

**MATERNAL GROUP B *STREPTOCOCCUS* RECTO VAGINAL
COLONIZATION IN HARAR, EASTERN ETHIOPIA: MAGNITUDE,
VERTICAL TRANSMISSION AND DRUG SUSCEPTIBILITY PATTERN**

Ph.D DISSERTATION

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Maternal Group B *Streptococcus* Recto-vaginal Colonization in Harar, Eastern Ethiopia: Magnitude, Vertical Transmission and Drug Susceptibility Pattern

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Dedication

This PhD work is dedicated to my mother Garitu Ayana and my father Assebe Yadeta.

Biographical Sketch

I was born 1978 GC in Horo Guduru Wollega Zone, at a particular village called “Abuna” from my mother Garitu Ayana and father Assebe Yadeta. Both my father and mother are agrarian. When my age reached school, I joined and attended at Sekela” primary and junior secondary school. Then I completed grade nine to twelve at Shambu Senior Secondary school which is 16 kilometres away from my families’ home.

I had a strong ambition to be a best health professional. In the secondary schooling, all my thought was to be a good health professional. This strong need and hope was conceived and grown in me as there was one non- professional person who was providing medicine in the form of injection and per mouth at my rural village. During this time, I was critically observing what he was doing. This further provoked my motives to attend health related education to become a health professional. When I was grade seven, I wrote on my exercise book saying “I will be a doctor”. This exercise book stayed with me until I completed grade 12. Having completed the high school, I joined Nekemt Nursing School and attended diploma program in comprehensive nursing. Then I joined Shambu Hospital and worked as a nurse director. After providing a one and half year services, I joined Amanuel Psychiatry Nursing School for advanced diploma in psychiatry nurse. Completing Psychiatry Nursing courses, I back to Shambu Hospital and served as a head Psychiatry unit for two years. In the year that followed, I joined Jimma University for BSC Degree in Nursing. Then I was recruited and employed by Haramaya University as assistant lecturer. I served for two years and joined Addis Ababa University School of Nurse to pursue my master degree in maternal and reproductive health nursing.

I have served for four years in Shamboo Hospital in different positions: as a head nurse, nurse directors, and head of psychiatry unity in the hospital. Shambu Hospital is one of the district hospitals in Oromiya regional State, Horo Guduru Wollega Zone. The hospital had no caesarean section services due to lack of trained professionals in the field of maternal health and adequate resources. There were plenty of maternal health’s related in service delivery challenge in the hospital. Owing to this, there was a high maternal mortality, stillbirth and neonatal mortality which could easily be preventable by the professionals in this field. This takes my soul to focus on my advanced study on maternal and neonatal health.

I am very lucky in joining Haramaya University and getting the opportunity to take part in contributing my professional service in resolving the national challenge of the scarcity of trained health professionals in mental health, maternal health and neonatal health. I am proud for contributing my professional service for opening and heading of Psychiatry Nurse Department at Haramaya University for two year. I also feel honoured and excited for opening MSc in Maternal and Neonatal Health Nursing. To further improve maternal and neonatal health services at national level, I have participated in the harmonization of midwifery curriculum. I have also assisted higher education relevance and quality office (HERQA) in evaluating higher education in both nurse and midwifery program to assure their standard at national level. I have also actively participated in training of Basic emergency obstetric and newborn care (BEmONC) for in-services health professionals working in Dire Dawa administration, Oromiya Regional State, and Harari Regional State and got certificate of appreciation. These all take me to work my PhD in public health focusing on maternal and neonatal health for better professional contribution in the field.

Currently, I am a team member of a prospective study entitled with “Which maternal infections are associated with stillbirth and early neonatal death in East Africa? The study is being conducted in Hiwot Fana Specialized University Hospital in Ethiopia and Kilifi county Hospital, Kenya jointly with London School of Hygiene and Tropical Medicine and Kenya Kilifi Project. This study will identify infections that are associated with stillbirth and newborn death. Test for infection, using traditional and new laboratory methods. This may help me to address the limitation of my PhD work.

I have published four articles on peer reviewed journals. I worked as reviewer of Elsevier, journal of obstetrics and gynaecology.

