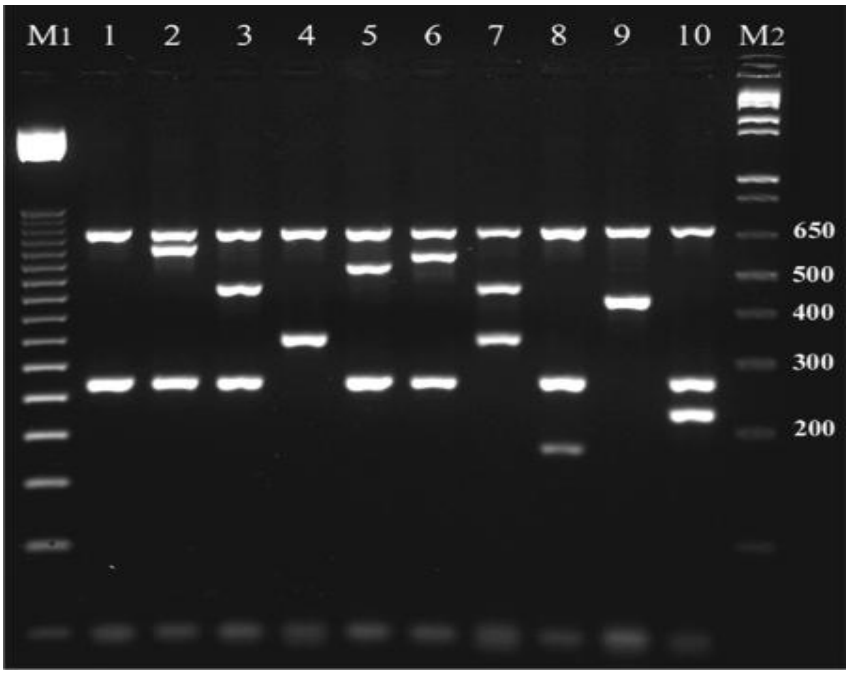


Figure 2.1. Gel electrophoresis of the multiplex PCR amplification products. Direct analysis of amplicon size and band pattern allowed the determination of the molecular serotypes of the GBS reference strains used as the following: lane 1: NCTC 11078 (A909, Ia); lane 2: NCTC 8187 (H36B, Ib); lane 3: NCTC 11079 (18RS21, II); lane 4: ATCC BAA22 (M781, III); lane 5: CNCTC 1/82 (IV); lane 6: ATCC BAA-611 (2603, V); lane 7: strain Prague 118754 (VI); lane 8: Prague 4/89 (7271, VII); lane 9: NCTC 9828 (M9, VIII); lane 10: strain ISS5611 (IX). M1: molecular marker 50 bp Step Ladder (Promega); M2: molecular marker 1 kb plus DNA Ladder (Life Technologies).(Taken from Imperi et al, 2010).

III. Multilocus Sequence Typing (MLST)

The purpose of Multilocus sequence typing is to see how molecular epidemiology of GBS from Ethiopia fit into the international GBS population structure. MLST was performed for all GBS isolates recovered as described by Jones *et al.* (2003). Briefly, fragments of the seven housekeeping loci were amplified with *Taq* polymerase and PCR systems. The amplified fragments were purified and sequenced with the BigDye terminator v1.1 (Applied Biosystems). The nucleotide sequences were determined with an ABI prism sequencer (Applied Biosystems) and were analyzed by the use of the DNASIS program (Hitachi Genetic Systems, Berlin, Germany). Alleles for seven loci were assigned on the MLST website (http://pubmlst.org/sagalactiae/) and each isolate was defined by the sequence ST based on the unique allelic profile. The new alleles was re-amplified and re-sequenced. The

program eBURST was used to group isolates into lineages or CCs, where each isolate had identical alleles at six or more loci with at least one another member of the group (Annex VII). Primers for amplification and seqeucning for MLST are listed in Table 2.4.

Table 2.4. List of Primers for seven housekeeping loci which were used for MLST (adopted from Jones *et al,* 2003).

locus	use	Forward (5'to3')	Reverse (5'to3')	Amplicon size (bp)
adhP	amplification	CTTGGTCATGGTGAAGCACT	ACTGTACCTCCAGCACGAAC	672
	Sequencing	GGTGTGTGCCATACTGATTT	ACAGCAGTCACAACCACTCC	498
pheS	amplification	GATTAAGGAGTAGTGGCACG	TTGAGATCGCCCATTGAAAT	723
	Sequencing	ATATCAACTCAAGAAAAGCT	TGATGGAATTGATGGCTATG	501
atr	amplification	CGATTCTCTCAGCTTTGTTA	AAGAAATCTCTTGTGCGGAT	627
	Sequencing	ATGGTTGAGCCAATTATTTC	CCTTGCTCAACAATAATGCC	501
glnA	amplification	CCGGCTACAGATGAACAATT	CTGATAATTGCCATTCCACG	589
	Sequencing	AATAAAGCAATGTTTGATGG	GCATTGTTCCCTTCATTATC	498
sdhA	amplification	AGAGCAAGCTAATAGCCAAC	ATATCAGCAACAAGTGC	649
	Sequencing	AACATAGCAGAGCTCATGAT	GGGACTTCAACTAAACCTGC	519
glcK	amplification	CTCGGAGGAACGACCATTAA	CTTGTAACAGTATCACCGTT	607
	Sequencing	GGTATCTTGACGCTTGAGGG	ATCGCTGCTTTAATGGCAGA	459
tkt	amplification	CCAGGCTTTGATTTAGTTGA	AATAGCTTGTTGGCTTGAAA	859
	Sequencing	ACACTTCATGGTGATGGTTG	TGACCTAGGTCATGAGCTTT	480

IV. Resistance gene detection

Whole genome sequencing (WGS) was done to screen antibiotic resistance gene and extract other data as described by Metcalf *et al.* (2017). Briefly, *Streptococcus agalactiae* strains were cultured on Trypticase soy agar supplemented with 5% sheep blood and incubated overnight at 37oc. Genomic DNA for short-read WGS was extracted manually using a modified QIAamp DNA mini kit protocol (Qiagen, Inc, Valenica, CA, USA). Nucleic acid concentration was quantified by nitrogen Qubit assay (Thermo Fisher Scientific Inc., Waltham, MA, USA) and samples were sheered using a Covaris M220 ultrasonicator (Covaris, Inc., Woburn, MA, USA) programmed to generate 500-bp fragments. Libraries were constructed on the SciCloneG3 (PerkinElmer Inc., Waltham, MA, USA) using a

TruSeq DNA PCR-Free HT library preparation kit with 96 dual indices (Illumina Inc., San Diego, CA, USA) and quantified by a KAPA qPCR library quantification method (Kapa Biossystem Inc., Wilmngton, MA, USA). WGS was generated with MiSeq v2 500 cycle kit (Illumina Inc). WGS-based identification of serotypes, MLST and antimicrobial resistance determinants were determined using CDC Streptococcus Laboratory GBS pipeline.

2.10. REFERENCE STRAINS

Enterococcus faecalis (ATCC 29212), *Staphylococcus aureus* (ATCC 24923), *Streptococcus pneumoniae* (ATCC 6305), *Streptococcus pyogenes* (ATCC 19615), and *Streptococcus agalactiae* (ATCC12403) were used as a quality control throughout the study.

2.11. STATISTICAL ANALYSIS

Data entry and analysis was done using computer with SPSS version 20 software. Prevalence figures were calculated for the total study population of each study site and separately by age group and risk factors. Chi-square and other test were used to compare the results obtained from pregnant women and their newborn with different age groups and risk factors. P value less than 0.05 was considered significant.

2.12. ETHICAL CONSIDERATION

The Project was approved and ethically cleared by the Department of Microbiology, Immunology & Parasitology research ethics committee, the Institutional Review Board of the College of Health Sciences, Addis Ababa University (ref no. 069/13/DMIP) and the National Ethics and Research Committee (ref no.3.10/795/06). Official permission from the study sites was obtained. Written informed consent was obtained from all study participants. The parents/guardians were consented for permission to collect samples from their new born. The laboratory findings were communicated to the attending physician/s for further treatment and management of neonates with culture proven GBS for sepsis and meningitis.

CHAPTER III: RESULTS

3.1. SOCIO-DEMOGRAPHIC DATA OF STUDY POPULATION

Overall a total of 1873 participants were involved in the present study; 840 of them were pregnant women; 857 of them were their newborn (17 of them were twins) and 176 were those suspected of early and late onset neonatal disease due to GBS. The study participants were from Adama Hospital Medical College (AHMC), Hawassa Referral Hospital (HRH) and Tikur Anbessa Specialized Hospital (TASH). Majority of study participnats were from Town/City, majority of them were house wives, and majority of them belong to age group of 21-25.

3.1.1. Adama Hospital Medical College (AHMC)

A total of 280 pregnant women along with their 282 newborn (two of them were twins) participated in the present study. Majority of the participants were from Adama city 193(68.9%) followed by Lume 17(6.1%) and Fentale 11(3.9%). Most of study participants were house wives 264(94.3%). Most of them belong to age group of 21-25 113(40.4%). The sex of their newborns, male accounted for 137(48.6%) and female 145(51.4%) (Table 3.1).

Table 3.1. Socio-demographic characteristics of pregnant women attending Adama Hospital Medical from June 2014 to October 2014.

Characteristics		No.	Percent (%)
Address	Adama	193	68.9
	Lume	17	6.1
	Fentale	11	3.9
	Boset	10	3.6
	Minjar	9	3.2
	Dodota	9	3.2
	Mojo	3	1.1
	Jiju	3	1.1
	Sire	3	1.1
	Awash melkasa	2	0.7
	Dhera	2	0.7
	Awash	2	0.7
	Others	4	1.4
	Total	**280**	**100**
Occupation	House wife	264	94.3
	Nurse	3	1.1
	Merchant	3	1.1
	Street dweller	2	0.7
	Others	4	1.4
	Total	**280**	**100**
Age	16-20	52	18.6
	21-25	113	40.4
	26-30	84	30
	31-35	26	9.3
	36-40	5	1.8
	Total	**280**	**100**
Sex of their newborn	Male	137	48.6
	Female	145	51.4
Total		**282**	**100**

3.1.2. Hawassa Referral Hospital (HRH)

A total of 280 pregnant women along with their 292 newborn (twelve of them were twins) were participated in the present study. Majority of the participants were from Hawassa city 147(52.5%), followed by Shashamane 40(14.3%), Wondogenet 17(6.1%), and Halaba 9(3.2%). Majority of the participants were house wives 232(82.9%); most of them belongs to the age group of 21-25 111(39.6%); the sex of their newborn, male accounted for 152(52.1%) and female 140(47.9%) (Table 3.2).

Table 3.2. Socio-demographic characteristics of pregnant women attending Hawassa Referral Hospital from 2014 to March 2015.

Characteristics		No.	Percent (%)
Address	Hawassa	147	52.5
	Shashamane	40	14.3
	Wondogenet	17	6.1
	Halaba	9	3.2
	Tula	9	3.2
	Dore	5	1.8
	Dodola	5	1.8
	Arsi negele	7	2.5
	Dore bafana	4	1.4
	Asasa	3	1.1
	Kokosa	3	1.1
	Dato	3	1.1
	Others	28	10
	Total	**280**	**100**
Occupation	House wife	232	82.9
	Nurse	12	4.3
	Civil servant	7	2.5
	Teacher	5	1.8
	Student	4	1.4
	Day worker	4	1.4
	Merchants	4	1.4
	Cleaner	2	0.7
	Private work	2	0.7
	NGO	2	0.7
	Others	6	2.14
	Total	**280**	**100**
Age	16-20	40	14.3
	21-25	111	39.6
	26-30	92	32.9
	31-35	31	11.1
	36-40	6	2.1
	Total	**280**	**100**
Sex of their newborn	Male	152	52.1
	Female	140	47.9
Total		**292**	**100**

3.1.3. Tikur Anbessa Specialized Hospital (TASH)

A total of 280 pregnant mothers along with their 283 newborn (three of them were twins) were participated in the present study. Majority of the participants were from Kolfe keranio sub-city 115(41.1%) and followed by Lideta 68(24.3%); majority of the participants were house wives 190(67.9%); most of the participants belongs to the age group of 26-30 115(41.1%); the sex of their newborn, male accounted for, 158(55.8%) female, 125(44.2%) (Table 3.3).

Table 3.3 Socio-demographic characteristics of pregnant women attending Tikur Anbessa Specialized Hospital from March 2015 to August 2015.

	Description	**No.**	**Percent (%)**
Address	Kolfe keranio	115	41.1
	Lideta	68	24.3
	Addis ketema	14	15
	Sebeta	8	2.9
	Bishoftu	8	2.9
	Lafto	8	2.9
	Arada	8	2.9
	Nifas silk	7	2.5
	Adama	7	2.5
	Yeka	5	1.8
	Bole	5	1.8
	Arsi	4	1.4
	Gulale	23	8.2
	Total	**280**	**100**
Occupation	House wife	190	67.9
	Merchants	29	10.4
	Civil servant	18	6.4
	Private work	14	5
	Student	7	2.5
	Cleaner	3	1.1
	Teacher	2	0.7
	Tailor	2	0.7
	others	15	5.4
	Total	**280**	**100**
Age	16-20	23	8.2
	21-25	103	36.8
	26-30	115	41.1
	31-35	27	9.6
	36-40	11	3.6
	41-45	1	0.4
	total	**280**	**100**
Sex of their newborn	Male	158	55.8
	Female	125	44.2
Total		**283**	**100**

3.2. OBSTETRICS AND HEALTH CHARACTERISTICS OF PREGNANT WOMEN AND THEIR NEWBORN

a. Obstetrics and Health Characteristics of Pregnant Mothers at AHMC

Among 280 pregnant women who participated in the present study, most of them delivered at gestational age of 37-42 weeks (86.1%), those with primigravida accounted for 55%, those with multigravida accounted for 45%, participants with history of newborn who developed EOD were 0.4%; those with previous vaginal delivery accounted for 42.8%, (Table 3.4).

Table 3.4. Gestational characteristics of 280 pregnant women attending Adama Hospital Medical College from June 2014 to October 2014.

Characteristics n=280		No.	Percent (%)
Gestational age in weeks	<37	28	10
	37-42	241	86.1
	>42	11	3.9
Gravida	Primigravida	154	55
	Multigravida	126	45
History of EOD	yes	1	0.4
	No	125	44.6
	NA	154	55
Previous mode of delivery	Vaginal Delivery	120	42.8
	Cesarean section	4	1.4
	Abortion	1	0.4
	NA	154	55
	Instrument	1	0.4
Duration of rupture of membrane	0-5hr	206	73.6
	6-10	45	16.1
	11-15	14	5
	16-20	5	1.8
	21-25	4	1.4
	>25	6	2.1
Premature Rupture of Membrane (PROM)	Yes	26	9.3
	No	254	90.7
Chorioamnionitis	Yes	3	1.1
	No	277	99.9
Meconium stained amniotic fluid	Yes	57	20.21
	No	223	79.08
Other illness	Yes	29	10.4
	No	251	89.6

NA: Not applicable for primigravida

b. Health Characteristics of their Newborn at AHMC

Among 282 newborn who participated in the present study, those who were alive during birth were 269 (95.4%), most of the newborn were in weight range of 2500-4000g 246 (87.2%). Newborn with APGAR score at 5 minutes >7 were 179 (63.5%), those with APGAR score at 10 minutes >7 were 247 (87.6%); Newborn with other abnormalities were 18 (6.4%). The prevalence of newborn who were not dead during birth was 13(4.6%) (Table 3.5).

Table 3.5. Clinical characteristics of new born delivered at Adama Hospital Medical College from June 2014 to October 2014.

Characteristics n=282		**frequency**	**percent**
Status of newborn during birth & immediate after birth	Alive	269	95.4
	Dead	13	4.6
Weight	<1500g	2	0.7
	1500-2499g	24	8.5
	2500-4000g	246	87.2
	>4000	10	3.5
APGAR** score at 5 minute	≤7	103	36.5
	>7	179	63.5
APGAR score at 10 minute	≤7	35	12.4
	>7	247	87.6
Developed EOD	Yes	4	1.4
	No	278	98.6
Other abnormality*	Yes	18	6.4
	No	264	93.6

*Hydrocepaly, Anacephaly, Down syndrome, Macerated IUFD

APGAR: Appearance, Pulse, Grimace, Activity, Respiration

c. Obstetrics and Health Characteristics of Pregnant Mothers at HRH

Among 280 pregnant women who participated in the study, most of them delivered at gestational age of 37-42 weeks 249 (88.9%), those with primigravida were 134 (47.9%), multigravida 146 (52.1%), those with previous vaginal delivery accounted for 115 (41.1%); most of them had duration of rupture of membrane of 0-5 hours 241 (86.1%); those with premature rupture of membrane (PROM) were 18(6.4%) (Table 3.6).

Table 3.6. Gestational characteristics of 280 pregnant women attending| Hawassa Referral Hospital from November 2014 to March 2015.

Characteristics n=280		No.	%
Gestational age in weeks	<37	25	8.9
	37-42	249	88.9
	>42	6	2.1
Gravida	Primigravida	134	47.9
	Multigravida	146	52.1
History of EOD	No	146	52.1
	NA*	134	47.9
Previous mode of delivery	Spontaneous Vaginal delivery (SVD)	115	41.1
	Cesarean section	30	10.7
	NA	134	47.9
	SVD-CS	1	0.4
Duration of rupture of membrane	0-5hr	241	86.1
	6-10	15	5.4
	11-15	10	3.60
	16-20	1	0.40
	21-25	4	1.40
	>25	9	3.20
Premature rupture of membrane (PROM)	Yes	18	6.4
	No	262	93.6
Chorioamnionitis	Yes	2	0.7
	No	278	99.3
Meconium stained amniotic fluid	Yes	36	12.33
	No	244	87.14
Other illness	Yes	11	3.9
	No	269	96.1

***NA: Not applicable for primigravida, SVD: spontaneous vaginal delivery, CS: Cesceral section**

d. Health Characteristics of their Newborn at HRH

Among 292 newborn participated in the present study, those who were alive during birth were 280 (95.9%); most of them were in weight range of 2500-4000g, 248 (84.9%); most of them had APGAR score at 5 minutes >7, 161 (55.1%), APGAR score at 10 minutes >7, 257 (88%); those with other abnormalities were 13 (4.5%). The prevalene of newborn who were dead during birth was 12/292(4.1%) (Table 3.7).

Table 3.7. Clinical characteristics of new born delivered at Hawassa Referral Hospital from 2014 to March 2015.

Characteristics		frequency	percent
Status of newborn during birth & immediate after birth	Alive	280	95.9
	Dead	12	4.1
Weight	<1500g	4	1.4
	1500-2499g	28	9.6
	2500-4000g	248	84.9
	>4000	12	4.1
APGAR** score at 5 minutes	≤7	131	44.9
	>7	161	55.1
APGAR score at 10 minutes	≤7	35	12
	>7	257	88
Developed EOD	Yes	5	1.7
	No	287	98.3
Other abnormality*	Yes	13	4.5
	No	279	95.5

*Cyanosis, Hydrocephaly, Spinabefida, Overweight, Underweight

**APGAR: Appearance, Pulse, Grimace, Activity, Respiration

e. Obstetrics and Health Characteristics of Pregnant Mothers at TASH

Among 280 pregnant mothers participated in the present study, most of them were delivered at gestational age of 37-42 weeks 223 (79.6%), those with primigravida accounted 133 (47.5%), those with multigravida accounted 147 (52.5%), participants with history of

newborn who developed EOD were 32 (11.4%); participants with previous vaginal delivery accounted for 129 (46.1%); most of them had duration of rupture of membrane of 0-5 hours 179 (63.9%); those with PROM were 14 (5%) (Table 3.8).

Table 3.8. Gestational characteristics of 280 pregnant mothers attending Tikur Anbessa Specialized Hospital from March 2015 to August 2015.

Characteristics n=280		**Frequency**	**Percent (%)**
Gestational age in weeks	<37	37	13.2
	37-42	223	79.6
	>42	20	7.1
Gravida	primigravida	133	47.5
	Multigravida	147	52.5
History of EOD	yes	32	11.4
	No	115	41.1
	NA	133	47.5
Previous mode of delivery	SVD	129	46.1
	CS	14	5
	Instrument	1	0.4
	SVD/CS	3	1.1
	NA	133	47.5
Duration of rupture of membrane	0-5hr	179	63.9
	6-10	66	23.6
	11-15	10	3.6
	16-20	10	3.6
	21-25	4	1.4
	>25	11	3.6
Premature rupture of membrane (PROM)	Yes	14	5
	No	266	95
Chorioamnionitis	Yes	1	0.4
	No	279	99.6
Meconium stained amniotic fluid	Yes	9	3.2
	No	271	96.8
Other illness	Yes	35	12.5
	No	245	87.5

f. Health Characteristics of their Newborn at TASH

Among 283 newborn participated in the present study, those who were alive during birth were 270 (95.4%); most of them were in weight range of 2500-4000g 236 (83.4%); most of them had APGAR score at 5 minutes >7, 200 (70.7%), APGAR score at 10 minutes >7, 257 (90.8%); those with other abnormalities were 18 (6.4%). The prevalene of newborn who were dead during birth was 13(4.6%) (Table 3.9).

Table 3.9. Clinical characteristics of new born delivered at Tikur Anbessa Specialized Hospital from March 2015 to August 2015.

Characteristics		frequency	percent
Newborn status during birth & immediate after birth	Alive	270	95.4
	Dead	13	4.6
Weight	<1500g	16	5.7
	1500-2499g	31	111
	2500-4000g	236	83.4
	>4000	0	0
APGAR score at 5 minute**	≤7	83	29.3
	>7	200	70.7
APGAR score at 10 minute	≤7	26	9.2
	>7	257	90.8
Developed EOD	Yes	6	2.1
	No	277	97.8
Other abnormality*	Yes	18	6.4
	No	265	93.7

*Cervical bone fracture, Cleft lip and palate, bradic cardiac, Respiratory distress **APGAR: Appearance, Pulse, Grimace, Activity, Respiration

3.3. GBS COLONIZATION RATE IN PREGNANT WOMEN AND THEIR NEWBORN

3.3.1. Overall GBS colonization rate in three selected hospitals

Out of 840 pregnant women screened for GBS, 146 (17.4%) 95% CI(14.9-20) were colonized with GBS. Out of 857 of their new born screened for GBS, 79 (9.2%) 95% CI(7.4-11.1) were colonized with GBS. The overall GBS colonization rate in both pregnant women and their new born was 225/1697 (13.3%). The overall vertical transmission rate of GBS from pregnant women to their new born was 79/146(54.1%) (Table 3.10).

3.3.2. Adama Hospital Medical college

Out of 280 pregnant women screened for GBS, 37 (13.2%) 95%CI(8.9-17.5) were colonized with GBS. Out of 282 new born 21 (7.4%) 95%CI(4.6-10.6) were colonized with GBS. Over all GBS colonization rate in both pregnant women and new born at AHMC was 58/562 (10.3%). Vertical transmission rate GBS from Pregnant women to newborn was 21/37 (56.8%) (Table 3.10).

3.3.3. Hawassa Referral Hospital

Out of 280 pregnant women screened for GBS, 44 (15.7%) 95% CI (11.8-20.0) were colonized with GBS. Out of 292 newborn, 26 (8.9%) 95% CI (5.8-12.3) were colonized with GBS. Overall GBS colonization rate in both pregnant women and new born at Hawassa Referral Hospital was 70/572 (12.2%). Vertical transmission rate GBS from Pregnant women to newborn was 26/44 (59.1%) (Table 3.10).

3.3.4. Tikur Anbessa Specialized Hospital

Out of 280 pregnant women screened for GBS, 65 (23.2%) 95%CI(18.6-28.9) were colonized with GBS. Out of 283 newborn, 32 (11.3%) 95%CI(7.8-15.2) were colonized with GBS. Over all GBS colonization rate in both pregnant women and new born at Tikur Anbessa Specialized Hospital was 97/563 (17.2%). Vertical transmission rate GBS from Pregnant women to newborn was 32/65 (49.2%) (Table 3.10).

Table 3.10. Site specific and overall GBS colonization Rate and Vertical transmission rate in three study sites (AHMC, HRH, TASH).

Characteristics	AHMC		HRH		TASH		Total	
	x/N	%	x/N	%	x/N	%	x/N	%
Maternal colonization rate	37/280	13.2	44/280	15.71	65/280	23.21	146/840	17.4
Newborn colonization rate	21/282	7.4	26/292	8.9	32/283	11.31	79/857	9.2
Overall colonization rate	58/562	10.3	70/572	12.24	97/563	17.23	225/1697	13.3
Vertical transmission rate	21/37	56.8	26/44	59.1	32/65	49.2	79/146	54.1

AHMC: Adama Hospital Medical College, HRH: Hawassa Referral Hospital, TASH: Tikur Anbessa Specialized Hospital

3.4. SEROTYPE DISTRIBUTION OF GBS ISOLATES IN PREGNANT WOMEN AND THEIR NEWBORN

3.4.1. Overall serotype distribution GBS isolates in three selected hospitals

The overall serotype distribution of GBS isolates (228) collected from pregnant women (146), their Newborn (79) and newborn with neonatal disease due to GBS (3) is as follows; II 69(30.3%), Ia 46(20.2%), V 41(17.9%), Ib 39(17.1%), III 23(10.1%), VII 2(0.9%), Non typable 8(3.5%) (Figure 3.1 and 3.2).

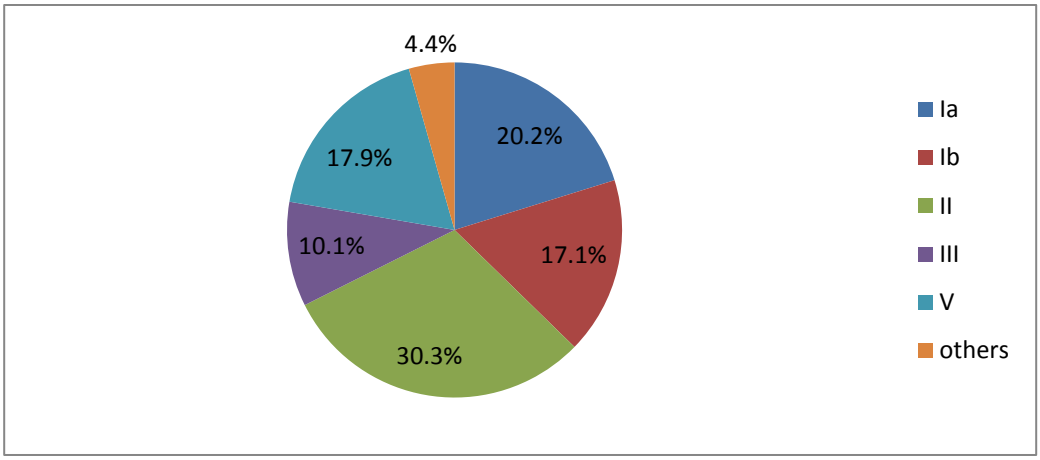


Figure 3.1 Overall serotype distributions of GBS collected from pregnant women, their newborn and newborn with neonatal disease (others include serotype VII and non typable).

The serotypes distribution of GBS isolates in pregnant women in three hospitals is as follows: out of 146, 29(19.9%) of GBS were serotype Ia; 23(15.8%) of them were serotype

Ib; 43(29.5%) of them were serotype II; 13(8.9%) of them were serotype III; 31(21.2%) of them were serotype V; 1(0.69%) of them was serotype VII; 6(4.1%) of them were non typable.

The serotypes distribution of GBS isolates in their new born in three hospitals is as follows, out of 79 GBS isolates 15(18.9%) of them were serotype Ia; 16(20.3%) of them were serotype Ib; 26(32.9%) of them were serotype II; 9(11.4%) of them were serotype III; 10(12.7%) of them were serotype V; 1(1.3%) of them was serotype VII; 2(2.5%) of them were non typable.

Overall serotype distribution of GBS isolates in both pregnant women and their newborn is as follows, out of 225 GBS isolates from pregnant mother and their new born, 69 (30.7%) of them were serotype II; 41(18.2%) of them were serotype V; 44 (19.6%) of them were serotype Ia; 39(17.3%) of them were serotype Ib; 22 (9.8%) of them were serotype III; 2 (0.9%) of them were type VII; 8 (3.6%) of them were Non typable. Over all GBS Vertical Transmission rate was 79/146(54.1%) (Table 3.11 and Figure 3.2).

Table 3.11. The overall serotypes distribution of GBS isolated from pregnant women and their new born and vertical transmission rate in three Hospitals (AHMC, HRH, TASH) (June 2014- August 2015).

Serotype	Pregnant women	Newborn	Pregnant women and Newborn	VTR*
Ia	29/146(19.9%)	15/79(18.9%)	44/225(19.6%)	15/29(51.7%)
Ib	23/146 (15.8%)	16/79(20.3%)	39/225(17.3%)	16/23(69.6%)
II	43/146 (29.5%)	26/79(32.9%)	69/225 (30.7%)	26/43(60.5%)
III	13/146 (8.9%)	9/79 (11.4%)	22/225(9.8%)	9/13(69.2%
V	31/146(21.2%)	10/79(12.7%)	41/225 (18.2%)	10/31(32.3%)
VII	1/146(0.7%)	1/79 (1.3%)	2/225 (0.9%)	1/1(100%)
NT	6/146(4.1%)	2/79 (2.5%)	8/225 (3.6%)	2/6(33.3%)
Total	**146/840 (17.4%)**	**79/857 (9.2%)**	**225/1697 (13.3%)**	**79/146(54.1%)**

***Vertical transmission rate**

3.4.2. Adama Hospital Medical College

The serotypes distribution of GBS isolates from in pregnant women from AHMC site is as follows, out of 37 GBS isolates 6 (16.2%) of them were serotype Ia, 8(21.6%) them were serotype Ib, 10(27%) of them were serotype II, 3(8.1%) of them were serotype III, 8(21.6%) of them were serotype V, 1(2.7%) of them was serotype VII, 1(2.7%) of them was non typable.

The serotypes distribution of GBS isolates in newborn were, out of 21 GBS isolates 3 (14.3%) of them were serotype Ia, 6(28.6%) of them were serotype Ib, 6(28.6%) of them were serotype II, 4(19%) of them were serotype III, 1(4.8%) of them was serotype V, and 1(4.8%) of them was serotype VII.

The overall serotype distribution of GBS isolates in pregnant women and newborn is as follows: out of 58 GBS isolates, 9 (15.5%) of them were serotype Ia, 14(24.1%) of them were serotype Ib, 16(27.6%) of them were serotype II, 7(12.1%) of them were serotype III, 9(15.5%) of them were serotype V, 2(3.5%) of them was serotype VII, 1(1.7%) of them was non typable. Vertical transmission rate of GBS was 21/37(56.8%) (Table 3.12).

Table 3.12. Serotypes distribution of GBS isolated from pregnant women and their new born and vertical transmission rate, Adama Hospital Medical College (June 2014-October 2014).

Serotype	Pregnant women	Newborn	Pregnant women & Newborn	VTR (%)
Ia	6/37(16.2%)	3/21(14.3%)	9/58(15.5%)	3/6(50%)
Ib	8/37(21.6%)	6/21(28.6%)	14/58(24.1%)	6/8(75%)
II	10/37(27%)	6/21(28.6%)	16/58(27.6%)	6/10(60%)
III	3/37(8.1%)	4/21(19%)	7/58(12.1%)	4/3(133%)
V	8/37(21.6%)	1/21(4.8%)	9/58(15.5%)	1/8(12.5%)
VII	1/37(2.7)	1/21(4.8)	2/58(3.5%)	1/1(100%)
NT	1/37(2.7)	0/20(0)	1/58(1.7%)	0/1(0)
Total	**37**	**21**	**58**	**21/37(56.8)**

3.4.3. Hawassa Referral Hospital

The serotypes distribution of GBS isolates in pregnant women is as follows: out of 44 GBS isolates 9 (20.5%) of them were serotype Ia, 5(11.36%) of them were serotype Ib, 14(31.8%) of them were serotype II, 6 (13.6%) of them were serotype III, 8(18.2%) of them were serotype V, 2(4.6%) of them were non typable.

The serotypes distribution of GBS isolates in newborn is as follows: out of 26 GBS isolates 4 (14.8%) of them were serotype Ia, 4(14.8%) of them were serotype Ib, 10(37%) of them were serotype II, 2(7.41%) of them were serotype III, 6(22.2%) of them was serotype V.

The overall serotype distribution of GBS isolates in pregnant women and newborn is as follows: out of 70 GBS isolates, 13 (18.6%) of them were serotype Ia, 9(12.9%) of them were serotype Ib, 24(34.3%) of them were serotype II, 8(11.4%) of them were serotype III, 14(20%) of them were serotype V, 2(2.9%) of them was non typable. Vertical transmission rate of GBS was 26/44(59.1%) (Table 3.13).

Table 3.13. Serotypes distribution of GBS isolated from pregnant women and their new born and vertical transmission rate, Hawassa Referral Hospital (November 2014-March 2015).

Serotype	Pregnant women	newborn	Pregnant women & newborn	VTR
Ia	9/44(20.5%)	4/27(14.8%)	13/70(18.6%)	4/9(44.4%)
Ib	5/44(11.4%)	4/27(14.8%)	9/70(12.9%)	4/5(80%)
II	14/44(31.8%)	10/27(37%)	24/70(34.3%)	10/14(71.4%)
III	6/44(13.6%)	2/27(7.4%)	8/70(11.4%)	2/6(33.3%)
V	8/44(18.2%)	6/27(22.2%)	14/70(20%)	6/8(75%)
NT	2/44(4.6%)	0/27(0)	2/70(2.9%)	0/4(0)
Total	**44**	**26**	**70**	**26/44(59.1)**

3.4.4. Tikur Anbessa Specialized Hospital

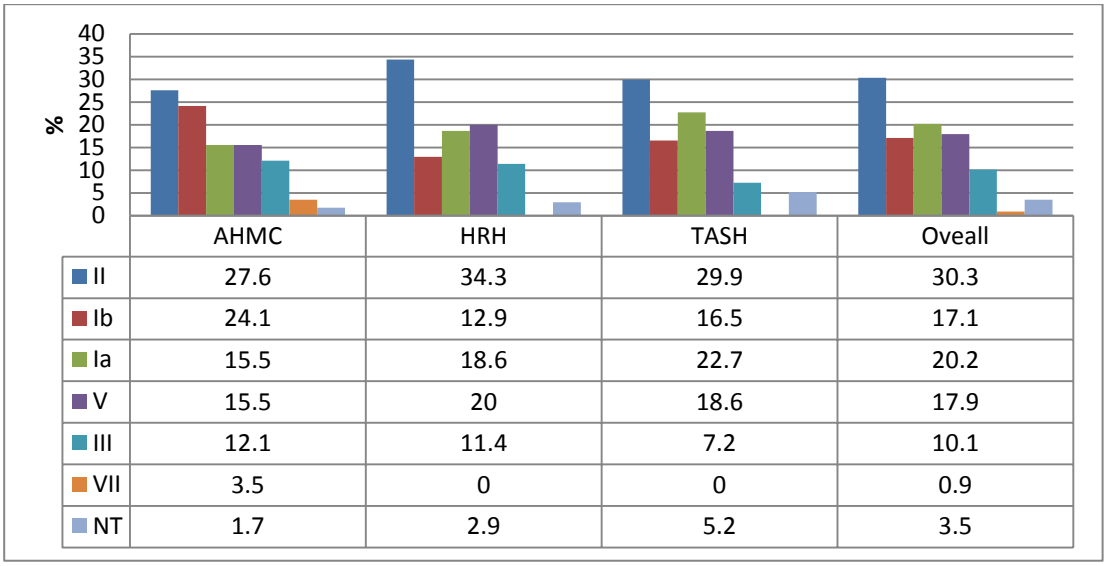
The serotypes distribution of GBS isolates in pregnant women were, out of 65 GBS isolates 14 (21.5%) of them were serotype Ia, 10(15.4%) of them were serotype Ib, 19(29.2%) of them were serotype II, 4 (6.2%) of them were serotype III, 15(23.1%) of them were serotype V, 3(4.6%) of them were non typable.

The serotypes distribution of GBS isolates in newborn were, out of 32 GBS isolates 8 (25%) of them were serotype Ia, 6(18.8%) of them were serotype Ib, 10(31.3%) of them were serotype II, 3(9.4%) of them were serotype III, 3(9.4%) of them was serotype V, 2(6.3%).

The overall serotype distribution of GBS isolates in pregnant women and newborn is as follows: out of 97 GBS isolates, 22 (22.7%) of them were serotype Ia, 16(16.5%) of them were serotype Ib, 29(29.9%) of them were serotype II, 7(7.2%) of them were serotype III, 18(18.6%) of them were serotype V, 5(5.2%) of them was non typable. Vertical transmission rate of GBS was 32/65(49.2%) (Table 3.14).

Table 3.14. Serotypes distribution of GBS isolated from pregnant women and their new born and vertical transmission rate, Tikur Anbessa Specialized Hospital (March 2015-August 2015).

Serotype	**Pregnant women**	**Newborn**	**Pregnant women & newborn**	**VTR**
Ia	14/65(21.5%)	8/32(25%)	22/97(22.7%)	8/14 (57.1%)
Ib	10/65(15.4%)	6/32(18.8%)	16/97(16.5%)	6/10 (60%)
II	19/65(29.2%)	10/32(31.3%)	29/97(29.9%)	10/19 (52.6%)
III	4/65(6.2%)	3/32(9.4%)	7/97(7.2%)	3/4 (75%)
V	15/65(23.1%)	3/32(9.4%)	18/97(18.6%)	3/15 (20%)
NT	3/65(4.6%)	2/32(6.3%)	5/97(5.2%)	2/3 (66.7%)
Total	**65**	**32**	**97**	**32/65(49.2)**



	AHMC	HRH	TASH	Oveall
II	27.6	34.3	29.9	30.3
Ib	24.1	12.9	16.5	17.1
Ia	15.5	18.6	22.7	20.2
V	15.5	20	18.6	17.9
III	12.1	11.4	7.2	10.1
VII	3.5	0	0	0.9
NT	1.7	2.9	5.2	3.5

Figure 3.2 Serotype distribution of GBS across study sites; AHMC, HRH, TASH and overall serotype distribution.

3.5. RISK FACTORS ANALYSIS FOR GBS COLONIZATION

3.5.1. Adama Hospital Medical College

a. Pregnant women

Risk factor analysis was performed for maternal GBS colonization from Adama Hospital Medical College is shown in Table 3.15. None of the variables have statistically significant associated with maternal GBS colonization. High colonization rate was observed in pregnant women whose gestation age is 37-42, whose duration of rupture is 0-5hr and those who are not experiencing PROM.

Table 3.15. Risk factors analysis for maternal GBS colonization, Adama Hospital Medical College AHMC (June 2014-October 2014).

Variables		Prevalence of GBS N=280, total GBS=37	OR(95%CI)	P-value*
Age group	15-27	27/201(13.4%)	1.2(0.54-2.69)	0.65
	≥28	10/79(12.7%)	1	
Gestational age	<37	2/27 (7.4%)	0.49(0.11-2.2)	0.35
	37-42+	35/253(13.8%)	1	
Gravida	Primigravida	21/150(14%)	1.1(0.54-2.2)	0.82
	Multigravida	16/130(12.3%)	1	
Pervious mode of delivery	Vaginal	16/125(12.8%)	0.9(0.47-1.93)	0.8
	CS, Instrument & abortion	1/5(20%)	1.6(0.17-15.2)	0.6
	NA	19/150(12.6%)	1	
Duration of rupture of membrane	0-5hr	28/206(13.6%)	0.98(0.32-3.03)	0.98
	6-10	5/45(11.1%)	0.78(0.19-3.19)	0.73
	11-15+	4/29(13.8%)	1	
Premature rupture of membrane (PROM)	Yes	4/26(15.4%)	1.22(0.39-3.76)	0.73
	No	33/254(12.9%)	1	
Meconium stained amniotic fluid	Yes	4/57(7.01%)	0.4(0.15-1.28)	0.13
	No	33/223(14.8%)	1	

*calculated by using binary logistic regression

b. Their New born

Risk factor analysis was performed for Newborn GBS colonization from Adama Hospital Medical College is shown in Table 3.16. None of the variables were statistically significant associated with maternal GBS colonization. Even though it is not significant, high colonization rate was observed in newborn whose weight is within 2500-4000g range. Out of 13 newborn who were born dead, 1(7.7%) of them was colonized with GBS. Out of 30 newborn who were born in <37 week of gestation 1(3.3%) of them were colonized with GBS. Out of 4 newborn who developed neonatal disease 1(25%) was colonized with GBS.

Table 3.16. Risk factors analysis for newborn GBS colonization, Adama Hospital Medical College AHMC (June 2014-October 2014).

Variables		Prevalence of total N=282 GBS=21	OR(95%CI)	P-value**
Week of birth	<37	1/30(3.3%)	0.4(0.05-3.1)	0.38
	37-42+	20/252 (7.9%)	1	
APGAR* score at 5'	≤7	5/103 (4.8%)	0.77(0.26-2.44)	0.63
	>7	16/179(8.9%)	1	
Status of new born during birth & immediate after birth	Dead	1/13 (7.7%)	1.03(0.12-7-8.4)	0.97
	Alive	20/269(7.4%)	1	
Developed EOD	Yes	1/4(25%)	3.89(0.38-38.7)	0.25
	No	20/278(7.2%)	1	
Other abnormality	Yes	1/18(5.6%)	0.78(0.9-5.6)	0.75
	No	20/264(7.6%)	1	

*APGAR: Appearance, Pulse, Grimace, Activity, Respiration

**calculated by using binary logistic regression

3.5.2. Hawassa Referral Hospital (HRH)

a. Pregnant women

Risk factor analysis was performed for maternal GBS colonization from Hawassa referral hospital is shown in Table 3.17. None of the variables have statistically significant associated with maternal GBS colonization. Even though it is not significant high colonization rate was observed in pregnant women whose gestation age is 37-42, whose duration of rupture is 0-5hr and those who are not experiencing PROM, whose previous mode of delivery is vaginal and those who are not experiencing chorioamnionitis.

Table 3.17. Risk factors analysis for maternal GBS colonization, Hawassa Referral Hospital (November 2014-March 2015).

Variables		Prevalence of GBS positive N=280 total GBS=44	OR(95%CI)	P-value*
Age group	15-27	28/190(14.7%)	0.79(0.41-1.56)	0.51
	≥28	16/90(17.8%)	1	
Gestational age	<37	4/25(16%)	0.9(0.08-10.5)	0.9
	37-42	38/249(15.3%)	0.93(0.1-8.1)	0.95
	>42	2/6(33.3%)	1	
Gravida	Primigravida	24/134(17.9%)	1.38(0.7-2.6)	0.34
	Multigravida	20/146(13.7)	1	
History of EOD	No	20/146(13.7%)	0.73(0.38-1.4)	0.34
	Not applicable	24/134(17.9%)	1	
Pervious mode of delivery	Vaginal	16/115(13.9%)	0.69(0.34-1.38)	0.29
	CS	4/31(12.9%)	0.88(0.31-2.53)	0.84
	Not applicable	24/134(17.9%)	1	
Duration of rupture of membrane	0-5hr	39/241(16.2%)	2.1(0.47-9.1)	0.34
	6-10hr	4/15(26.7%)	4(0.6-25.3)	0.14
	11-15hr+	1/24(4.2%)	1	
Meconium stained amniotic fluid	Yes	4/36(11.1%)	0.85(0.3-2.3)	0.75
	No	40/244(16.4%)	1	
Other illness	Yes	2/11(18.2%)	1.2(0.25-5.7)	0.82
	No	42/269(15.6%)	1	

≥15 hours *calculated by using binary logistic regression

b. Their newborn

Risk factor analysis was performed for Newborn GBS colonization from Hawassa referral hospital is shown in Table 3.18. None of the variables were statistically significant associated with maternal GBS colonization. Even though it is not significant high colonization rate was observed in Even though it is not significant high colonization rate was observed in newborn whose weight is within 2500-4000g range and whose APGAR score at 5 and 10 minute ≥7.