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I would like to express my heartfelt gratitude to my main supervisor Professor Alemayehu Worku who has taught me so much and has guided this work. I have no words for his dedication, encouragement and support throughout the study. My deepest gratitude goes to my supervisor Professor Yemane Berhane who has provided me with meticulous, extensive and invaluable guidance and support during the course this thesis. My acknowledgment also goes to Dr. Gudina Egata who closely supervised my work, and provided timely advice throughout my study. I am grateful to Dr. Berhanu Seyoum who advised me on the laboratory procedures, facilitated the laboratory training, and helped me to acquire the necessary materials for the lab works.

Ethiopian Public Health Institute provided all reference strains used for the quality control of the study. Hiwot Fana Hospital, Jugal Hospital and Arategn Health Center offered treatment and care during the study period. Harari Regional Health Bureau facilitated the study in the health facilities. I thank you all for your invaluable contributions.

My special gratitude is to Dadi Marami who provided unreserved support in completing the laboratory work, you were exceptionally diligent. I would like to express my heartfelt gratitude to all friends in Haramaya University, Adama Regional Laboratory, and Assela University for your kind support.

This thesis would be incomplete without a mention of the support given me by Gadise Lemma who kept my spirits up. Without her lifting me up, I doubt it should ever have been completed.

I dedicate my thesis to my families who have supported me all the way since the beginning of my studies of three of my children.

Abbreviations and Acronyms

ANC	Antenatal Care
APR	Adjusted Prevalence Ratio
CAMP	Christie-Atkins-Munch-Petersen
CDC	Centers for Disease Control
CI	Confidence Interval
EDHS	Ethiopian Demographic and Health Survey
EOND	Early Onset of Neonatal Disease
EONGD	Early Onset of Group B Streptococcus Disease
EOS	Early Onset of Sepsis
GBS	Group B Streptococcus
GDP	Growth Domestic Product
HEP	Health Extension Programme
HSTP	Health Sector Transformation Plan
IRR	Incidence Rate Ratio
IAP	Intrapartum Antibiotic Prophylaxis
LOND	Late Onset of Neonatal Disease
LMIC	Low and Middle Income Countries
MDG	Millennium Development Goal

MICs	Minimum Inhibition Concentration
MLS	Macrolides, Lincosamides and Streptogramin
MMR	Maternal Mortality Ratio
MOH	Ministry of Health
MOI	Ministry of Information
NGO	Non-Governmental Organization
NMR	Neonatal Mortality Rate
OR	Odds Ratio
PDVAC	Product Development for Vaccine Advisory Committee
PROM	Prolonged Ruptures of the Membrane
PPROM	Preterm Premature Rupture of the Membrane
SDG	Sustainable Development Goal
SOP	Standard Operating Procedure
TPROM	Term Pre-Labour Rupture of the Membrane
UN	United Nation
UNICEF	United Nations Children's Fund
USAID	United States Agency for International Development
WHO	World Health Organization
VIF	Variance Inflation Factor

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Original Paper and Manuscripts

This dissertation is based on the following original papers which are referred in the text by their roman number

- I** Yadeta T, Worku A, Egata G, Seyoum B, Marami, D, Berhane Y. Magnitude of Group B *Streptococcus* Recto-Vaginal Colonization among pregnant Women: a cross-sectional study, Eastern Ethiopia (*Ready for submission*).
- II** Yadeta T, Worku A, Egata G, Seyoum B, Marami, D, Berhane Y. Vertical transmission of group B *Streptococcus* and associated factors among pregnant women: a cross-sectional study, Eastern Ethiopia (*Published in dove infectious and drug resistance*).

III Yadeta T, Worku A, Egata G, Seyoum B, Marami, D, Berhane Y. Maternal Group B *Streptococcus* Recto-Vaginal Colonization increases the Odds of Stillbirth: Evidence from eastern Ethiopia (*under peer review in BMC pregnancy and childbirth*).

IV Yadeta T, Worku A, Egata G, Seyoum B, Marami, D, Berhane Y. Erythromycin and Clindamycin Resistance and associated factors of *Streptococcus agalactiae* isolated from pregnant women in Harar, East Ethiopia (*under peer review in BMC pregnancy and childbirth*).