Out of 12 newborn who were born dead, 2(16.7%) of them were colonized with GBS. Out of 28 newborn who were born in <37 week of gestation 3(10%) of them were colonized with GBS. Out of 5 newborn who developed neonatal disease 1(20%) was colonized with GBS.

Table 3.18. Risk factors analysis for newborn GBS colonization, Hawassa Referral Hospital (November 2014-March 2015).

Variables		Prevalence of GBS, N=292 Total GBS=26	OR(95%CI)	P-value*
Weight	1000-2499g	3/32(9.4%)	1.5(0.27-9.11)	0.9
	2500-4000g+	23/260(8.8%)	1	
APGAR score at 5’	≤7	11/131(8.4%)	0.83(0.37-1.9)	0.65
	>7	15/161(9.3%)	1	
Week of birth	<37	3/28(10.7%)	0.6(0.05-7)	0.68
	37-42	22/258(8.5%)	0.47(0.05-4.37)	0.55
	>42	1/6(16.7%)	1	
APGAR score at 10’	≤7	5/35(14.3%)	1.78(0.62-5)	0.28
	>7	21/257(8.6%)	1	
Status of new born during birth & Immediate after birth	Dead	2/12(16.7%)	2.0(0.42-9.8)	0.37
	Alive	24/280(8.6%)	1	
Developed EOD	Yes	1/5(20%)	2.5(0.27-23.3)	0.42
	No	25/287(8.7%)	1	
Other disease	yes	4/13(30.8%)	4.95(1.41-17.3)	0.012
	No	22/279(7.9%)	1	

*calculated by using binary logistic regression +weight Greter than 4000g

3.5.3. Tikur Anbessa Specialized Hospital

a. Pregnant women

Risk factor analysis was performed for maternal GBS colonization from Tikur Anbessa Specialized Hospital is shown in Table 3.19. None of the variables were statistically significant associated with maternal GBS colonization. Even though it is not significant high colonization rate was observed in pregnant women whose gestation age is 37-42, whose duration of rupture of membrane is 0-5hr and those who are not experiencing PROM. There

is also high colonization rate among those whose duration of rupture of membrane is >5 hours.

Table 3.19. Risk factors analysis for maternal GBS colonization, Tikur Anbessa Specialized Hospital (March 2015-August 2015).

Variables		Prevalence of GBS N=280, Total GBS=65	OR(95%CI)	P value*
Age group	15-27	40/171(23.4%)	1.03(0.58-1.81)	0.93
	≥28	25/109(22.9%)	1	
Gestational age	<37	8/37(21.6%)	1.1(0.29-4.24)	0.87
	37-42	53/223(23.8%)	1.25(0.4-3.89)	0.70
	>42	4/20 (20%)	1	
Gravida	Primigravida	33/133 (24.8%)	1.18(0.68-2.1)	0.55
	Multigravida	32/147 (21.8%)	1	
History of EOD	Yes	10/32 (31.2%)	1.38(0.59-3.21)	0.46
	No	22/115 (19.1%)	0.72(0.39-1.32)	0.29
	NA	33/133(24.8%)	1	
Pervious mode of delivery	Vaginal	30/129 (23.3%)	0.92(0.5-1.62)	0.77
	CS, instrument	2/18(13.3%)	0.38(0.08-1.74)	0.9
	NA	33/133(24.8%)	1	
Duration of rupture of membrane	0-5hr	43/179(24%)	1.2(0.49-2.98)	0.68
	6-10hrs	15/66(22.7%)	1.13(0.41-3.12)	0.81
	11-15hrs	7/35(10%)	1	
PROM	Yes	3/14(21.4%)	0.89(0.24-3.32)	0.87
	No	62/266(23.3%)	1	-
Meconium stained amniotic fluid	Yes	2/9(22.2%)	0.94(0.19-4.66)	0.94
	No	63/271(23.2%)	1	
Other illness	Yes	5/33(15.2%)	0.55(0.21-1.51)	0.25
	No	60/247(24.3%)	1	

******calculated by using binary logistic regression

b. Their newborn

Risk factor analysis was performed for Newborn GBS colonization from Tikur Anbessa Specialilzed Hospital is shown in Table 3.20. None of the variables were statistically significant associated with maternal GBS colonization. Even though it is not significant high colonization rate was observed in newborn whose weight is within 2500-4000g range and whose APGAR score at 5 and 10 minute ≥7. Out of 13 newborn who were born dead, 2(15.4%) of them were colonized with GBS. Out of 16 newborn those whose weight is less

than <1500g, 2(12.5%) were colonized with GBS. Out of 40 newborn who were born in <37 week of gestation 4(10%) of them were colonized with GBS.

Table 3.20. Risk factors analysis for new born GBS colonization, Tikur Anbessa Specialized Hospital (March 2015-August 2015).

Variables		Prevalence of GBS, N=283 Total GBS=32	OR(95%CI)	P-value*
Weight	<1500g	2/16(12.5%)	1.2(0.24-5.33)	0.86
	1500-2499g	4/31(12.9%)	1.2(0.38-5.3)	0.38
	2500-4000g	26/235(11.1%)	1	
Week of birth	<37	4/40(10%)	1.0(0.17-5.98)	1.0
	37-42	26/223(11.66%)	1.2(0.27-5.6)	0.78
	>42	2/18(11.1%)	1	
APGAR score at 5minute	≤7	14/84(16.67%)	2.0(0.95-4.26)	0.06
	>7	19/199(9.55%)	1	
APGAR score at 10minute	≤7	4/27(14.8%)	1.42(0.46-4.4)	0.54
	>7	28/256(10.94%)	1	
Status of new born during birth	Dead	1/13 (7.7%)	0.6(0.081-5.13)	0.67
	Alive	32/270(11.8%)	1	
Developed EOD	Yes	0/6(0)	-	0.99
	No	32/277(11.5%)	1	
Other disease	Yes	1/18(5.6%)	0.4(0.057-3.45)	0.44
	No	31/265(11.7)	1	

*calculated by using binary logistic regression

3.6. BURDEN OF GBS IN NEONATAL DISEASES

3.6.1. Socio-demographic characteristics

A total of 176 newborn were with neonatal sepsis (n=170) and meningitis (n=6) were investigated with GBS. Most of them were from Addis Ababa 163(92.6%). Out of 176 infants less than 90 days participated in the present study, 163(92.6%) of them were those who developed EOD and 13(7.4%) were those who developed LOD. Out of 176 infants less than 90 days participated in the present study those whose age was less than 7 days accounted for 163(92.6%); male accounted for 73(41.5%); those whose weight was less than 1500g accounted for 19(10.8%); those whose APGAR score at 5 minutes less than 7 accounted for 61(34.7%) and those whose APGAR score at 10 minutes less than 7 accounted for 138(78.4%) (Table 3.21).

Table 3.21. Socio-demographic characteristics of newborn with sepsis and meningitis, Tikur Anbessa Specialized Hospital (March, 2015-September, 2015).

Characteristics		Frequency	percent
Address	Addis Ababa	163	92.6
	Oromia	12	6.8
	Amhara	1	0.6
Sex	Male	73	41.5
	Female	103	58.5
Age	<7	163	92.6
	≥7	13	7.4
Week of birth	<37	69	39.2
	37-42	95	54.0
	>42	12	6.8
Weight	<1500	19	10.8
	1500-2499g	60	34.1
	2500-4000g	94	53.4
	>4000g	3	1.7
APGAR score at 5 minute	≥7	115	65.3
	<7	61	34.7
APGAR score10 minute	≥7	38	21.6
	<7	138	78.4
Clinical diagnosis	Sepsis	170	96.6
	Meningitis	6	3.4

3.6.2. Clinical characteristics of newborn with sepsis and meningitis

Out of 176 newborn who were suspected of having neonatal disease 163(92.6%) of them were hypothermic, 151(85.8%) of them experienced breathing problem (Table 3. 22).

Table 3.22. Clinical characteristics of newborn with sepsis and meningitis, Tikur Anbessa Specialized Hospital (March, 2015-September, 2015).

Clinical Presentation		**Frequency**	**Percent**
Hypothermia	Yes	163	92.6
	No	13	7.4
Breathing problem	Yes	151	85.8
	No	25	14.2
Diarrhea	Yes	65	36.9
	No	111	63.1
Reduced movement	Yes	62	35.2
	No	114	64.8
Reduced suckling	Yes	129	73.3
	No	47	26.7
Seizure	Yes	79	44.9
	No	97	55.1
Slow heart rate	Yes	36	20.5
	No	140	79.5
Swollen belly	Yes	69	39.2
	No	107	60.8
Vomiting	Yes	97	55.1
	No	79	44.9
Yellow skin color	Yes	115	65.3
	No	61	34.7

3.6.3. Burden of GBS in neonatal diseases and Risk factor analysis

Out of 176 new born with sepsis and meningitis 3 (1.7%) were positive for GBS. All of the GBS were isolated from blood sample collected from new born whose ages were less than 7

days. The burden of EOD-GBS and associated risk factors is shown in Table 3.23. Even though it not statistically significant, all of the three GBS was isolated from new who came from Addis Ababa are, and those who were female, whose age is in between 2500-4000g, whose APGAR score at 5 minute is < 7 and those who were hypothermic.

Table 3.23. Burden of GBS and Risk factors analysis in neonatal diseases in Tikur Anbessa Specialized Hospital (March, 2015-September, 2015).

Characteristics		**Prevalence of GBS N=176, total GBS=3**	**OR (95%CI)**	**P-value***
Week of birth	<37	1/69(1.4)	0.72(0.06-8.6)	0.83
	37-42+	2/107(1.8)	1	
APGAR score10	≥7	2/38(5.3)	7.6(0.6-86)	0.17
	<7	1/138(0.7)	1	
Diarrhea	Yes	2/65(3.1)	3.5(0.3-39.3)	0.3
	No	1/111(0.9)	1	
Reduced movement	Yes	1/62(1.6)	0.9(0.08-10.3)	0.9
	No	2/114(1.8)	1	
Reduced suckling	Yes	2/129(1.6)	1.4(0.12-15.6)	0.8
	No	1/47(2.1)	1	
Seizure	Yes	2/79(2.5)	2.5(0.2-28)	0.4
	No	1/97(1.0)	1	
Slow heart rate	Yes	0/36(0)	0.9	-
	No	3/140(2.1)	1	
Swollen belly	Yes	1/69(1.4)	0.7(0.07-8.68)	0.8
	No	2/107(1.9)	1	
Vomiting	Yes	1/97(1.0)	0.4(0.06-4.0)	0.4
	No	2/79(2.5)	1	
yellow skin and eye	Yes	1/115(0.9)	0.3(0.02-2.9)	0.2
	No	2/61(3.3)	1	

***calculated by using binary logistic regression**

3.6.4. Serotype distribution of GBS isolates from neonatal diseases

Out of three GBS isolated from newborn with sepsis and meningitis, two of them were serotype Ia and one of them was serotype III.

3.7. ANTIBIOTIC SUSCEPTIBILITY DATA

3.7.1. Antibiotic susceptibility pattern of GBS isolates

Of the 228 GBS isolates, antibiotic susceptibility test was performed using micro dilution test on 125 isolates against nine antibiotics (Table 3.24). All of them were susceptible to Penicillin, MIC range was 0.03-0.12µg/ml: 72/125(57.6%) of them were sensitive at MIC of 0.06µg/ml, 6/125 (4.8%) of them were sensitive at MIC of 0.03µg/ml, 47/125 (37.6%) were sensitive at MIC of 0.12µg/ml. All of them were also susceptible to Linezolid (MIC range, 0.25-2µg/ml), Cefotaxime (MIC range, 0.03-0.25µg/ml) and Vancomycin (MIC range, 0.5-1µg/ml). High level resistance was observed to Tetracycline 114(91.2%) (MIC range, 1-8µg/ml) and low level of resistance was observed against Clindamycin 1(0.8%) (MIC range, 0.06-32µg/ml), Erythromycin 8(6.4%) (MIC range, 0.03-32µg/ml), Daptomycin 3(2.4%) (MIC range, 0.25-2µg/ml), and Levofloxacin 9(7.2%) (MIC range, 0.5-8µg/ml).

Table 3.24. Susceptibility pattern of GBS isolated from pregnant women and newborn from three study sites: AHMC, HRH and TASH (June, 2014 to September, 2015).

S. Nº	Antibiotics	Susceptible		Intermediate		Resistant n (%)	
		No.	%	No.	%	No.	%
1	Penicillin(PEN)	125/125	100	-	-	-	
2	Levofloxacin(LEVO)	116/125	92.8	-	-	9/125	7.2
3	Linezolid (LZD)	125/125	100	-	-	-	-
4	Tetracycline(TET)	10/125	8.0	1/125	0.8	114/125	91.2
5	Clindamycin(CL)	123/125	98.4	1/125	0.8	1/125	0.8
6	Erythromycin(ERY)	114/125	91.2	3/125	2.4	8/125	6.4
7	Cefotaxime(FOT)	125/125	100	-	-	-	-
8	Vancomycin(VAN)	125/125	100	-	-	-	-
9	Daptomycin (DAP)	122/125	97.6	-	-	3/125	2.4

3.7.2. Antibiotic susceptibility pattern of GBS vs. serotype distribution

The susceptibility pattern of 125 GBS isolates varies across different serotypes. Serotype V showed high level of resistance to Levofloxacin, 6 (66.7%); serotypes II showed high level of resistance to Tetracycline, 51(44.7%) and to Erythromycin, 2(66.7%). (Table 3.25).

Table 3.25. Susceptibility pattern of GBS isolates based on serotype distributions

Antibiotics Tested		Serotype distribution				
		Ia	Ib	II	III	V
Penicillin(PEN)	S	29/125 (23.2%)	11/125 (8.6%)	51/125(40.8%)	10/125(8%)	24/125(19.2%)
	R	0	0	0	0	0
Levofloxacin(LEVO)	S	29/125(23.2%)	10/116(8.6%)	50/116(43.1%)	9/116(7.8%)	18/116(15.5%)
	R	0	1/9(11.1%)	1/9(11.1%)	1/9(11.1%)	6/9(66.7%)
Linezolid (LZD)	S	29/125(23.2%)	11/125(8.8%)	51/125(40.8%)	10/125(8.0%)	24/125(19.2%)
	R	0	0	0	0	0
Tetracycline(TET)	S	8/10(80%)	2/10(20%)	0	0	0
	I	1/1(100%)	0	0	0	0
	R	21/114(18.4%)	9/114(7.9%)	51/114(44.7%)	9/114(7.8%)	24/114(21.1%)
Clindamycin(CL)	S	29/125(23.2%)	11/125(8.9%)	50/124(40.7%)	10/125(8.1%)	23/124(18.6%)
	I	0	0	1/1(100%)	0	0
	R	0	0	0	0	1/1(100)
Erythromycin(ERY)	S	29/125(23.2%)	10/117(8.6%)	49/122(40.2%)	6/114(5.3%)	20/117(17.1%)
	I	0	0	2/3(66.7%)	1/3(33.3%)	0
	R	0	1/8(12.5%)	0	3/8(37.5%)	4/8(50%)
Cefotaxime(FOT)	S	29/125(23.2%)	11/125(8.8%)	51/125(40.8%)	10/125(8.0%)	23/125(19.2%)
	R	0	0	0	0	0
Vancomycin(VAN)	S	29/125(23.8%)	11/125(8.8%)	49/125(40.8%)	10/125(8.0%)	24/125(19.2%)
	R	0	0	0	0	0
Daptomycin(DAP)	S	29/125(23.2%)	10/122(8.2%)	49/122(40.2%)	10/125(8%)	24/125(19.2%)
	R	0	1/3(33.3%)	2/3(66.7%)	0	0

3.7.3. Antibiotic susceptibility pattern of GBS isolated across three Hospitals

Out of 125 GBS isolates tested for antibiotic susceptibility testing 28(22.4%) of them were collected at Adama Hospital Medical College, 62(49.6%) of them were collected at Hawassa Referral Hospital, 35(28%) of them were collected at Tikur Anbessa specialized Hospital. All of the GBS isolates from the three study sites were susceptible to Penicillin, Vancomycin, Linezolid and Cefotaxime. Out of 35 of GBS isolates from TASH, 35/114(30.7%) of them were resistant to Tetracycline. All of the GBS isolates from TASH were susceptible to Daptomycin (Table 3.26).

Table 3.26 Antimicrobial susceptibility pattern of 125 GBS across three study sites: AHMC, HRH and TASH (June, 2014 to September, 2015).

Antibiotics		AHMC No. of isolates tested= 28	HRH No. of isolates tested=62	TASH No. of isolates tested=35
Penicillin(PEN)	S	28/125(22.4%)	62/125(49.6%)	35/125(28.7%)
	R	0	0	0
Levofloxacin(LEVO)	S	23/116 (19.8%)	59/116(50.8%)	34/116(29.3%)
	R	5/9(55.6%)	3/9(33.3%)	1/9(11.1%)
Linezolid (LZD)	S	28/125(22.4%)	62/125(49.6%)	35/125(28%)
	R	0	0	0
Tetracycline(TET)	S	6/10(60%)	4/10(40%)	0
	I		1/1(100%)	0
	R	22/114(19.3%)	57/114(50%)	35/114(30.7%)
Clindamycin(CL)	S	28/125(22.4%)	61/124(49.2%)	34/124(27.4%)
	I	-	1/1(100%)	-
	R	0	0	1/1(100%)
Erythromycin(ERY)	S	26/117(22.2%)	57/114(50.0%)	30/114(26.3%)
	I	0	2/3(66.7%)	1/3(33.3%)
	R	2/8(25%)	2/8(25%)	4/8(50%)
Cefotaxime(FOT)	S	28/125(23%)	62/125(49.6%)	35/125(28%)
	R	0	0	0
Vancomycin(VAN)	S	28/125(22.4%)	62/125(49.6%)	35/125(28%)
	R	0	0	0
Daptomycin(DAP)	S	27/122(22.1%)	60/122(49.2%)	35/125(28%)
	R	1/3(33.3%)	2/3(66.7%)	0

*calculated by using chi-square, likelihood ratio was taken

3.7.4. Antibiotic susceptibility pattern of GBS isolated from Neonatal Diseases

All of three GBS strains isolated from 176 newborn with neonatal disease were susceptible to all antibiotic tested except to Tetracycline.

3.8. MOLECULAR CHARACTERIZATION DATA

3.8.1. Multiplex PCR (m-PCR) serotype data

a. Overall data

Out of 228, 123 GBS strains characterized by multiplex PCR and WGS pipeline prediction were grouped into 6 serotypes: II 49(39.8%), Ia 27(22%), Ib 14(11.4%), V 23(18.7%), III 9(7.3%), and IV 1(0.8%) (Table 3.27 and Figure 3.3).

b. Adama Hospital Medical College

Out of 28 GBS strains from AHMC 8(28.6%) of them were serotype Ia; 6(21.4%) of them were serotype Ib; 4(14.3%) of them were serotype II; 1(3.6%) of them were III; 9(32.1%) of them were serotype V (Table 3.27 and Figure 3.3).

c. Hawassa Referral Hospital

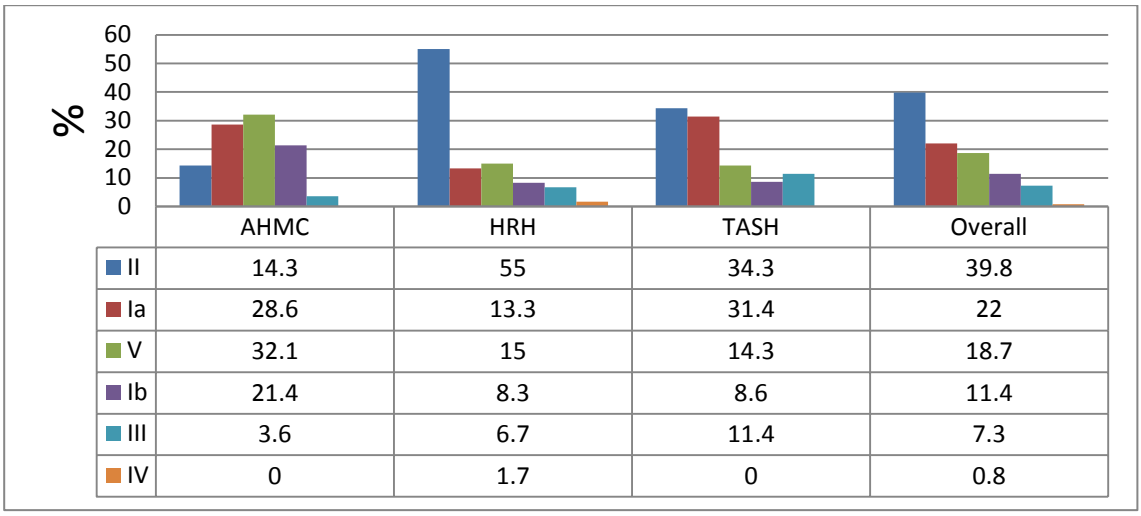
Out of 60 GBS strain from HRH 8(13.3%) of them were serotype Ia; 5(8.3%) of them were serotype Ib; 33(55%) of them were serotype II; 4(6.7%) of them were serotype III; 1(1.7%) of them were serotype IV; 9(15%) of them were serotype V (Table 3.27 and Figure 3.3).

d. Tikur Anbessa Specialized Hospital

Out of 35 GBS strains from TASH 11(31.4%) of them were serotype Ia; 3(8.6%) of them were serotype Ib; 12(34.3%) of them were serotype II; 4(11.4%) of them were serotype III; 5(14.3%) of them were serotype V (Table 3.27 and Figure 3.3).

Table 3.27. Serotype distribution of 123 GBS strains collected from three study sites: AHMC, HRH and TASH (June, 2014 to September, 2015), method was multiplex PCR.

Serotypes	Origin of GBS strains n (%)						Total
	Ia	Ib	II	III	IV	V	
AHMC	8(28.6)	6(21.4)	4(14.3)	1(3.6)	-	9(32.1)	28 (22.8)
HRH	8(13.3)	5(8.3)	33(55)	4(6.7)	1(1.7)	9(15)	60 (48.8)
TASH	11(31.4)	3(8.6)	12(34.3	4(11.4)	-	5(14.3)	35 (28.4)
Total	27(22)	14(11.4)	49(39.8)	9(7.3)	1(0.8)	23(18.7)	123 (100



	AHMC	HRH	TASH	Overall
II	14.3	55	34.3	39.8
Ia	28.6	13.3	31.4	22
V	32.1	15	14.3	18.7
Ib	21.4	8.3	8.6	11.4
III	3.6	6.7	11.4	7.3
IV	0	1.7	0	0.8

Figure 3.3. Serotype distribution of selected 123 GBS strains among GBS collected from different parts of Ethiopia by Imeri *et al* (2010) mPCR method (AHMC, HRH and TASH).

3.8.2. MLST profile

a. Over all MLST profile of GBS isolates from three study sites

Out of 228 GBS isolates 123 were further characterized by using MLST and were grouped in to 17 STs. Out of 17 STs identified, the proportion of each ST is as follows: ST-10 38(30.9%), ST-110 1(0.8%), ST-12 8(6.5%), ST-167 1(0.8%), ST-19 14(11.4%), ST-196 3(2.4%), ST-2 13(10.6%), ST-23 9(7.3%), ST-249 3(2.4%), ST-3 2(1.6%), ST-5 5(4.1%), ST-8 6(4.9%), ST-932 4(3.3%), ST-933 7(5.7%), ST-934 3(2.4%), ST-935, 2(1.6%), and ST-936 4(3.3%). ST-932, ST-933, ST-934, ST-935 and ST-936 are new GBS strains from Ethiopia. ST-932 occurred four times, all of them were from TASH. ST-933 occurred seven time, all of them were from AHMC. ST-934, 935 and ST-936 were detected from HRH. ST-10, ST-19 and ST-2 were found in three study sites. ST-3, ST-8 and ST-933 were detected only from AHMC; ST-196, ST-167, ST-934, ST-935 and ST-936 were detected only from HRH; ST-110, ST-5, ST-2, and ST-932 were detected only from TASH (Table 3.28). The allelic profile of 123 GBS strains is shown in Table 3.29.

b. MLST profile from Adama Hospital Medical College

Out of 28 GBS isolates 2(7.1%) of them were ST-10, 3(10.7%) of them were ST-19, 7(25%) of them were ST-2, 1(3.6%) of them was ST-249, 2(7.1%) of them were ST-3, 6(21.1%) of them were ST-8, and 7(25%) of them were ST-933 (Table 3.28).

c. MLST profile from Hawassa Referral Hospital

Out of 60 GBS isolates 28(46.7%) of them were ST-10, 5(8.3%) were ST-12, 1(1.67%) were ST-167, 6(10%) were ST-19, 3(5%) were ST-196, 3(5%) were ST-2, 3(5%) were ST-23, 2(3.3%) were ST-249, 3(5%) were ST-934, 2(3.3%) were ST-935, and 4(6.7%) were ST-936 (Table 3.28).

d. MLST profile from Tikur Anbessa Specialized Hospital

Out of 35 GBS isolates 8(22.9%) of them were ST-10, 1(2.9%) of them were ST-110, 3(8.6%) of them were ST-12, 5(14.2%) of them were ST-19, 3(8.6%) of them were ST-2, 6(17.1%) of them were ST-23, 5(14.3%) of them were ST-5, and 4(11.4%) of them were ST-932 (Table 3.28).

Table 3.28. Seqeunce type profiles of GBS isolates along with source of origin: GBS were collected from three different study sites: AHMC, HRH and TASH (June, 2014 to September, 2015).

	Study sites , total number of GBS=123			
STs	**AHMC**	**HRH**	**TASH**	**Total (%)**
ST-10	2/28(7.1%)	28/60(46.7%)	8/35(22.9%)	38 (30.9)
ST-110	-	-	1/35(2.9%)	1 (0.8)
ST-12	-	5/60(8.3%)	3/35(8.6%)	8 (6.5)
ST-167	-	1/60(1.7%)	-	1 (0.8)
ST-19	3/28(10.7%)	6/60(10%)	5/35(14.3%)	14 (11.4)
ST-196	-	3/60(5%)	-	3 (2.4)
ST-2	7/28 (25%)	3/60(5%)	3/35(8.6%)	13 (10.6)
ST-23	-	3/60(5%)	6/35(17.1%)	9 (7.3)
ST-249	1/28(3.6%)	2/60(3.3%)	-	3 (2.4)
ST-3	2/28(7.1%)		-	2 (1.6)
ST-5	-	-	5/35(14.3%)	5 (4.1)
ST-8	6/28(21.4%)	-	-	6 (4.9)
ST-932*	-	-	4/35(11.4%)	4 (3.3)
ST-933*	7/28(25%)	-	-	7 (5.7)
ST-934*	-	3/60 (5%)	-	3 (2.4%)
ST-935*	-	2/60(3.3%)	-	2 (1.6)
ST-936*	-	4/60(6.7%)	-	4 (3.3)
Total	28	60	35	123

* New ST entry from Ethiopia is available at http://pubmlst.org/sagalactiae

Table 3.29 Allelic profile of 123 GBS strains collected from three study sites: AHMC, HRH and TASH (June, 2014 to September, 2015).

STs	CCs	Allelic profile							No (%)
		adhp	*phes*	*atr*	*glnA*	*sdhA*	*glcK*	*tkt*	
249	249	5	4	6	1	2	1	3	3(2.4)
933	249	5	4	6	1	2	1	82	7(5.7)
23	249	5	4	6	3	2	1	3	9(7.3)
5	2	12	1	4	1	1	3	4	5(4.1)
934	singleton	41	1	44	98	1	33	2	3(2.4)
8	2	4	1	4	1	3	3	2	6(4.9)
12	2	10	1	4	1	3	3	2	8(6.5)
3	2	1	1	4	1	1	3	2	2(1.6)
932	2	1	1	4	1	2	3	4	4(3.3)
936	2	1	1	4	99	2	3	4	4(3.3)
167	2	1	1	6	1	1	2	2	1(0.8)
10	2	9	1	4	1	3	3	2	38(30.9)
19	2	1	1	3	2	2	2	2	14(11.4)
196	2	1	1	3	1	1	12	2	3(2.4)
2	2	1	1	3	1	1	2	2	13(10.6)
100	2	1	1	3	2	2	2	9	1(0.8)
935	2	174	1	3	1	1	2	2	2(1.6)
Total alleles		**7**	**2**	**4**	**5**	**3**	**5**	**5**	

e. Clonal complexes of GBS strains

123 GBS strains' MLST profiles were separated into three groups defined by eBURST when compared to GBS allelic profiles from http://pubmlst.org/sagalactiae/: two clonal complexes (CC-249, n=19 and CC-2, n=101) and one singleton (ST-934, n=3). eBURST analysis is shown in Figure 3.2.

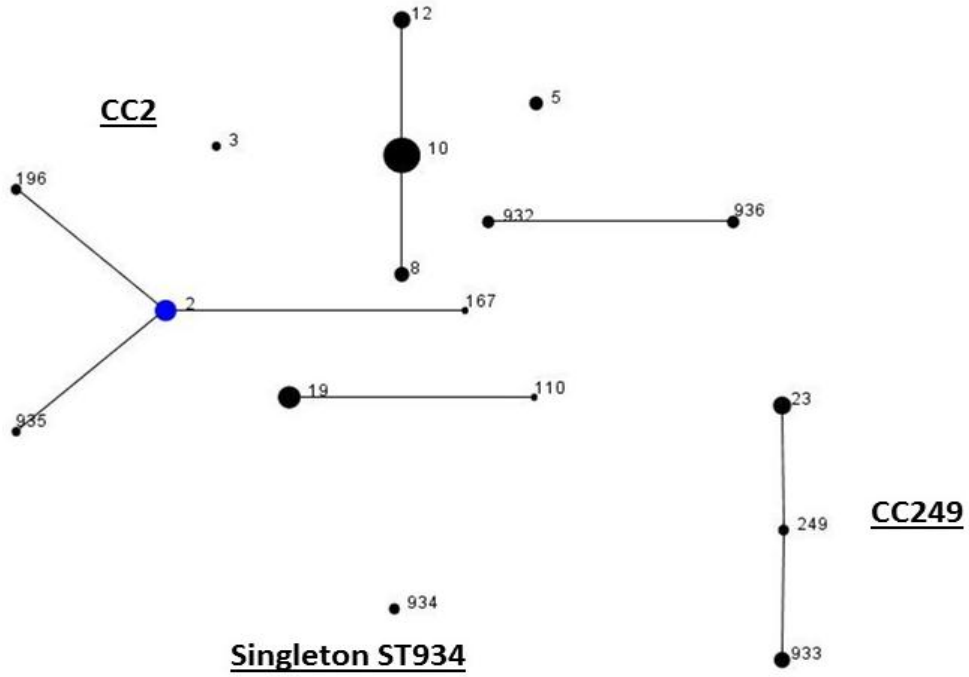


Figure 3.4 EUBURST diagrams of closely related isolates (Clonal complexes) of GBS strain from three sites of Ethiopia. The blue circle indicates the founder of ancestral types which have given rise to descendants sequence type strain black dots. The ST those which are directly connected to founder differ from the founder by only one single locus or allele are called Single locus variants (SLV). STs that do not cluster in to clonal complexes are called singleton STs. The diameter of the circles is illustrating the number of strains.

From the three clonal complexes, the number of GBS strains included under CC-2 was 101(82.1%); it contains the following STs; ST-10 which accounted 38(37.6%); ST-110 which accounted 1(0.9%), ST-12 which accounted 8(7.9%); ST-167 which accounted 1(0.9%); ST-19 which accounted 14(13.9%); ST-196 which accounted 3(2.9%); ST-2 which accounted 13(12.9%); ST-3 which accounted 2(1.9%); ST-5 which accounted 5(4.9%); ST-8 which accounted 6(5.9%), ST-932 which accounted 4(3.9%); ST-935 which accounted 1(1.9%); and ST-936 which accounted 4(3.9%).

The number of GBS strain included under CC-249 is 19(15.5%); it contains the following STs; ST-23 accounted for 9(47.4%); ST-249 accounted for 3(15.8%); ST-933 accounted for 7(36.8%); ST-23 accounted for 7/19(36.8%); ST-249 accounted for 3/19 (15.8%). The proportion of singleton is 3(2.4%). There are three sequence types (ST-934) (2.4%) which were included under singleton (Table 3.30 and Figure 3.4).

Table 3.30. Sequence types and Clonal complexes of 123 GBS strains collected from three study sites: AHMC, HRH and TASH (June, 2014 to September, 2015).

Sequence types	Clonal complexes (CC) N=123		
	CC-249	**CC-2**	**Singleton**
ST-10	-	38/101 (37.6%)	-
ST-110	-	1/101 (0.9%)	-
ST-12	-	8/101 (7.9%)	-
ST-167	-	1/101 (0.9%)	-
ST-19	-	14/101 (13.9%)	-
ST-196	-	3 /101(2.9%)	-
ST-2	-	13/101 (12.9%)	-
ST-23	9/19 (47.4%)	-	-
ST-249	3/19 (15.8%)	-	-
ST-3	-	2/101 (1.9%)	-
ST-5	-	5/101 (4.9%)	-
ST-8	-	6/101 (5.9%)	-
ST-932	-	4/101 (3.9%)	-
ST-933	7/19(36.8%)	-	-
ST-934	-	-	3/3 (100%)
ST-935	-	2/101 (1.9%)	-
ST-936	-	4/101 (3.9%)	-
Total	19 /123(15.5%)	101 (82.1%)	3 (2.4%)

f. Distribution of GBS serotypes along sequence types

Out of 17 STs , ST-10 contains thirty-eight serotype II, ST-110 contains one serotype V, ST-12 contains eight serotype Ib, ST-167 contains one serotype II, ST-19 contains nine serotype III and five serotype V, ST-196 contains two serotype V and one serotype IV (may indicate serotype switching), ST-2 contains thirteen serotype V, ST-23 contains nine serotype Ia, ST-249 contains three serotype Ia, ST-3 contains two serotype II, ST-5 contains five Ia, ST-8

contains six serotype Ib, ST-932 contains four serotype II, ST-933 contains seven serotype Ia, ST-934 contains three serotype Ia, ST-935 contains two serotype V, ST-935 contains four serotype II (Table 3.31).

Table 3.31 Sequence type distribution with in different serotypes of GBS collected from three study sites: AHMC, HRH and TASH (June, 2014 to September, 2015).

ST	Serotypes N=123					
	Ia	Ib	II	III	IV	V
ST-10	-	-	38/38(100%)	-	-	-
ST-110	-	-	-	-	-	1/1(100%)
ST-12	-	8/8(100%)	-	-	-	-
ST-167	-	-	1/1(100%)	-	-	-
ST-19	-	-	-	9/14(64.3%)		5/14(35.7%)
ST-196	-	-	-	-	1/3(33.3%)	2/3(66.7%)
ST-2	-	-	-	-		13/13(100%)
ST-23	9/9(100%)	-	-	-	-	-
ST-249	3/3(100%)	-	-	-	-	-
ST-3	-	-	2/2(100%)	-	-	-
ST-5	5/5(100%)	-	-	-	-	-
ST-8	-	6/6(100%)	-	-	-	-
ST-932	-	-	4/4(100%)	-	-	-
ST-933	7/7(100%)	-	-	-	-	-
ST-934	3/3(100%)	-	-	-	-	-
ST-935	-	-	-	-		2/2(100%)
ST-936	-	-	4/4(100%)	-	-	-
Total	27(21.9%	14(11.4%)	49(39.9%)	9(7.3%)	23(18.7%)	1(0.8%)

g. Distribution of Clonal complex across study sites

Clonal complex distribution of GBS strains across study sites is as follows: 8(42.1%) GBS strains of CC-249, 20(19.8%) strain of CC-2 was found from AHMC. 5(26.3%) strains of CC-249, 52(51.5%) strains of CC-2 and 3(100%) strains of singleton was found from HRH. 6(31.6%) strains of CC-249, 29(28.7%) strains of CC-2 was detected from TASH (Table 3.32).

Table 3.32. Distribution of Clonal complexes across three study sites: AHMC, HRH and TASH (June, 2014 to September, 2015).

CCs	Study sites, No of GBS=123			Total	P-value
	AHMC	HRH	TASH		
CC-249	8/19(42.1%)	5/19(26.3%)	6/19(31.6%)	19	0.065
CC-2	20/101(19.8%)	52/101(51.5%)	29/101(28.7%)	101	
Singleton	0	3/3(100%)	0	3	
Total	28	60	35	123	

3.8.3. Antibiotic resistance gene data

a. Overall ST profile of GBS isolates from three study sites

Out of 123 GBS strains 13(10.6%) of them contained no antibiotic resistance gene; 63(51.2%) of them contains *tet*(M-1) gene, 39 (31.7%) of them contains *tet*(L-1) and *tet*(M-1), 2(1.6%) of them contains *erm*(TR-1) and *tet*(M-1), 5(4.1%) of them contains *erm* (TR-1), *gyr*A (GBS-1), *Par* C (GBS-1) and *tet*(M-1), 1(0.8%) of them contains *erm*(B-1, *tet*(O-1).

Out of 9 GBS isolates which were resistant to Levofloxacin 5 (55.6%) of them contains *Gyr*A (GBS-1) and *Par*C (GBS-1). Out of 115 GBS isolates which were resistant and intermediate to Tetracycline, 111(96.5%) of them contains at least one tetracycline resistance genes: *tet* (M-1), *tet* (L-1), and *tet* (O-1). Out of 11 GBS isolates which showed resistance or intermediate to Erythromycin **7 (**63.6%) of them contains *erm* (TR-1) gene; out of 8 GBS which showed resistance to Erythromycin 1(9%) of them contains *erm* (B-1); one of them contains *erm* (TR-1) resistance gene but it was sensitive to Erythromycin (Table 3.33).

b. Adama Hospital Medical College

Out of 28 GBS strains from AHMC 8(28.7%) of them contains no antibiotic resistance gene, 16(57.1%) of them contains *tet* (M-1) gene, 2(7.1%) of them contains *tet* (L-1) and *tet*(M-1), 2(7.1%) of them contains *erm* (TR-1), *gyrA* (GBS-1), *Par* C (GBS-1) and *tet*(M-1) (Table 3.32) (Table 3.33).

c. Hawassa Referral Hospital

Out of 60 GBS strains from AHMC 5(8.3%) of them contains no antibiotic resistance gene, 25(41.7%) of them contains *tet* (M-1) gene, 28(46.7%) of them contains *tet (*L-1) and *tet* (M-1), 2(3.3%) of them contains *erm* (TR-1), *gyr*A (GBS-1), *par* C (GBS-1) and *tet* (M-1) (Table 3.33).

d. Tikur Anbessa specialized Hospital

Out of 35 GBS strains from TASH 22(62.9.%) of them contains *tet* (M-1) gene, 9(25.7%) of them contains *tet* (L-1) and *tet*(M-1), 1(2.9%) of them contains *erm* (TR-1), *gyr*A (GBS-1), *par* C (GBS-1) and *tet* (M-1), 1(2.9%) contains *erm* (B-1), *tet* (O-1) (Table 3.33).

Table 3.33. Distribution of antibiotic resistance gene among GBS collected from three study sites: AHMC, HRH and TASH (June, 2014 to September, 2015).

Resistance gene	Study sites No of GBS=123			Total
	AHMC	HRH	TASH	
No resistance gene	8/13(61.5%)	5/13(38.5%)	-	13(10.6%)
tet(M-1)	16/63(25.4%)	25/63(39.7%)	22(34.9%)	63(51.2%)
tet(L-1), *tet*(M-1)	2/39(5.1%)	28/39(71.8%)	9/39(23.1%)	39 (31.7%)
erm(TR-1),*tet* (M-1)	-	-	2/2(100%)	2(1.6%)
erm(TR-1), *gyr*A(GBS-1), *par* C(GBS-1), *tet*(M-1)	2/5(40%)	2/5(50%)	1/5(20%)	5(4.1%)
erm(B-1, *tet*(O-1)	-	-	1/1(100%)	1(0.8%)
Total	28	60	35	123

e. Distribution of resistance gene across sequence types

Out of 38 strains of ST-10, 1(2.6%) of them contains *tet*(M-1) and 37(97.4%) of them contains *tet*(L-1) and *tet*(M-1), 1(100%) of ST-110 contains *erm* (B-1) *and tet*(O-1) genes; out of 8 strains of ST-12, 5(62.5%) of them contains *tet*(M-1) genes; ST-19 contains all type of resistance gene except *erm*(B-1) and *tet*(O-1) ST-933 does not contain any antibiotic resistance gene (Table 3.34).

Table 3.34 Seqeunce type profile of 123 GBS isolates along with serotype (mPCR), antibiotic resistance gene: GBS collected from three different study sites: AHMC, HRH and TASH (June, 2014 to September, 2015).

STs	**Antibiotic Resistance genes**					
	No Resistance gene	***tet*(M-1)**	***tet*(L-1), *tet*(M-1)**	***erm*(TR-1),tet (M-1)**	***erm*(TR-1), gyr*A*(GBS-1), *par* C (GBS-1), *tet*(M-1)**	***erm*(B-1, *tet*(O-1)**
ST-10	-	1/38(2.6%)	37/38(97.4%)	-	-	-
ST-110	-	-	-	-	-	1/1(100%)
ST-12	3/8(37.5%)	5/8(62.5%)	-	-	-	-
ST-167	-	1/1(100%)	-	-	-	-
ST-19	-	5/14(35.7%)	2/14(14.3%)	2/14(14.3%)	5(35.7%)	-
ST-196	-	3/3(100%)	-	-	-	-
ST-2	-	13/13(100%)	-	-	-	-
ST-23	-	9/9(100%)	-	-	-	-
ST-249	1/3(33.3%)	2/3(66.7%)	-	-	-	-
ST-3	-	2(3.2%)	-	-	-	-
ST-ST-5	-	5/5(100%)	-	-	-	-
ST-8	-	6/6(100%)	-	-	-	-
9ST-32	-	4/4(100%)	-	-	-	-
ST-933	7/7(100%)	-	-	-	-	-
ST-934	2/3(66.7%)	1/3(33.3%)	-	-	-	-
ST-935	-	2/2(100%)	-	-	-	-
ST-936	-	4/4(100%)	-	-	-	-
Total	13/123(10.6%)	63/123(51.2%)	39/123(31.7%)	2/123(1.6%)	5/123(4.1%)	1/123(1.6%)

f. Distribution of resistance gene across GBS serotypes

The distribution of antibiotic resistance gene in different serotypes is as follows: out of 27 serotype Ia, 10(37%) of them did not contain any resistance gene; 17(62.9%) of them contains *tet* (M-1) resistance gene. Out of 14 serotype Ib 3(21.4%) of them contains no resistance gene; 11(78.6%) of them contains *tet* (M-1) resistance gene. Among 49 serotype II, 12(15.2%) of them contains *tet* (M-1) resistance gene; 37(75.5%) of them contains *tet*(L-

1), *tet*(M-1). Out of 9 serotype III, 5(55.6%) of them contains *tet*(M-1), 2(22.2%) of them contains *tet*(L-1), *tet*(M-1) and 2(22.2%) of them contains *erm*(TR-1), *tet*(M-1). One serotype IV contains *tet*(M-1). Among of 23 serotype V, 17(73.9%) of them contains *tet*(M-1), 5(21.7%) contains *erm*(TR-1), *gyr*A(GBS-1), *par* C (GBS-1), *tet*(M-1) and 1(4.3%) of them contains *erm*(B-1), *tet*(O-1). All GBS serotypes in this study contains *tet*(M-1) gene (Table 3.35).

Table 3.35 Antibiotic resistance gene profile of GBS serotypes (mPCR): GBS collected from three different study sites: AHMC, HRH and TASH (June 2014, to September, 2015).

Resistance genes	Serotypes, N=123						Total
	Ia	Ib	II	III	IV	V	
No resistance gene	10/27(37%)	3/14(21.4%)	-	-	-	-	13(10.6%)
tet(M-1)	17/27(62.9%)	11/14(78.6%)	12/49(24.5%)	5/9(55.6)	1/1(100%)	17/23(73.9%)	63(51.2%)
tet(L-1), *tet*(M-1)	-	-	37/49(75.5%)	2/9(22.2%)	-	-	39(31.7%)
erm(TR-1), *tet*(M-1)	-	-	-	2/9(22.2%)	-	-	2(1.6%)
erm(TR-1), *gyr*A(GBS-1), *par C*(GBS-1), *tet*(M-1)	-	-	-		-	5/23(21.8%)	5(4.1%)
erm(B-1), *tet*(O-1)	-	-	-	-	-	1/23(4.3%)	1(0.8%)
Total	27	14	49	9	1	23	123(100%)

CHAPTER IV: DISCUSSION

In present study a total of 840 pregnant women and their 857 newborn from Adama Hospital Medical College (AHMC), Hawassa Referal Hospital (HRH) and Tikur Anbesa Sepicalized Hospital (TASH) and 176 infants less than 90 days with EOD and LOD from Tikur Anbessa Speiclized Hospital were included.

The overall maternal GBS colonization rate in this study (17.4%) is comparable with studies done in other parts of the world, which is in the range of 10-30% (Verani *et al.,* 2010; Edmond *et al.,* 2012; Patras *et al.,* 2015a; Hansen *et al.,* 2004; van deer *et al.,* 2008; Russell *et al.,* 2017). It is also relatively comparable to colonization rate reported from several countries such as Thai-Myanmar border (12%) (Turner *et al.,* 2012), Brazil (14.6%) (Simoes *et al.,* 2007), Germany (16%) (Brimil *et al.,* 2006), Italy (17.9%) (Sensini *et al.,* 1997), New Zealand (20%) (Grimwood *et al.,* 2002), Congo (20%) (Mitima *et al.,* 2014), Ethiopia (20.7%) (Mohammed *et al,* 2012), The Netherlands (21%) (Valkenburg *et al.,* 2006), USA (21%) (Regan *et al.,* 1996), and Malawi (21.7%) (Gray *et al.,* 2011).

Compared to colonization rate reported from China (6.5%) (Wang *et al.,* 2015), India (2.5%) (Kulkarni *et al.,* 2001), and Nigeria (8.3%) (Donbraye-Emmanuel *et al.,* 2010), the overall maternal colonization rate in the present study is high. The observed difference in colonization rate among pregnant women can be due to geographical differences, different ethnic back ground and laboratory methods used.

Maternal colonization rate is not uniform across the three study sites, the highest maternal colonization rate was found from TASH (23.2%) and the lowest from AHMC (13.2%). The colonization rate from three study sites is in the range of 13-23%. Like this study, variation in maternal GBS colonization with in a country was reported from other countries (Nishihara *et al.,* 2016; Schrag *et al.,* 2002). Collectively, maternal colonization rate from specific study sites and the overall maternal colonization rate revealed the existence of the primary risk factor for early onset neonatal disease due to GBS in Ethiopia.

The overall newborn colonization rate (9.2%) and the overall vertical transmission rate (54.1%) found in the present study are comparable with earlier reports when GBS emerged in developed countries for the first time (Baker and Kasper, 1976). Vertical transmission rate

reported at that time was in the range of 40-70% (Baker and Kasper, 1976; Boyer and Gotoff, 1985). In contrast to this study relatively high newborn GBS colonization rate (38%) and low vertical transmission rate (39.5%) was reported from Bangladesh (Saha *et al.,* 2017). Similar to Bangladesh, low vertical transmission rate (16.7%) was also reported from China (Yang *et al.,* 2011). Factors which determine vertical transmission of GBS from colonized mother to newborn is not clearly identified.

The highest newborn colonization rate was detected from TASH (11.3%) followed by HRH (8.9%) and AHMC (7.4%). The highest vertical transmission rate was from HRH (59.1%), followed by AHMC (56.8%) and TASH (49.2%). Similarly, prevous studies showed that about 50% of newborn from colonized mother are GBS positive during birth. Among colonized newborn only 1-2% develop invasive disease (Berardi *et al.,* 2015; Anthony *et al.,* 1981; Heath 2016).

There is no data on neonatal disease due to GBS from Ethiopia. Previous study conducted by Shitaye *et al*. (2010) on etiology of neonatal disease at TASH did not find GBS. The major organisms Shitaye *et al.* (2010) identified were *Klebsiella* (39.2%) and *Staphylococcus aures* (22.2%). Unlike their study from Ethiopia, 1.7% prevalence of GBS among infants less than 3 months was detected in this study. Moreover, invasive GBS disease among infants less than 3 months was reported from Kenya (0.76/1000lb) (Seale *et al.,* 2016), Thailand (0.2/1000lb) and Philippines (0.3%) (Villanueva-Uy *et al.,* 2015).

There is scarce or no data regarding EOD-GBS and LOD-GBS in Sub-Saharan countries including Ethiopia. The incidence of EOD-GBS in developing countries is estimated to be 0-3.06/1000 lb (Dagnew *et al.,* 2012). Studies from Malawi and S. Africa reported the incidence of EOD-GBS of 0.92/1000lb (Gray *et al.,* 2007) and 2.06/1000lb (Madhi *et al.,* 2003) respectively. Even though it is difficult to compare the result of this study directly with incidence of neonatal disease caused by GBS from other countries due to differences in study design, sample size and lack of denominator, this study not only revealed the presence of primary risk factor but also detected invasive GBS disease in Ethiopia for the first time.

Group B streptococcus vaccine, which is in advanced stage, is based on capsular polysaccharide. Data on GBS serotype from different geographic location is critical for

development of appropriate vaccine formulation. Epidemiology of GBS serotype varies from country to country and from time to time (Russel *et al.,* 201: Seale *et al.,* 2017). The prevalent serotype reported from Japan are; VIII (35.6%), VI (24.6%) followed by serotype III (11%) (Lachenauer *et al.,* 1999). The predominant GBS serotypes reported from Iran are serotype V (19.6%), II (12%), and IV (12.5%) (Jannati *et al.,* 2012). Recently, Slotved *et al.,* (2017) reported high prevalence of serotype VII-IX from Ghana. This indicates epidemiology of GBS serotype varies across different geographic location of Africa.

The overall serotype distribution of 228 GBS strains collected from pregnant women, their newborn, and infants with early and late onset neonatal disease, II (30.3%), Ia (20.2%), V (17.9%), Ib (17.1%), III (10.1%), VII (0.9%), non typeable (3.5%), is in partial agreement with distribution of GBS serotypes reported from other countries such as China, USA, Malaysia, Brazil, S. Africa (Wang *et al.,* 2015; Ippolito *et al.,* 2010; Eskandarian, 2015; Dutra *et al.,* 2014; Madzivhandila *et al.,* 2011).

In this study, serotype distribution of GBS was assessed in three different sites of Ethiopia which are far away from each other with a minimum of 100km. The overall (10.3%) and site specific distribution of serotype II in the present study is high compared to reports from other countries whch is in the range of 5.4-14% (Fluegge *et al.,* 2006, Eskandarian, 2015; Ipplito *et al,* 2010). Site specific prevalence of serotype II in the present study is not uniform across the three study sites; the highest was found from HRH (34.3%) and the lowest was from AHMC (27.6%). However, unlike reports from many other countreis, prevalance of serotype II found from the three sties was consistently high. (Wang *et al.,* 2015; Madhi *et al.,* 2003).

In contrast to reports from several studies, the overall (10.1%) and site specific prevalence of serotype III in the present study is low; the highst was from from AHMC (12.1%) and the lowest was from TASH (7.2%). The prevalence of serotype III from other countries is in the rage of 40-60% (Brzychczy-Wolch *et al.,* 2014; Fluegge *et al.,* 2006; Madhi *et al.,* 2003; Strakova and Motlova, 2004; Wang *et al.,* 2015; Shet and Ferrier, 2004). On the other hand, similar to the present study, low prevalence of serotype III was reported from few countries such Brazil (6.7%) (Dutra *et al.,* 2014) and Japan (11%) (Madzivhandila *et al.,* 2011). As

serotype III is belived to be the most virulent, this area requires further investigation in Ethiopia setting.

The overall (20.2%) and site specific prevalence of serotype Ia, the highst was from TASH (22.7%), is in line with study done in several countreis such as China (17.9%) (Wang *et al.,* 2015), Germany (17%) (Fluegge *et al.,* 2006), Malaysia (17.5%) (Eskandarian, 2015), Switzerland (19.2%) (Frohlicher *et al.,* 2014) and Poland (20%) (Brzychczy-Wolch *et al.,* 2014). However, high serotype Ia was reported from USA (32.8%) (Shet and Ferrier, 2004), S. Africa (30.1%) (Madzivhandila *et al.,* 2011) and Brazil (27.6%) (Dutra *et al.,* 2014). On the other hand, low prevalence of serotype Ia was reported from Japan (6.8%) (Lachenauer *et al.,* 1999).

Serotype V has been identified as an important neonatal and adult colonising and disease causing serotype globally (Le Doare *et al.,* 2016). In this study, site spesfic and overall distribution of serotype type V (17.9%) identified is comparable to proportion of serotype V reported from USA (19%) (Shet and Ferrier, 2004) and it is higher than reports from several countries such as; Germany (9%) (Fluegge *et al.,* 2006), S. Africa (5.6%) (Madhi *et al.,* 2003), and Malaysia (9.7%) (Eskandarian, 2015). Serotype V is the second prevlanet serotype at HRH (20%) followed by TASH (18.56%).

The overall (17.1%) and site specific proportion of serotype Ib found in this study is high compared to Germany (5%) (Fluegge *et al.,* 2006), USA (9.2%) (Shet and Ferrier, 2004), S. Africa (6.7%) (Madzivhandila *et al.,* 2011), Poland (5%) (Brzychczy-Wolch *et al.,* 2014) and Japan (8.2%) (Lachenauer *et al.,* 1999). Serotype Ib is the second prevalent serotype at AHMC (24.1%), where as at HRH, it is the fourth prevalent serotype (12.9%) The finding of the present study is comparable to serotype Ib reported from China (16.1%) (Wang *et al.,* 2015) and Brazil (18.7%) (Dutra *et al.,* 2014).

Serotyping of GBS using commercially available antisera is not perfect as a result some non typeability is expected (Kong *et al.,* 2008). This can be due to several reasons such as lack of capsule, expression of low amount of capsule, mutation in capsule coding gene, uncharacterized strains and inherent problem of antsiera used for serotyping . Some of GBS

strains (3.5%) collected in this study were non typeable. This finding is in line with reports from other countries (Imperi *et al.,* 2010; Weisner *et al.,* 2004; Ferrieri *et al,* 2004). On the other hand, high non typeability rate was found from China (14.3%) (Wang *et al.,* 2015) and Japan (9.6%) (Lachenauer *et al.,* 1999). Moreover, other studies reported high non typeability rate of up to 19.4% (Zeng *et al.,* 2006) and 20% (Pinto *et al.,* 2013). Currently, different molecular methods have been developed to minimize non typeability rate (Imperi *et al.,* 2010; Kong *et al.,* 2005).

Serotype distribution of invasive GBS disease identified in this study (two of them were serotype Ia and one of them was serotype III) is similar with reports from other countries (Mahdi *et a.l,* 2003; Gray *et al.,* 2007; Weisner *et al.,* 2004). Among ten different GBS serotypes, serotype Ia and III are commonly associated with invasive neonatal disease (Dagnew *et al.,* 2012).

Unlike the overall and site specific distribution of GBS serotypes, in which serotype II is the most prevalent, Edmond *et al.* (2012) and Dagnew *et al.* (2012) reported that GBS serotype III to be the most common in many countries, followed by serotypes Ia, Ib, II, and V. According to Edmond *et al.* (2012) altogether these five GBS serotypes (III, Ia, Ib, II and V) account for more than 94% of neonatal diseases caused by GBS worldwide. The present study also showed vaccine formulation containing the above GBS stereotypes (II, Ia, V, Ib and III) will be suitable for Ethiopia. According to this study, the current vaccine formulation in clinical trial (Madhi *et al.,* 2016), which is comprised of only serotype Ia, Ib and III, will not cover all GBS serotypes circulating in Ethiopia, particularly the dominant serotypes (II and V) are missed from the vaccine formulation. In addition, vaccine on last phase of clinical trial may cover only about 47.4% of neonatal diseases caused by GBS in Ethiopia. Based on this study, vaccine consisting of serotype II, Ia, Ib, V and III will cover about 95.6% of neonatal diseases caused by GBS in Ethiopia.

In the present study risk factors associated with maternal and newborn GBS colonization rate and burden of neonatal disease among new born with early and late onset neonatal disease was determined; however, no significant association between maternal and neonatal GBS colonization rate and measured risk factors was found ($P>0.05$).

Even though it was not statistically significant, high colonization rate was found among pregnant women whose gestational age was greater than 37 weeks in all the three study sites. Colonization rate was high among pregnant women whose duration of rupture of membrane was >10 hours at AHMC (13.8%), 6-10 hours at HRH (26.7%) and 0-5 hours at TASH (24%). Among pregnant women who had premature rupture of membrane (PROM) from AHMC, 15.4% of them were colonized with GBS and from TASH 21.4% of them were colonized with GBS. 13.4% of pregnant women from AHMC and 23.4% from TASH whose age was less than 28 years were colonized with GBS (P>0.05). This may indicate young maternal age is a risk factor for GBS colonization, similar finding was reported from other countries (Hiller *et al.,* 2005; El Beitune *et al.,* 2005; Slotved *et al.,* 2017).

Newborn who were colonized with GBS was followed using telephone interview to determine the proportion of new born who were going to develop EOD-GBS. Among all newborn from AHMC and HRH which were colonized with GBS, 4.8% and 3.8% of them developed sign and symptoms of EOD respectively. Out of all newborn (from three sites) colonized with GBS, 2.5% of them developed sign and symptom of EOD, but no attempt was made to isolate GBS from their blood and CSF specimen. The overall finding (2.5%) is similar with previous reports; however, site specific finding is higher than previous report (Berardi *et al.,* 2015; Boyer and Gotoff, 1986; Boyer and Gotoff, 1985).

The overall prevalence of newborn those who were not alive during birth in this study was 4.4%. Relatively equal rate of newborn those who were dead during birth was found from AHMC (4.61%), HRH (4.1%) and TASH (4.6%). Moreover, among newborn who were dead or died immediately after delivery 7.7% from AHMC, 16.7% from HRH, 7.7% from TASH were colonized with GBS. Overall prevalence of GBS among newborn who were alive during birth accounted 10.52%. Studies from other countreis such as Keneya and S. Africa indicated GBS as important cause of still birth, even though it is difficult to qunitify the impact of GBS (Nan *et al.,* 2015; Seale *et al.,* 2016; Seale *et al.,* 2017). As a result this area may need further investigation in Ethiopia.

Among newborn who aspirated meconium stained amniotic fluid 7% from AHMC, 11.1% from HRH and 22.2% from TASH were colonized with GBS. Most of GBS in this study

were detected from newborn whose weight was in between 2500-4000 gram. 9.4% of new born who were colonized at HRH had weight less than 2500g. 4.8% of newborn from AHMC, 8.4% from HRH and 16.6% from TASH who had APGAR score at 5 minutes $\leq$ 7 were colonized with GBS. The APGAR score for all newborn was improved when measured at 10 minutes.

In the present study no significant association was found between prevalence of GBS among newborn with neonatal disease and measured risk factors. However out of 3 GBS isolated two of them were detected from newborn who were presented with reduced suckling motility, seizure, with no swollen belly, week of birth greater than 37 and those with no reduced movement.

The susceptibility pattern of GBS isolates to Penicillin (all of them were susceptible), Vancomycin (all of them were susceptible), Linezolid (all of them were susceptible), Cefotaxime (all of them were susceptible), Daptomycin (97.6% of them were susceptible), and Levofloxacin (92.8% of them were susceptible) is comparable with previous studies conducted in different countries (Dutra *et al.*, 2014; Eskandarian *et al.*, 2015; Yamada *et al.*, 2015; Garland *et al.*, 2011; Bolukaoto *et al.*, 2015).

In the present study, GBS strains resistant to or with reduced Penicillin susceptibility have not been detected. Most of GBS strains (37.6%) in the present study showed MIC of 0.12µg/ml, only some of them (4.8%) were sensitive at MIC of 0.03µg/ml µg/ml (Range: 0.03-0.12µg/ml). Penicillin is the first agent for prevention and treatment of GBS infections; however, starting from 1994, GBS strains with reduced susceptibility to Penicillin (0.25µg/ml to 1µg/ml) have been reported periodically (Jannati *et al.*, 2012; Kimura *et al.*, 2008; Wang *et al.*, 2015; Longitin *et al.*, 2011; Dahesh *et al.*, 2008; Gaudreau *et al.*, 2010).

Complicating the problem, GBS with reduced Penicillin sensitivity tends to be resistant to other drugs such as Fluoroquinolones and Macrolides (Kimura *et al.*, 2013). Clinical GBS strains with reduced Penicillin sensitivity became highly cephalosporin resistant by acquiring amino acid substitutions in PBP1A and PBP2X (Kimura *et al.*, 2013; Nagano *et al.*, 2012). Even though GBS strains resistant or with reduced susceptibility to Penicillin

were not detected from Ethiopia, it is significant public health concerns which requires further investigation and follow up. Unlike reports from other countries, GBS strains resistant to Linezolid, Cefotaxime and Vancomycin were not detected in this study (Teatero *et al.,* 2017; Park *et al.,* 2014; Sendi *et al.,* 2016).

Clindamycin and Erythromycin are alternative antibiotics to prevent EOD-GBS for Penicillin-allergic pregnant women with high risk of anaphylaxis. Rising of GBS strains resistant to Erythromyicn and Clindamycin from time to time is complicating management of pregnant women who are allergic to Penicillin (Seo *et al.,* 2010). In contrast to reports from many other countries, majority of GBS strains in the present study were susceptible to Erythromycin (91.2%) and Clindamycin (98.4%) (Verani *et al.,* 2010; Eskandarian *et al.,* 2015; Frohlicher *et al.,* 2014; Berg *et al.,* 2014; Seo *et al.,* 2010). Unlike the present study, high level of resistance to Erythromycin (60-75%) (Wang *et al.,* 2013) and to Clindamycin (50-55%) (Wenjing *et al.,* 2017) were reported from China. Similar to the present study (6.4%), low level of resistance to Erythromycin was reported from Australia (6.4%) (Garland *et al.,* 2011), Brazil (4.1%) (Dutra *et al.,* 2014), Thai-Myanmar border (8.5%) (Turner *et al.,* 2012) and France (4%) (Weisner *et al.,* 2004). In this study, only one GBS strain was resistant to Clindamycin. Similar to this study low resistance rate to Clindamycin was reported from Japan (1%) (Matsubara *et al.,* 2001), Australia (4.2%) (Garland *et al.,* 2011), and Brazil (3%) (Dutra *et al.,* 2014). Generally, in contrast to this study, worldwide studies reported high resistance rate to Erythromycin which ranges from 18 to 54% (Fitoussi *et al,* 2001; Dipersio and DiPersio JR *et al,* 2006) and to Clindamycin which ranges from 12 to 62.8% (Fitoussi *et al.,* 2001; Berg *et al.,* 2014).

The difference in antibiotic resistance rate observed to Erythromycin and Clindamycin can be due to IAP is not standard of care in Ethiopia indicating minimum selective pressure which drives emerging of Erythromycin and Clindamycin resistant GBS strains. Acquisition of resistance against these antibiotics by horizontal gene transfer has been detected in GBS (Phares *et al.,* 2008; Pearlman *et al.,* 1998). Absence or low antibiotic resistance of GBS strains in the present study may indicate the suitability of Penicillin, Erythromycin, and Clindamycin for Ethiopia to prevent EOD-GBS until vaccine is available on the market.

Group B Streptococcus with high level of resistance to Fluoroquinolones was described in Japan in 2003 for the first time (Kawamura *et al.,* 2003) and after that time, it has been reported from other countries (Tazi *et al.,* 2008). In this study, relatively low level of resistance to Levofloxacin (7.2%) was identified. In contrast to the present study, high level of resistance to Levofloxacin which ranges from 25.9 to 39.3% was reported from China (Wenjing *et al.,* 2017; Wang *et al.,* 2015). Moreover, resistance rate to Levofloxacin found in this study is low compared to report from Canada (12%) (Neemuchwala *et al.,* 2016a). On the other hand, compared to this study, low level of resistance rate to Levofloxacin was reported form Taiwan (1.3%) (Wu *et al.,* 2008), Italy (1.4%) (Piccinelli *et al.,* 2015) and Brazil (1%) (Nakamura *et al.,* 2011).

Majority of GBS strains (91.2%) in this study, similar to reports from other countries, were resistant to Tetracycline (Wang *et al.,* 2013; Da Cunha *et al.,* 2014; Dutra *et al.,* 2014; Wang *et al.,* 2015; Weisner *et al.,* 2004). This may strengthen the suggestion which states, the current circulating GBS strains in the world were selected by Tetracycline usage in 1940s (da Cunha *et al.,* 2014). Comparable to this study, high level of resistance to Tetracycline was also reported from Brazil (83%) (Nakamura *et al.,* 2011), Australia (85.9%) (Zhao *et al.,* 2008), Kuwait (89.5%) (Boswihi *et al.,* 2012), Canada (89%) (Teatero *et al.,* 2017), and Island (85%) (Heelan *et al.,* 2004).

Antibiotic resistance pattern observed in this study varied across the three study sites. Among GBS strains tested, most of GBS strains which were resistant to Levofloxacin were from AHMC (55.6%) followed by HRH (33.3%) and TASH (11.1%). Studies from China also reported variation of antibiotic resistance to Levofloxacin in different parts of China (Wang *et al.,* 2013; Wang *et al.,* 2015). Most of GBS from HRH were resistant to Tetracycline (50%), followed by those GBS collected from TASH (30.7%) and AHMC (19.3%). 25% of GBS strains from AHMC and HRH, and 50% of GBS strains from TASH were resistant to Erythromycin. 33.3% of GBS strains from AHMC and 66.7% of GBS strains from HRH were resistant to Daptomycin. Like this study, different pattern of antibiotic resistance has been reported from different countries (Da Cunha *et al.,* 2014).

The present study attempted to assess antibiotic resistance pattern among different GBS serotypes. Compared to other serotype, high proportion of serotype II (44.7%) and V (21.1%) showed resistance to Tetracycline. Majority of serotype V also showed resistance to Levofloxacin (66.7%) compared to other GBS serotypes. In addition, high proportion (50%) of GBS serotype V strains were resistant to Erythromycin. Variation of antibiotic resistance rate among different serotype of GBS was also reported from other countries (Von *et al.,* 2003; Jannati *et al.,* 2012; Wang *et al.,* 2013; Usein *et al.,* 2014; Zhao *et al.,* 2008).

Out of 228 GBS strains collected in the present study, 123(53.9%) of them were further characterized by mPCR. Serotyping by mPCR showed similar pattern of GBS distribution compared to serotyping performed by using type specific antisera. GBS serotype distribution identified by mPCR, II (39.8%), Ia (22%), V (18.7%), Ib (11.4%), III (7.3%), and IV (0.8%), is comparable to overall GBS serotype distribution performed by type specific antisera. The prevalence of serotype II detected by group specific antisera (30.3%) is relatively low compared to serotyping performed by mPCR (39.8%). The prevalence of serotype II by mPCR is high compared to the prevalence of serotype II reported from other countries (Ipplito *et al.,* 2010; Madzivhandila *et al.,* 2011). Magnitude of serotype III detected by mPCR is relatively low compared to serotype III detected by group specific antisera (7.3% vs. 10.1%) and reports from other countries (Imperi *et al.,* 2010; Wang *et al.,* 2015). Serotype IV was not detected originally by type specific antisera, but it was detected by mPCR among non typeable GBS strains (one isolate). Recently GBS serotype IV is emerging among pregnant women as a colonizer and in neonatal disease in USA, Canada and Iran (Teatero et *al.,* 2015; Teatero *et al.,* 2017; Diedrick *et al.,* 2010; Jannati *et al.,* 2012). Generally, GBS serotype VI, VIII and IX were not detected in this study by both methds: serotype specific antisera and multiplex PCR.

Multi locus sequence typing (MLST) grouped 123 GBS strains collected from various parts of Ethiopia in to 17 STs. In contrast to the present study, which indicated high prevalence of ST-10 (30.9%), ST-19 (11.4%) and ST-2(10.6%), study from Bucharest area reported ST-1, ST-17 and ST-28 to be the prevalent STs (Usein *et al.,* 2014). Study from Sweden also identified five major genetic lineages such as; ST-19, ST-23, ST-17, ST-1 and ST-9 in

which majority of them are different from predominant STs of the present study (Luan *et al.*, 2005). Similar to the present study, high proportion of ST19 was reported from China (Wang *et al.*, 2015). Moreover, from globally collected GBS strains, ST-1, ST-19, and ST-23 were found to be the prevalent STs (Jones *et al.*, 2003). In contrast this study, high proportion of ST-1 (20%), and ST-22 (18%) were reported from USA (Manning *et al.*, 2008). Sequence types such as ST-1, ST-17, ST-9, ST-22 and ST-28 were not identified in this study.

Among 17 STs detected in this study 5 of them were Nobel GBS strains from Ethiopia. These new sequence types were identified from three different sties of Ethiopi; 3 of them from HRH, one of them from AHMC and one from TASH. The most predominant new ST was ST-932. As some STs are linked with invasive disease; it important to moniter ST profile of GBS strains circulating within a given country (Teatero *et al.*, 2016).

The population structure of GBS strains collected in this study is different from other countries both in quantity and diversity. 123 GBS strains collected from different parts of Ethiopia were grouped only in to two clonal complexes and one singleton: CC-2 and CC-249. Majority of GBS strains 82.2% belongs to CC-2, only 15.5% of them categorized under CC-249. Unlike this study, GBS strains collected from Poland were grouped in to five different clonal complexes such as CC23, CC19, CC17, CC10 and CC1 (Brzychczy-wloch *et al.*, 2014). Unlike this study, Meehan *et al.* (2014) reported CC1, CC17 and CC23 as major Clonal complexes from Ireland. Additionally, the predominant CCs reported from Bucharest are CC19, CC10, and CC17 (Usein *et al.*, 2014). This may indicate the STs and population structure of GBS stains collected from Ethiopia is different from other geographic areas and need further investigation to understand how population structure of GBS from Ethiopia fit in to the global population structure of GBS.

There is little variation of STs distribution across the three study sites. ST-10, ST-19 and ST-2 were detected from all three study sites; whereas ST-110, ST-932 and ST-5 were detected only from TASH. ST-8, ST-3, and ST-933 were detected only from AHMC. ST-934, ST-167, ST-196, 935 and ST-936 were detected only from HRH. Majority of STs in present study were represented by single serotype except ST-19 and ST-196. ST-19 contains

serotype III (64.3%) and serotype V (35.7%); ST-196 contains serotype IV (33.3%) and V (66.7%). This may indicate serotype switching. It requires further investigation to confirm if there is capsular switching between different serotypes. Capsular switching among GBS has also been reported from somewhere else (Neemuchwala *et al.,* 2016b; Bellais *et al.,* 2012).

Majority of GBS serotypes identified in this study are heterogeneous in terms of STs. Among different GBS serotype identified in this study, serotype Ia, II and V are the most hetrogeneous. All of them were grouped in to five different STs; serotype Ia were grouped in to ST-23, ST-249, ST-5, ST-933 and ST-934. Serotype II was grouped in to ST-110, ST-167 ST-3, ST-932, and ST- 936. Serotype V was grouped in to ST-110, ST-, ST-19, ST-196, and ST-2. The next heterogeneous serotype was Ib, it was grouped in to two STs (ST-12 and ST-8). Serotype III is homogenous; it was grouped in to only one STs (ST-19). Unlike the present study, among invasive GBS strains collected by Wang *et al,* (2015) serotype III was grouped in to three STs (ST-17, ST-19 and ST-650). Comparable to this study, all of the GBS strains collected from Bucharest area were grouped in to more than one STs (Usein *et al.,* 2014).

Out of 123 GBS strains collected in this study, 89.4% of them harboured at least one antibiotic resistance gene. Similar to this study, Boswihi and Colleagues (2012) identified antibiotic resistance gene among majority of GBS they collected from Kuwait. The frequent antibiotic resistance gene detected among GBS collected in this study was Tetracycline resistance genes such as *tet* (M-1), *tet* (L-1) and *tet* (O-1). Likewise, majority of GBS strains from other places also harbored Tetracycline resistance genes (Usein *et al.,* 2014; Da Cunha *et al.,* 2014; Boswihi *et al.,* 2012).

Among GBS strains tested in the present study, 88.6% of them harboured *tet* (M-1) gene alone and or in combination with other antibiotic resistance genes such as *tet* (L-1), *erm* (TR-1), *gyr*A (GBS-1), *par*C (GBS-1); 31.7% of them harboured *tet* (L-1) gene in combination with *tet* (M-1) gene; and 0.8% of them harboured *tet* (O-1) gene in combination with *erm* (B-1) gene. The total *tet* (M-1) resistance gene identified in the present study (88.6%) is comparable with study done in Kuwait (94.5%) (Boswihi *et al.,* 2012). The proportion of *t*et (L-1) gene detected in this study (31.7%) is high compared to report from

Kuwait (1.6%) (Boswihi *et al.,* 2012). The proportion of *tet* (O-1) resistance gene in this study is low (0.8%) compared to Kuwait (3.9%) (Boswihi *et al.,* 2012). Additionally another study from Egypt reported comparable level of *tet* (M-1) resistance gene (83.7%), low level of *tet* (L-1) resistance gene (12.2%) and relatively high level of *tet* (O-1) resistance gene (1%) among GBS strains collected from Egypt (Shabayek and Abdela, 2014b).

Among GBS strains collected from different parts of Italy, low proportion of them contains *tet* (M) resistance genes (46%) compared to this study (88.6%); however, high proportion of them contains *tet* (O) resistance genes (7.7%) compared to the present study (0.8%)(Gherardi *et al.,* 2007).

In contrast to other studies, most of GBS strains in the present study contained few antibiotic resistance genes that confer resistance to Erythromycin and Clindamycin (Wang *et al.,* 2015). Two types of Erythromycin resistance genes were detected among GBS strains collected in this study such as *erm* (TR-1) and *erm* (B-1). Out of total GBS tested, 5.7% of them contained *erm* (TR-1) and 0.8% of them contained *erm* (B-1). Unlike this study in which the level of *erm* (B-1) and *erm* (TR-1) resistance gene is low, high proportion of *erm* B (61.1%) (Boswihi *et al.,* 2012) and *erm* TR (38.9%) (Pinto *et al.,* 2013) was detected among GBS strains collected from Kuwait and Brazil respectively. Moreover study from S. Africa reported high proportion of *erm* B gene (55%) among GBS they collected (Bolukaoto *et al.,* 2015).

In the present study two GBS isolates which showed intermediate activity to Erythromycin (MIC=0.5µg/ml) did not contain any Erythromycin resistance gene. In contrast to this study, Heelan *et al.,* (2004) identified Erythromycin resistance gene among all GBS strains which showed resistance and intermediate activity to Erythromycin. The mechanism of antibiotic reisistance of these two GBS strain is not clear. Moreover, similar to USA and China *erm* (TR-1) resistance genes were detected from almost all Erythromycin resistant GBS strain (Heelan *et al.,* 2004; Wang *et al.,* 2015). In this study, Erythromycin resistance gene were detected among majority of GBS serotype V (26.1%) followed by serotype III (22.2%). Similar finding was reported from Italy (Gherardi *et al.,* 2007). Unlike this study, relatively

high proportion of GBS serotype III from Kuwait was found to harbour Erythromycin resistance gene (Boswihi *et al.,* 2014).

Gene mutation in the quinolone resistance-determining regions (QRDRs) of gyrase and topoisomerase IV were identified among 4.1% of GBS strains collected in this study, the detected genes were *gyr*A (GBS-1) and *Par*C (GBS-1). Five GBS strains in this study contain both *gyr*A (GBS-1) and *par*C (GBS-1) together along with other antibiotic resistance genes. Unlike this study, study from Taiwan, Canada and China identified Levofloxacin resistance genes among majority of GBS strains collected in their respective countries (Wu *et al.,* 2008; Piccinelli *et al.,* 2015; Neemuchwala *et al.,* 2016a; Wang *et al.,* 2013). Most of quinolone resistance determining regions from Italy and China were detected from GBS serotype Ib, which belongs ST-19 and serotype III which belongs to ST-19 respectively (Piccinelli *et al.,* 2015; Wang *et al.,* 2013). However, all GBS strains which contained fluoroquinoles (Levofloxacin) resistance gene in this study were detected from serotype V that belongs to ST-19. Similar to this study, Usein *et al.* (2014) detected fluoroquinolones resistance genes among serotype V that belongs to ST-19 (Usein *et al.,* 2014).

The pattern of antibiotic resistance genes in this study showed slight difference among STs. Antibiotic resistance genes which confer resistance to Levofloxacin, *gyr*A (GBS-1) and *par*C (GBS-1) were detected only from ST-19. Antibiotic resistance gene such as *erm* (B-1, *tet* (O-1) were detected only from ST-110. The *tet* (L-1) was detected from ST-10 and ST-19. Similar pattern of antibiotic resistance genes among STs were reported from various countries (Usein *et al.,* 2014; Wang *et al.,* 2013). Unlike GBS strains collected from other countries, *mef* (A/E), *mef* (A), and *mef* (E) antibiotic resistance gene were not detected among GBS strains collected for this study (Nakamura *et al.,* 2011; Zhao *et al.,* 2008; Garland *et al.,* 2011; Arana *et al.,* 2014; Nagano *et al.,* 2012) .

CHAPTER V: CONCLUCION AND RECOMMENDATION

5.1. CONCLUSION

In this study, a total of 1873 participants involved from three study sites, AHMC, HRH and TASH; 840 of them were pregnant women, 857 were their newborn, and 176 were infants less than 90 days with early and late onset neonatal disease. Majority of pregnant women participants were in gestational age of 37-42 weeks, all of them delivered vaginally. The overall prevalence of GBS among pregnant women, their new born, newborn with early onset neonatal disease and vertical transmission rate is 17.4%, 9.2%, 1.7% and 54.11% respectively. The overall serotype distribution of GBS collected from pregnant women, their newborn, and infants less than 90 days with early onset neonatal disease is as follows: serotype II (30.3%), serotype Ia (20.2%), serotype V (17.9%), serotype Ib (17.1%), serotype III (10.1%), VII (0.9%) and non-typeable (3.5%). In this study no significant association was observed between GBS colonization among pregnant women and newborn and risk factors measured. All of the GBS isolates were susceptible to Penicillin, Linezolid, Cefotaxime and Vancomycin. High level resistance was observed to Tetracycline (91.2%) on the other hand, low level resistance was observed to Clindamycin (0.8%), Erythromycin (6.4%), Daptomycin (2.4%) and Levofloxacin (7.2%). GBS strains collected from different parts of Ethiopia were grouped in to 17 STs by MLST. The prevalent STs in decreasing order is as follows; ST-10 (30.9%), ST-19 (11.4%), ST-2 (10.6%), ST-23 (7.3%), ST-12 (6.5%), ST-933 (5.7%), ST-8 (4.9%), ST-5 (4.1%), and ST-932 (3.3%). ST-932, ST-933, ST-934, ST-935 and ST-936 are new GBS strains entered to http://pubmlst.org/sagalactiae online GBS data base. 123 GBS strains collected in the present study was resolved in to two clonal complexes: CC-2 and CC-249 and one singleton (ST-934). Antibiotic resistance genes were detected from 89.4% of GBS strains collected this study. 51.2% of them contains *tet*(M-1) antibiotic resistance gene alone, 31.7% of them contains *tet(*L-1) and *tet*(M-1), 1.6% of them contains *erm*(TR-1) and *Tet*(M-1), 4.1% of them contains *erm (*TR-1), *gyr*A (GBS-1), *Par*C (GBS-1) and *tet*(M-1), 1(0.8%) of them contains *erm*(B-1, *tet*(O-1). Overall this study demonstrated the presense of primary risk factor for early onset disease due to GBS and neonatal disease due to GBS in three sites of Ethiopia. In the present study large number of infants with disease was not included because of shortage of logistics and limited

fund; therefore, it is difficult to compare the finding of this study of neonatal disease due to GBS with those reported from other countreis.

5.2. RECOMMENDATIONS

Maternal GBS colonization late in pregnancy is the necessary risk factor for development of EOD. In the present study, the overall and site specific maternal GBS colonization rate is comparable to colonization rate reported from developed countries. Neonatal sepsis due to GBS was shown in this study for the first time from Ethiopia setting. Based on these finding the following recommendation is made;

- Large scsle and multicenter study to determine EOD and LOD due to GBS from Ethiopia at large.

All of GBS strains tested in this study were susceptible to penicillin, the first choise of antibiotic for IAP and majority of them were susceptible to erythromycin and clindamycin, the second choise of antibiotic if pregnant women are allergic to penicillin and there is high risk of anaphlyxis. The existing strategy (IAP), which is widely used in developed countreis, is currently facing a problem because of high level of resistance to erythromycin and clindamycin. Most importantly, penicillin is challenged by emergence of GBS with reduced suscpetiblity to penicillin from several countries. Based on these findings the following recommendations are made;

- As all or most of GBS strains collected in this study were susceptible to penicillin, clindamycin and erythromycin, risk factor based IAP strategy should be considered until vaccine is available.
- Monitering antimicrobial susceptibility pattern of GBS to penicillin, erythromycin, clindamycin, cefotaxime, vancomycin and other antibiotics that can possibly used for IAP or management of GBS disease in Ethiopia.

This study identified the prevalent GBS serotypes to be serotype II, Ia, V, Ib, and III. Few capsular polysaccharide based GBS vaccines are entering advanced stage clinical trial. It was indicated that GBS serotypes vary from country to country and from time to time. The vaccine formulation for one country may not be suitable for the other country and it is

probably may not work all the time as GBS serotype can change over time. Moreover, the prevalene of serotype III, the one which is assumed to be most virulent, is low in this study. Therefore, based on these findings the following are recommended;

- MOH-Ethiopia or other stake holder along with international organization should consider working together on vaccine that considers GBS serotypes circulating in Ethiopia; according to this study, serotype Ia, Ib, II and V are the most prevalent.
- Monitering GBS serotypes circulating among pregnant women and newborn with EOD and LOD over time in Ethiopia.
- As GBS serotype III, which is highly virulent, is low in this further study is needed to see how this will affect burden of disease due to GBS among infants less than 90 days in Ethiopia.

Molecular characterization of GBS strains in this study by MLST and wholde genome sequencing revealed partially different Sequence types, Clonal complexes and antibiotic resistance genes compared to other countreis. The population structure does not fit to global GBS population structure. Most importantly, ST-17 and CC-17 (hyper virulent ST and CC) was not detected in this study. Almost all GBS strains tested harboured antibiotic resistance genes, majority of them were tetracycline resistance gene; even though few in number, antibiotic resistance genes to second option antibiotics for IAP; erythromycin and clindamycin were detected. Based on these findings the following are forwarded;

- Further study should be done to determine how the absence of hypervirulent ST and CC will affect EOD and LOD caused by GBS in Ethiopia.
- Additional large scale study covering wider geographic location of the country and long time span is required to assess how GBS strains from Ethiopia will fit to global population structure of GBS.
- As antibiotic resistance gene can commonly transfer among GBS strains by horizontal mechanism, monitoring of antibiotic resistance genes among GBS strain circulating in Ethiopia to commonly used antibiotics for management of GBS disease and IAP should be considered.

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ANNEX I: INFORMATION SHEET FOR PREGNANT WOMEN AND THEIR NEW BORN

Annex 1.1. Information sheet for pregnant women and their new born (English version)

You are invited to participate in this study, which involves mothers and their newborn from Tikur Anbessa Specilized Hospital, Hawassa Referral Hospital, and Adama Hospital Medical College. The aim of this study is to determine the colonization rate among pregnant women and their newborn by bacteria known as group B *Streptococcus* and the burden of neonatal disease caused by this microbe in Ethiopia and to give recommendation to concerned bodies so as to take appropriate measures to prevent this disease. Group B *Streptococcus* is recognized as a major cause of disease among newborn in different parts of the world. Transmission of group B *Streptococcus* from colonized mother to their newborn is vertical, it occurs during birth.

a. **Purpose**: the purpose of this study is to determine colonization rate of GBS among pregnant women, their newborn and burden of neonatal disease in selected hospitals of Ethiopia: Tikur Anbessa Specilaized Hospital, Hawassa Referral Hospital and Adama Teaching Hospital.

b. **Duration**: the duration of this study depend on the availability of study subjects and it can take about one year. However, specimen from you and your child is collected only once. If infection is suspected in your child more samples will be collected.

c. **Procedure to be carried out:** the procedure is easy and simple; sample from pregnant women will be collected from vagina and anorectal area using sterile cotton swab by attending physician. Sample will also be taken from your new child from external ear, nasal nares, and umbilical area using cotton swab by attending physician. For newborns suspected of sepsis blood or for newborn suspected of meningitis CSF sample will by collected by attending physician. All samples will be transported to Laboratory for analysis.

d. **Risk and discomfort:** There will be minor discomfort during collection of samples. During collection of samples from your child appropriate precaution will be taken and all samples will be collected by trained health professionals. Appropriate medical care will be provided to your child.

e. Expected benefits: Mothers are not directly benefited from this study, however; in the future newborn in Ethiopia will be benefited at large, as this study contribute for consideration of antibiotic prophylaxis and vaccine development as a prevention strategy in Ethiopia.

f. Confidentiality: We respect yours and your child's privacy and confidentiality. Any information that identifies you or your child will not be shared with anyone else outside the study team. If a research article or publication comes from this study, you and your child will not be identified by name. The information we collect from you and your child as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study.

g. Voluntary Participation and Withdrawal from the Study: Your participation is completely voluntary and you have the right to refuse to participate in this study. You can stop participating and prevent your child's participation in the study at any time after giving your consent. This decision will not affect in any way your and your child's current or future medical care in the health facility.

h. Contact information: If you have any questions about this study you can contact the following investigators and the ethics committee for further information. Mussa Mohammed Tel 0926044997; Dr. Yimtubezinash Woldeamanuel Tel 0911225832; Dr. Daniel Asrat Tel 0911223019 ; IRB-CHS tel : 0118961396

Annex 1.2 Amharic translation information sheets

የመረጀ ቅጽ ለነፍሰ ጡር ሴቶች እና ለህጻናቶቻቸዉ (Amharic version)

በዚህ እናቶችን እና አዳዲስ የሚወለዱ ህጻናትን በሚያካትት ጥናት እንዲሳተፉ ተጋብዟል። ይህ ጥናት የሚካሄደዉ በጥቁር አንበሳ ዩኒቨርሲቲ ሆስፒታል ፣ በሀዋሳ ሪፋራል ሆስፒታል እና በአዳማ ሆስፒታል ሜዲካል ኮሌጅ ዉስጥ ነው። የዚህ ጥናት ዋና አላማ ግሩፕ ቢ እስትሪፕቶኮከስ የሚባል ባክቴርያ በእናቶች እና በ ህጻናት ላይ የለዉን ስርጭት ለማወቅ፤ በዚህ ባክቴርያ የሚመጠውን የህጻናት ህመም በኢትዮጵያ የለዉን መጠን ለማወቅ፤ እንዲሁም አስፈላጊው የመከላከል እርምጃ እንዲወሰድ ለሚመለከተው አካል ለማሳወቅ ነው። ግሩፕ ቢ እስትሪፕቶኮከስ በአለማችን ላይ ዋና የህጻናት ህመም አምጪ እንደሆነ ተውቆዋል። ግሩፕ ቢ እስትሪፕቶኮከስ ከእናት ወደ ልጅ የሚተላለፈው በወሊድ ግዜ በቀጥታ ነው።

<u>ህ አላማ:</u> የዚህ ጥናት አላማ በኢትዮጲያ በተመረጡ ሆስፒታሎች ዉስጥ፡ጥቁር አንበሳ ዩኒቨርሲቲ ሆስፒታል፤ ሃዋሳ ሪፈራል ሆስፒታል እና አዳማ ሆስፒታል ሜድካል ኮሌጅ፤ የግሩፕ ቢ እስትሪፕቶኮከስ በእናቶች እና በህጻናት ላይ ያለውን ስርጭት ለማወቅ እና እንዲሁም በዚህ ባክቴርያ የሚመጠውን የህጻናት ህመም መጠን ለማወቅ ነው።

<u>ለ የሚፈጀው ጊዜ:</u> ይህ ጥናት የሚፈጀው ጊዜ በጥናቱ ተሳታፊ የመገኘት ሁኔታ ላይ ይወሰናል ፤ ሆኖም እስከ አንድ አመት ጊዜ ሊፈጅ ይችላል። ነገርግን ከእርሶ እና ከልጆት ናሙና የሚወሰደው አንድ ግዜ ብቻ ነው። ልጆት የህመም ምልክት ካሳየ ተጨማሪ ናሙና ልወሰድ ይችላል።

<u>ሐ የአሰራር ሁኔታ</u>: ለዚህ ጥናት የሚወሰደው ናሙና በቀላል መንገድ የሚከናወን ነው። ናሙና የሚወሰደው በሀኪም ከእናቶች ከሀፍረታ ስጋ እና ከፍንጢጣ ላይ ጥጥ በመጠቀም ነው። ከሚወለደው ልጅ ላይ ከጆሮ የዉጭ አካል ፡ ከአፍንጫ ዉስጥ እና ከእንብርት አከባቢ ጥጥ በመጠቀም ናሙና ይወሰዳል። ከታመሙት ህጻናት የትኩሳት ምልክት ከላቸዉ ደም ወይንም የማጅራት ገትር ምልክት ከላቸዉ ከጀርባ አከርካሪ የሚገኝ ፈሳሽ እንደ አስፈላጊነቱ በሀኪም ይወሰዳል፡ ከዚያም ሁሉም ናሙና ለምርመራ ወደ ላብራቶሪ ይላካል።

<u>መ የሚያስከትለው አደጋ:</u> ናሙና በሚወሰድበት ወቅት አነስተኛ አለመመቸት ሊኖር ይችላል። ናሙና ከልጆት በሚወሰድበት ጊዜ አስፈላጊው ጥንቃቄ ይደረጋል: እንዲሁም ሁሉም ናሙና የሚወሰደው በሰለጠኑ የጤና በለሙያዎች ነው። አስፈላጊ የህክምና ክትትል ለልጆት ይደረጋል።

<u>ሠ የሚያስገኘው ጥቅም:</u> እናቶች ከዚህ ጥናት በቀጥታ ተጣቃሚ አይደሉም። ነገር ግን ወደ ፊት ኢትዮጲያ ዉስጥ የሚወለዱ ህጻናት በሰፊው ተጠቃሚ ይሆናሉ። ምክንያቱም ይህ ጥናት ለዚህ ህመም የሚሆን ቅድመ ህክምና ብሎም ክትባት እንደመከላከያ ዘዴ ወደፊት ኢትዮጲያ ዉስጥ እንዲታሰብ ትልቅ አስተዋጽኦ ስለለው ነው።

<u>ረ . ሚስጥራዊነት:</u> የእርሶ እና የልጆትን ሚስጥር እናከብራለን። እርሶን እና ልጆትን የምያመላክት ማንኛዉንም መረጃ ከተመራማሪዎች በስተቀር ለማንም ታልፎ አይሰጥም። ከዚህ ጥናት በመጻሄት ላይ የሚታተም ነገር ካለ እርሶም ሆነ ልጆት በስም አትጠቀሱም። ለዚህ ጥናት ተብሎ ከርሶም ሆነ ከልጆት የተገኘ ማንኛዉንም መረጃ ሳጥን ዉስጥ ይቆለፍበታል ወይንም በሚስጥር ቁጥር ኮምፒተር ዉስጥ ይቀመጣል። ይህንን መረጀ ማግኘት የሚችሉት ከምርምሩ ጋር ግኑኝነት ያላቸዉ ሰዎች ብቻ ናቸዉ።

ሰ. በፈቃደኝነት የመሳተፍ እና ከጥናቱ ስለመቋረጥ: በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በፈቃደኝነት ላይ የተመሰረተ ነው። ከልጆት ጋር በጥናቱ ያለመሳተፍ መብት አሎት። እርሶ እና ልጆት በማንኛውም ግዜ የዚህ ጥናት ተሳታፊነቶን የስምምነት ቅፁንም ከፋረሙ ቦኋላ ቢሆን ማቋረጥ ይችላሉ። ይህን በመወሰኖ ከልጆት ጋር አሁን የሚያገኙትም ሆነ ወደፊት የምያገኙት የጤና አገልግሎት በምንም አይነት መልኩ አይስትጓገልም።

ሸ. አድራሻ: ይህን ጥናት በተመለከተ ጥያቄ ከሎት በሚከተለው አድራሻ ተመራማሪዎችን እና የስነምግባር ኮሚቴ በመጠየቅ ዝርዝር ማብራርያ ማግኘት ይችላሉ። ሙሳ መሀመድ ስልክ 0926044997፤ ዶ/ር ይምጡበዝናሽ ወልደአማኑኤል ስልክ 0911223019፤ ዶ/ር ዳንኤል አስራት ስልክ 0911223019፤ አይ አር ቢ– ጤ ና ሳይንስ ኮሌጅ ስልክ 0118961396

ANNEX II: CONSENT FORM

Annex- 2.1 Consent form for mother and newborn

Consent form for pregnant women and their new born (English version)

Serial no_____

Card no_____

Name of study participant: _____

I have been requested to participate in this study which involves collection of specimen from me and from my new child. The purpose of the study and sample collection procedure has been explained to me. I have also read the information sheet (or it has been read to me); I have understood that this study is about Group B *streptococcus* in selected Hospitals of Ethiopia, and this bacteria is one of leading cause of morbidity and mortality among newborn in the world. I have asked some questions and clarification has been given to me. I have given my consent freely to participate in the study with my child and I hereby confirm my agreement with my signature.

	Date	Signature
Mother		
Investigator signature		
Witness signature		
1. 2.		

Annex 2.2 Amharic translation of consent form for pregnant mothers

የስምምነት ቅጽ ለነፍስ ጡርሴቶች እና ለህጻናቶች (Amharic version)

ተራ ቁጥር_____

የካርድ ቁጥር_____

የተሳታፊ ስም_____

በዚህ ጥናት፣ ናሙናው ከ እኔ እና አዲስ ከሚወለደዉ ልጄ በሚወሰድበት እንድሳተፍ ተጋብዥያለው። የዚህ ጥናት አስፈላጊነት እና የናሙና አወሳሰድ ተብራርቶልኛል። የመረጃ ቅጹንም አንብቢያለው ወይንም ተነቦልኛል። ይህ ጥናት ስላ ግሩፕ ቢ እስትሪፕቶኮከስ በተመረጡ የኢትዮጲያ ሆስፒታሎች ዉስጥ ያለውን ስርጭት ለማወቅ እንደሆነም ተረድቻለው። ይህ ባክቴርያ በአለም ላይ ዋና የህጻናት ህመም አምጪ እና ገዳይ እንደሆነም ተገንዝብያለው። አንድንድ ያልተረደውትን ነገር ጠይቄ ማብራርያ ተሰቶኛል። በዚህ ጥናት ከሚወለደው ልጄ ጋር ለመሳተፍ ፍቃደኛ መሆኔን በፊርማዬ አረጋግጣለው።

	ቀን	ፊርማ
የጥናቱ ተሳታፍ እናት		
ተመራማሪ		
ምስክሮች 1. . 2.		

ANNEX- III INFORMATION SHEET FOR NEWBORN WITH NEONATAL DISEASE

Annex 3.1 Information sheet Parents and/ or guardians of new born with EOD and LOD (English version)

You are invited to let your child participate in this study. This study involves newborn with disease at Tikur Anbessa Specilized Hospital. The aim of this study is to determine the burden of early and late onset disease due to Group B *Streptococcus* at Tikur Anbessa Specilized Hospital and to give recommendation to concerned bodies so as to take appropriate measures to prevent this disease. Group B *Streptococcus* is recognized as a major cause of disease among newborn in different parts of the world.

a. Purpose: the purpose of this study is to determine the burden of early and late onset disease due to GBS at Tikur Anbessa Specilzed Hospital.

b. Duration: the duration of this study depend on the availability of study subjects and it can take about one year. However, specimen from your child is collected only once.

c. Procedure to be carried out: the procedure is easy and simple; in children suspected of sepsis blood or CSF for newborn suspected of meningitis will by collected by attending physician. All samples will be transported to Laboratory for analysis.

d. Risk and discomfort: There will be minor discomfort during collection of samples. During collection of samples from your child appropriate precaution will be taken and all samples will be collected by trained health professionals. Appropriate medical care will be provided to your child.

e. Expected benefits: The information gained from yours and others child will help to consider prevention strategy for neonatal disease caused by GBS in Ethiopia, if your baby is positive for GBS appropriate medical care will be provided to him.

f. Confidentiality: We respect your child's privacy and confidentiality. Any information that identifies your child will not be shared with anyone else outside the study team. If a research article or publication comes from this study, your child will not be identified by name. The information we collect from your child as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study.

g. **Voluntary Participation and Withdrawal from the Study:** The participation is completely voluntary and you have the right not to late your child to participate in this study. You can stop your child participating in the study at any time after giving your consent. This decision will not affect in any way yours or your child's current or future medical care in the health facility.

h. **Contact information:** If you have any questions about this study you can contact the following investigators and the ethics committee for further information. Mussa Mohammed Tel 0926044997; Dr. Yimtubezinash Woldeamanuel Tel 0911225832; Dr. Daniel Asrat Tel 0911223019 ; IRB-CHS tel : 0118961396

Annex 3.2 Amharic translation information sheets for diseased newborn

የመረጀ ቅጽ ለታመሙት ህጻናት ወላጆች ወይንም አሳዳጊዎች (Amharic version)

በዚህ ጥናት ልጆትን እንዲያሳትፉ ተጋብዟል። ይህ ጥናት የሚካሄደዉ ታመዉ ወደ ጥቁር አንበሳ ዩኒቨርሲቲ ሆስፒታል በመጡ ህጻናት ላይ ነው። የዚህ ጥናት ዋና አላማ ግሩፕ ቢ እስትሪፕቶኮከስ በሚባል ባክቴርያ የሚመጠውን የጨቅላ ህጻናት ህመም እና እስከ 3 ወር ያሉትን ህጻናት ህመም መጠን ለማወቅ ነው። እንዲሁም አስፈላጊው የመከላከል እርምጃ እንዲወሰድ ለሚመለከተው አካል ለማሳወቅ ነው። ግሩፕ ቢ እስትሪፕቶኮከስ በተለያዩ የአለማችን ክፍል ላይ ዋና የህጻናት ህመም አምጪ እንደሆነ ተውቆዋል።

<u>ህ አላማ:</u> የዚህ ጥናት አላማ በጥቁር እንበሳ ዩኒቨርሲቲ ሆስፒታል በግሩፕ ቢ እስትሪፕቶኮከስ የሚመጣዉን የጨቅላ ህጻናት ህመም እና እስከ 3 ወር ያሉትን ህጻናት ህመም መጠን ለማወቅ ነው።

<u>ለ የሚፈጀው ጊዜ:</u> ይህ ጥናት የሚፈጀው ጊዜ በጥናቱ ተሳታፊ የመገኘት ሁኔታ ላይ ይወሰናል ። ሆኖም እስከ እንድ አመት ጊዜ ሊፈጅ ይችላል ፡ነገርግን ከልጆት ናሙና የሚወሰደው አንድ ግዜ ብቻ ነው።

<u>ሐ የአሰራር ሁኔታ</u>: ለዚህ ጥናት የሚወሰደዉ ናሙና በቀላል መንገድ የሚከናወን ነው። ናሙና የሚወሰደው ታመው ትኩሳት ካለባቸዉ ልጆች ደም ወይንም የማጅራት ገትር ምልክት ካለባቸዉ ልጆች ከጀርባ አከርካሪ የሚገኝ ፈሳሽ እንደ አስፈላጊነቱ በሀኪም ይወሰዳል ።

<u>መ የሚያስከትለው አደጋ:</u> ናሙና በሚወሰድበት ወቅት አነስትኛ አለመመቸት ሊኖር ይችላል። ናሙና ከልጆት በሚወሰድበት ጊዜ አስፈላጊዉ ጥንቃቄ ይደረጋል። እንዲሁም ናሙና የሚወሰደው በሰለጠኑ የጤና በለሙያዎች ነው። አስፈላጊዉ የህክምና ክትትልም ለልጆት ይደረጋል።

<u>ሠ የሚያስገኘው ጥቅም:</u> ይህ ከእርሶ ልጅ እና ከ ሌሎች ልጆች የሚገኝ መረጃ ወደፊት በግሩፕ ቢ እስትሪፕቶኮከስ የሚመጣዉን የህጻናት ህመም የመከላከል ዘዴን ለማሰብ ይጠቅማል። የእርሶ ልጅ ግሩፕ ቢ እስትሪፕቶኮከስ ከተገኘበት ተገቢው የህክምና እርዳታ ይደረግለታል።

<u>ረ . ሚስጥራዊነት:</u> የልጆትን ሚስጥር እናከብራለን። ለጆትን የሚያመላክት ማንኛዉንም መረጃ ከተመራማሪዎች በስተቀር ለማንም ታልፎ አይሰጥም። ከዚህ ጥናት በመጼሄት ላይ የሚታተም ነገር ካለ ልጆት በስም አይጠቀስም። ከልጆት የተገኘ ማንኛዉም መረጃ ሳጥን ዉስጥ ይቆለፍበታል ወይንም በሚስጥር ቁጥር ኮምፒተር ዉስጥ ይቀመጣል። ይህንን መረጀ ማግኘት የሚችሉት ክምርምሩ ጋር ግኙኝነት ያላቸዉ ሰዎች ብቻ ናቸዉ።

<u>ሰ. በፈቃደኝነት የመሳተፍ እና ከጥናቱ ስለመቋረጥ:</u> በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በፈቃደኝነት ላይ የተመሰረተ ነው። ለጆትን ያለማሳተፍ መብት አሎት። በማንኛውም ግዜ የልጆትን የጥናት ተሳታፊነት የስምምነት ቅፁንም ከፋረሙ ቦኋላ ቢሆን ማቋረጥ ይችላሉ። ይህን በመወሰኖ ከልጆት ጋር አሁን የሚያገኙት ሆነ ወደፊት የምያገኙት የጤና አገልግሎት በምንም አይነት መልኩ አይስትጓገልም።

<u>ሸ. አድራሻ:</u> ይህንን ጥናት በተመለከተ ጥያቄ ከሎት በሚከተለው አድራሻ ተመራማሪዎችን እና የስነምግባር ኮሚቴ በመጠየቅ ዝርዝር ማብራርያ ማግኘት ይችላሉ። ሙሳ መሀመድ ስልክ 0926044997፤ ዶ/ር ይምጡበዝናሽ ወልደአማኑኤል ስልክ 0911223019፤ ዶ/ር ዳንኤል አስራት ስልክ 0911223019፤ አይ አር ቢ–ጤና ሳይንስ ኮሌጅ ስልክ 0118961396

ANNEX IV CONSENT FORM FOR NEWBORN WITH NEONATAL DISEASE

Annex 4.1 Consent form for Parents and or guardians of new born with EOD and LOD (English version)

Serial no_____

Card no_____

Name of study participant: _____

I have been requested to let my child participate in this study which involves collection of specimen from my child. The purpose of the study and sample collection procedure has been explained to me. I have also read the information sheet (or it has been read to me); I have understood that this study is about burden of Group B *streptococcus* at Tikur Anbessa Specilzed Hospital, Ethiopia, which is one of leading cause of morbidity and mortality among newborn in the world. I have asked some questions and clarification has been given to me. I have given my consent on behalf of my child to let him participate in the study and I hereby confirm my agreement with my signature.

	Date	Signature
Parents or guardian signature		
Investigator signature		
Witness signature 1.		
2.		

Annex 4.2 Amharic consent form translation for Guardians

የስምምነት ቅጽ ለወላጆች ወይንም ላእሳዳጊዎች (Amharic version)

ተራ ቁጥር_____

የካርድ ቁጥር_____

የተሳታፊ ስም_____

በዚህ ጥናት፡ ናሙናው ክልጇ በሚወሰድበት፣ ልጇን እንዳሳተፍ ተጋብዣለው። የዚህ ጥናት አስፈላጊነት እና የናሙና አወሳሰድ ተብራርቶልኛል። የመረጃ ቅጹንም አንብቢያለው ወይንም ተነቦልኛል። ይህ ጥናት ስላ ግሩፕ ቢ እስትሪፕቶኮከስ በጥቁር አንበሳ ዩኒቨርሲቲ ሆስፒታል ኢትዮጲያ ያለውን መጠን ለማወቅ እንደሆነም ተረድቻለው። ይህ ባክቴርያ በአለም ላይ ዋና የህጻናት ህመም አምጪ እና ገዳይ እንደሆነም ተገንዝቢያለው። አንዳንድ ያልተረደውቱን ነገር ጠይቄ ማብራርያ ተሰቶኛል። በዚህ ጥናት ልጇ እንዲሳተፍ ፍቃደኛ መሆኔን በፊርማዬ አረጋግጣለው።

	ቀን	ፊርማ
ውላጅ ወይንም አሳዳጊ		
ተመራማሪ		
ምስክሮች 1.		
2.		

ANNEX V: QUESTIONNAIRE FOR PREGNANT WOMEN AND NEWBORN

5.1. Questionnaire for collection of socio-demographic and delivery realted information from women and their new born

Socio-demographic data of the mother and status of newborn

a. Participant Identification (pregnant women)

1. Serial No_____ 2. Card no_____
3. Address _____ 4. Participant name_____
5. Age_____ 6. Phone number_____
7. Occupation_____
9. History of primigravida or multigravida: Primigravida _____
   Multigravida_____
10. Number of prenatal visit_____
11. History of previous new born developed EOD_____
13. Rupture of membranes before labor onset _____
14. Duration of rupture of membrane_____
15. Presence of intrapartum fever (temperature) oC_____
17. Any other illness_____
17. Week of delivery_____
18. Date and time of rectovaginal specimen collection _____

b. Participant Identification (new born)

Mother Name_____

Serial number_____

Week of birth_____

Weight of newborn on delivery_____

Any abnormality observed with the new born_____

Date and time of collection of swab from external ear, nasal nares, and umbilical area of the newborn_____

5.2. Intervew question with colonized mother

1. Telephone interview questions format with mother colonized by GBS (English version)
2. We are calling you from (Hawassa, Adama, Tikur Anbesa specialized Hospital)
3. How is your child? _____
4. Is your child looks fine? _____
5. Does your child show a sign of pain? _____
6. Does your child have fever? _____
7. Does your child vomit repeatedly? _____
8. Does your child show sign of irritability/ unusual movements? _____
9. Does your child have bulged anterior fontanelle? _____
10. Does your child feed on breast milk? _____
11. Does your child breathe fast? _____
12. Do you see any other abnormalities with your child? _____
13. If you see what is it? _____
14. Would you explain it more? _____

If your child is sick within 7 days after birth, please bring your baby to our hospital so that he will get medical follow up. We really appreciate for your time, thank you very much goodbye.

የስልክ ቃለ መጠይቅ ማድረግያ ቅጽ ግሩፕ ቢ እስትሪፕቶኮከስ ከተገኘባቸው እናት ጋር (Amharic version)

ከ (ሀዋሳ፡ አዳማ፡ ጥቁር አንበሳ ሆስፒታል) ነዉ የምንደውልሎት

1. ልጆት እንዴት ነዉ?_____
2. ልጆት ስታይ ደህና ይመስላል?_____
3. ልጆት የህመም ምልክት አለዉ? _____
4. ልጆት ትኩሳት አለው?_____
5. ልጆትን ደጋግመዉ ያስመልሰዋል? _____
6. ልጆት ይወራጫል ወይንም የልተለመደ እንቅስቃሴ ያሰያል?_____
7. የልጆት ኣናት አብጠዋል?_____
8. ልጆት ጡት ይጠባል ?_____
9. ልጆት ፈጠን ፈጠን እያለ ይተነፍሳል?_____
10. ሌላ ልጆት ላይ የልተለመደ ምልክት አይተዋል?_____
    a. ካዩ ምንድነዉ እሱ ያዩት_____
    b. ትንሽ ብያብራሩልኝ_____

ልጆት ከተወለደ እስከ ሰባት ቀን ድረስባለ ግዜ የጤና መታወክ ከገጠመው ልጆትን ወደ ሆስፒታላችን አምጥተው ተገቢዉን የህክምና ክትትል እንዲያገኝ ያስደርጉ።

ANNEX-VI QUESTIONNAIRE FOR PARENTS/GUARDIANS FOR NEW BORN WITH EOD AND LOD

6.1. Socio-demographic data

1. Serial number_____

2. Age (days) _____

3. Card number_____

4. Week of birth_____

5. Weight_____

6. Clinical diagnosis_____

6.2. Clinical data

a. Meningitis

Signs and symptoms

SNº	**Sign and symptoms**	**Yes**	**No**	**comments**
1.	Does the child have a fever?			
2.	Does the child have poor appetite?			
3.	Does the child have a bulged anterior fontanelle?			
4.	Does the child have a seizure?			
5.	Does the child have dyspnea?			
6.	Is the child irritable?			
7.	Does the child have anorexia?			
8.	Does the child have vomiting?			
9.	Does the child have diarrhea?			
10.	Does the child have abdominal distention?			
11.	Does the child have neck rigidity?			
12.	Does the child have cyanosis?			
13.	Does the child have jaundice?			
14.	Does the child have downward gaze of the eyes?			
15.	Does the child have abnormal body temperature?			
16.	Does the child have change of activity or irritability?			

b. Sepsis

Signs and symptoms

SN^{o}	**Sign and symptoms**	**Yes**	**No**	**comments**
1.	Is the child body temperature change hypo or hyperthermia?			
2.	Does the child have berating problem?			
3.	Does the child have diarrhea?			
4.	Does the child have reduced movements?			
5.	Does the child has reduced suckling?			
6.	Does the child have a seizure?			
7.	Does the child have slow heart rate?			
8.	Does the child have a swollen belly?			
9.	Does the child have vomiting?			
10.	Is the child skin and whites of the eyes turned yellow?			

Others_____

ANNEX VII LABORATORY IDENTIFICATION OF GBS

Type specimen collected from the mother: Rectovaginal swab

Type of specimen collected from the new born: Swab from External ear, nasal mares and umbilical area

7.1. Culture and identification of GBS from the mother's sample

1. Growth of GBS in Lim broth _____
2. Growth of GBS on Sheep blood agar and hemolysis pattern_____
3. Result of Gram stain_____
4. Catalase test result_____
5. Result of Bile esculin_____
6. Result of CAMP test_____
7. Result of Strep B Grouping Latex antisera: positive/Negative_____

7.2 Culture and identification of GBS from the new born

1. Growth of GBS in Lim broth _____
2. Growth of GBS on Sheep blood agar and hemolysis pattern_____
3. Result of Gram stain_____
4. Catalase test result_____
5. Result of bile esculin _____
6. Result of CAMP test_____
7. Result of Slides of Strep B Grouping Latex antisera: positive/Negative_____

7.3 Capsular serotyping result of the GBS isolates and Antimicrobial sensitivity test

a. Serotyping of GBS isolates from mother

GBS isolates (Serial number of mother)	Serotypes										
	Ia	Ib	II	III	IV	V	VI	VII	VIII	IX	NT

b. Serotyping GBS isolate from new born

GBS isolates (Serial number of newborn)	Serotypes										
	Ia	Ib	II	III	IV	V	VI	VII	VIII	IX	NT

c. Antimicrobial sensitivity test result of the GBS isolates

SN	Drug	Susceptible n (%)		Intermediate n (%)		Resistant n (%)	
		n	%	N	%	N	%
1	Penicillin(PEN)						
2	Levofloxacin(LEVO)						
3	Linezolid (LZD)						
4	Tetracycline(TET)						
5	Clindamycin(CL)						
6	Erythromycin(ERY)						
7	Cefotaxime(FOT)						
8	Vancomycin(VAN)						
9	Daptomycin(DAP)						

Comments_____

Name of principal investigator _____

Signature _____ Date _____

7.4. Molecular characterization result

a. Serotypng GBS by using multiplex PCR

GBS isolates from mother

NT GBS isolates (Serial number of mother)	Serotypes									
	Ia	Ib	II	III	IV	V	VI	VII	VIII	IX

GBS isolates from newborn

NT GBS isolates (Serial number of newborn)	Serotypes									
	Ia	Ib	II	III	IV	V	VI	VII	VIII	IX

b. Result of MLST molecular characterization

SN^{o}	ST	Allelic profile	number of isolates in ST	CC

c. Dummy table for determining vertical transmission of GBS from mother to their new born

Serial N^{o}	Lab result of mother		Lab result of new born			New born (from GBS+ve) developed EOD	Lab result of suspected newborn with EOD
	GBS+/-ve	GBS serotype	Serial N^{o}	GBS+/-ve	GBS serotype	Interview by Phone	
						,,	
						,,	
						,,	
						,,	

7.5. Laboratory data collection sheet for newborn with suspected neonatal disease

a. Lab result recording format

2. Type of specimen collected from the new born
    a. Blood_____
    b. CSF_____
3. Growth in Tryptone Soy Broth Growth (For blood sample)_____
4. Growth on blood agar (for both the blood and CSF sample)_____
5. Gram staining result_____
6. Catalase test result_____
7. CAMP test result_____
8. Strep B Grouping kit agglutination result: positive/negative_____

b. Capsular serotyping result of the GBS isolates from newborn

GBS isolates (Serial number newborn)	Serotypes										
	Ia	Ib	II	III	IV	V	VI	VII	VIII	IX	NT

c. Molecular characterization result of GBS isolates from new born

Genotyping result of NT GBS by using multiplex PCR

NT GBS isolates (Serial number of newborn)	Serotypes									
	Ia	Ib	II	III	IV	V	VI	VII	VIII	IX

Result of MLST molecular characterization

S.N^{o}	ST	Allelic profile	No. of isolates in ST	CC

d. Distribution of resistance gene

SN^{o}	Types Antibiotic resistance genes	Frequency (n)		Percent (%)	
		n	%	N	%
1					
2					
3					
4					

Comments_____

Name of principal investigator _____

Signature _____ Date _____

e. Classification of the new born based on the clinical findings for early onset disease (EOD) and GBS status of the new born

Clinical condition	**Yes**	**No**	**GBS +/-ve**	**GBS serotype**
Meningitis (CSF)				
Probable meningitis (CSF)				
Sepsis (Blood)				

Undefined				
Low birth weight				
Premature				

f. Classification of the new born based on the clinical findings for late onset disease (LOD) and GBS status of the new born

Clinical condition	Yes	No	GBS +/ -ve	GBS serotype
Meningitis (CSF)				
Probable meningitis (CSF)				
Sepsis (Blood)				
Undefined				
Low birth weight				
Premature				

g. Protocol for multiplex PCR and MLST

Chelex DNA preparation of GBS isolates

GBS multiplex PCR-Imperi *et al,* 2010

I. Make Master mix (follow handout) in the PCR room

1. Use 1.5 ml tube
2. Add PCR grade water
3. Add master mix (red cap)
4. Add primers (Tube 1-19)

Primer list

SN^{o}	Primer Name	Sequence 5’ to 3’
1.	Cpsl-Ia-6-7-F	GAATTGATAACTTTTGTGGATTGCGATGA
2.	Cpsl-6-R	CAATTCTGTCGGACTATCCTGATG
3.	Cpsl-7-R	TGTCGCTTCCACACTGAGTGTTGA
4.	CpsL-F	CAATCCTAAGTATTTTCGGTTCATT
5.	CapsL-R	TAGGAACATGTTCATTAACATAGC

6.	CpsG-F	ACATGAACAGCAGTTCAACCGT
7.	CpsG-R	ATGCTCTCCAAACTGTTCTTGT
8.	CpsG-2-3-6-R	TCCATCTACATCTTCAATCCAAGC
9.	CpsN-5-F	ATGCAACCAAGTGATTATCATGTA
10.	Cps-N-5-R	CTCTTCACTCTTTAGTGTAGGTAT
11.	CpsJ-8-F	TATTTGGGAGGTAATCAAGAGACA
12.	CpsJ-8-R	GTTTGGAGCATTCAAGATAACTCT
13.	CpsJ-2-4-F	CATTTATTGATTCAGACGATTACATTGA
14.	CpsJ-2-R	CCTCTTTCTCTAAAATATTCCAACC
15.	CpsJ-4-R	CCTCAGGATATTTACGAATTCTGTA
16.	CpsI-7-9-F	CTGTAATTGGAGGAATGTGGATCG
17.	CpsI-9-R	AATCATCTTCATAATTATCTCCCATT
18.	CpsJ-Ib-F	GCAATTCTTAACAGAATATTCAGTTG
19.	CpsJ-Ib-R	GCGTTTCTTTATCACATACTCTTG

II. Load PCR plate in PCR room

1. Take PCR plate and label A-H and column with Marker
2. Add 23 µl to each well
3. Cover the plate with white cover

III. Add DNA in outside PCR lab

1. Take plate to outer lab area and place in PCR hood
2. Get DNA from the fridge and centrifuge the DNA at 80,000 rmp for 3 min
3. Remove white cover
4. Add 2ul of DNA to each well as per sheet
5. Re-seal plate with cover

IV. Place in PCR machine

1. Take plate and put in the thermal cycler
2. Switch on at the back
3. Select program (under user Sopio and program is *GBS*-sero)
4. Make use that volume is 25µl

5. Select start and program will run for 3 hours.

MLST protocol for Streptococcus agalactiae

I. Introduction

The molecular characterization of bacterial pathogens of clinical significance is increasingly important. Methods, such as multilocus sequence typing (MLST), allow bacterial strains to be characterized during case clusters, for antibiotic-resistant strains to be monitored, and for the impact of new vaccine to be assessed.

Multi locus sequence typing (MLST) is highly discriminatory molecular typing method that defined isolates of bacterial pathogens using the sequences of approximately 450-bp internal fragment of seven housekeeping genes. Clonal complexes are typically represented by a singly group of isolates sharing identical alleles at all seven loci, plus single-locus variants that differ from this group at only one out of the seven loci. As MLST is highly discriminatory, the members of each clonal complex can be assumed to have a recent common ancestor, and the molecular events that give rise to the single-locus variants can be used to estimate the relative contribution of recombination and mutation to clonal divergence.

II. Equipment and supplies

- Thermal cycler
- Pipettman and pipette tips
- PCR plates
- Taq DNA polymerase (+buffer and MgCL2)
- DNTPs (A, C, G and T) (1.25mM)
- Sterile PCR grade H2O
- Primers/Oligos for assay
- DNA from strains
- 1% agarose gel
- 1x TAE buffer
- ExoSAP-IT (USB Corporation)

- Big Dye version 1.1 sequencing kit (Applied Biosystems)
- UNIFITER plates
- Sephadex G-50

III. Primer for MLST

locus	use	Forward (5'to3')	Reverse (5'to3')	Amplicon size (bp)
adhP	amplification	CTTGGTCATGGTGAAGCACT	ACTGTACCTCCAGCACGAAC	672
	Sequencing	GGTGTGTGCCATACTGATTT	ACAGCAGTCACAACCACTCC	498
pheS	amplification	GATTAAGGAGTAGTGGCACG	TTGAGATCGCCCATTGAAAT	723
	Sequencing	ATATCAACTCAAGAAAAGCT	TGATGGAATTGATGGCTATG	501
atr	amplification	CGATTCTCTCAGCTTTGTTA	AAGAAATCTCTTGTGCGGAT	627
	Sequencing	ATGGTTGAGCCAATTATTTC	CCTTGCTCAACAATAATGCC	501
glnA	amplification	CCGGCTACAGATGAACAATT	CTGATAATTGCCATTCCACG	589
	Sequencing	AATAAAGCAATGTTTGATGG	GCATTGTTCCCTTCATTATC	498
sdhA	amplification	AGAGCAAGCTAATAGCCAAC	ATATCAGCAACAAGTGC	649
	Sequencing	AACATAGCAGAGCTCATGAT	GGGACTTCAACTAAACCTGC	519
glcK	amplification	CTCGGAGGAACGACCATTAA	CTTGTAACAGTATCACCGTT	607
	Sequencing	GGTATCTTGACGCTTGAGGG	ATCGCTGCTTTAATGGCAGA	459
tkt	amplificatio	CCAGGCTTTGATTTAG	AATAGCTTGTTGGCT	859

	n	TTGA	TGAAA	
	Sequencing	ACACTTCATGGTGAT GGTTG	TGACCTAGGTCATGA GCTTT	480

4. PCR setup

1. Setup PCR for each of the 7 genes per isolates (25ul PCR's for sequencing)

H2O……..15.8ul

PCR Buffer w/MgCl2 (10x)…….2.5ul

DNTP (10mM)……..0.5ul

Primer 1 (20uM)……0.5ul

Primer 2 (20uM)……0.5uM)

DNA …….5Um (from chelex extraction)

Total …….25ul

Use of Master Mix (Qiagen multiplex kit cat# 206143), which will include all reagents except the DNA. Pipette 20 ul into each well and then add 5 ul of DNA.

2. PCR condition (see Excel template for cycling changes for ddI):

94oC/1min

94oC/15sec

54oC/30sec x30cyles

72oC/45sec

72oC/10min

3. Run 2ul on a 1% agarose gel to check whether PCR worked and concentration.

5. PCR product clean-up

Use ExoSAP-IT (USB Corporation) according to manufacturer's instruction

4ul PCR product (use clean 0.2ml tubes)

3ul ExoSAP-IT

Place in thermal cycler

15 min/37oC

15 min/80oC

Hold/4oC

6. DNA sequencing

4ul Sequencing buffers 5X

1ul Big Dye version 1.1

0.25ul primer (forward or reverse) stock 20 uM/pmol)

14.75 H2O

Pipette 20 ul into ech tube and then add 1ul of cleaned PCR product

Place tubes in thermal cycler

1min/96oC

10s/96oC

5s/55oC

4min/60oC

7min/4oC

At 4oc

7. Sample preparation and sequencing

Use Whatmn UNFILTER plates and Sephadex G-50 cleanup as per protocol.

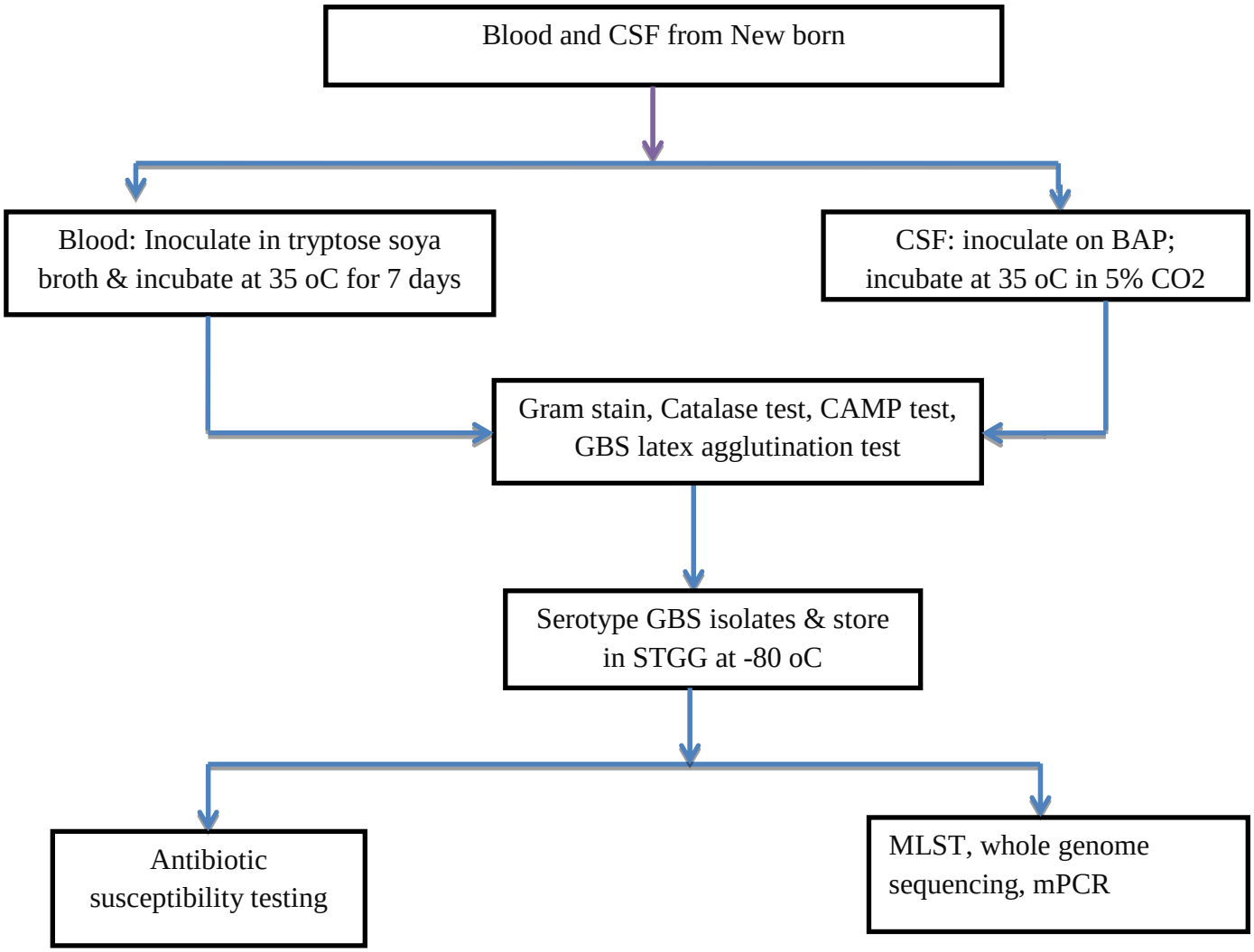
8. Sequencing

Outscore or sequencing in-house

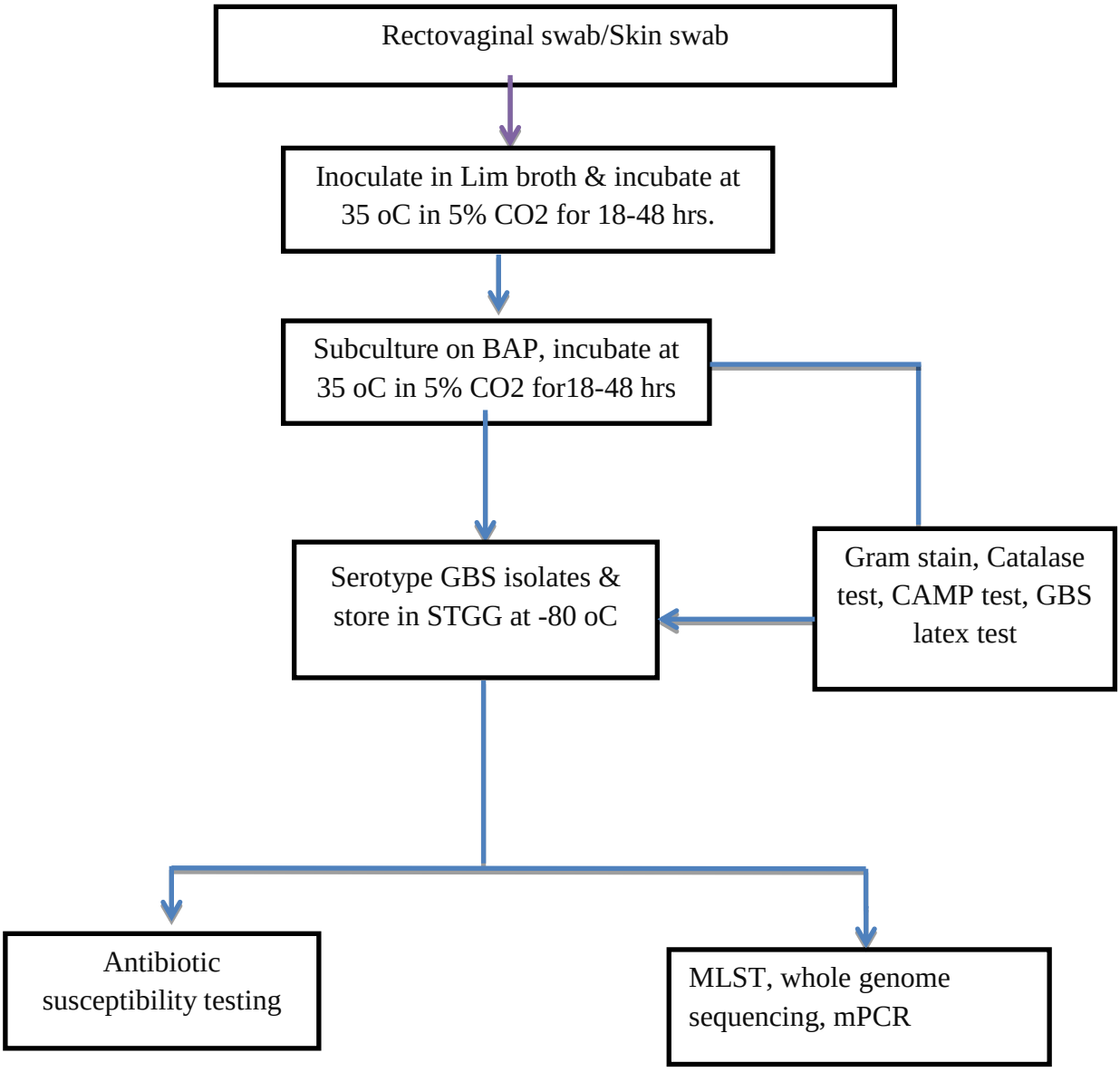
9. Sequence analysis

Sequences are analyzed either by FRET program at CDC or by DNA Star (EditSeq and Seqman) and Codoncode Aligner software. You can also use the BioEdit software available free in the internet. Assign allele numbers, if the sequence is novel, then sequence reverse strand and submit for allele assignment through www.mls.net. If 7 loci show novel ST, then submit to database for new ST number assignment as per instruction.

Flowchart for identification of GBS from newborn with neonatal disease



Flowchart for identification of GBS from Mother and new born



DECLARATION

I the undersigned declare that this PhD Thesis is my own original work and has not been presented for a degree in any other university and all sources of materials used for the Thesis have been duly acknowledged.

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