Abstract

Background: Group B *streptococci* are a major cause of foetal infection and neonatal pneumonia, septicaemia and meningitis in developing countries due to vertical transmission from mother to the fetus and newborn. This vertical transmission is largely preventable using intrapartum antibiotic prophylaxis (IAP). However, high number of fetus and infants in developing countries are dying due to lack of information and thus lack of appropriate interventions.

Aim: The aim of this dissertation was to assess the magnitude of Maternal Group B *streptococcal* Recto-vaginal colonization, vertical transmission and drug susceptibility pattern among pregnant women in Eastern Ethiopia.

Methods: Pregnant women admitted at labour and delivery room were studied. Samples were taken proportional to the client volume of the Hiwot Fana Specialized University Hospital, Jugal Hospital and Arategna health Center. They were interviewed, their clinical record reviewed, and laboratory test done. Maternal group B *streptococcal* recto-vaginal colonization was investigated by collecting swabs from both rectum and vagina, cultured on to Todd-Hewitt broth medium supplemented with Gentamicin and Nalidixic acid, and subsequently sub-cultured on 5 % sheep blood agar followed by identification of isolates based on colonial morphology, gram stain, catalase reaction, hippurate hydrolysis and Christie, Atkins, Munch-Petersen (CAMP) test. Vertical transmission was examined by collecting swabs specimen from the ear canal, umbilical, axilla, groin or nose within six hours after birth, similar culture and biochemical test done to identify group B streptococcus bacteria. Stillbirth was explored from medical record by using checklist. Antibiotic susceptibility testing was performed by disk diffusion method, then inducible clindamycin resistance by erythromycin investigated by D-test. The data were collected via structured questionnaire and checklist and double entered in to EpiData software (version- 3.1) and analyzed using STATA software (version-14). Log binomial, Logistic, and Robust Poisson regression models were used for the analysis, as deemed appropriately. Association was expressed as prevalence ratios (PR), Odds ratio (OR), and their 95% confidence interval.

Results: Overall, the magnitude of recto-vaginal group B *streptococcus* colonization in pregnant women was 13.68% (95% CI: 12.04-15.32). GBS recto-vaginal colonization was more prevalent among adolescent, illiterate and lower wealth index women.

The vertical transmission rate of GBS from colonized pregnant women was 45.02% (95% CI: 38.49, 51.68). Pre-labor rupture of membranes at term, prolonged rupture of the membrane ≥ 18 hours, and intrapartum maternal fevers were significantly associated with vertical transmission of GBS. Intrapartum antibiotic Prophylaxis (IAP) received ≥ 4 hours before delivery were reduce

vertical transmission by 83% (APR= 0.17; 95% CI; 0.09-0.30). The odds of having a stillbirth was 8.93 times higher among recto- vaginal GBS colonized pregnant women (AOR= 8.93; 95% CI; 5.47, 14.56)

Double disk diffusion test for susceptibility to GBS strain to currently used antibiotics was very high resistance; 56 (24.24%) to Erythromycin and 53 (22.94%) to Clindamycin. Reported antibiotics used, in the index pregnancy two weeks preceding the data collection was significantly associated with erythromycin and clindamycin resistance.

Conclusion: Vertical transmission of GBS from colonized pregnant women is very high. Resistance to erythromycin and clindamycin antibiotics are also high. Screening pregnant women for maternal GBS colonization and use of IAP for colonized women is imperative to prevent vertical transmission and reduce stillbirth and neonatal morbidity and mortality.

Key words

Group B streptococcus, vertical transmission, stillbirth, drug susceptibility, Risk factor, and neonate

1.

INTRODUCTION

1.1 Maternal and Children Health

The world has made substantial progress in maternal and child survival since 1990. The global maternal mortality ratio declined by 44 percent (Alkema *et al.*, 2016 ;United Nations, 2018) and the global under-five mortality rate was declined by 56 % in 2016. Despite substantial progress, improving maternal and child survival remains a matter of urgent concern. In the year 2015, worldwide 303,000 maternal deaths occur. In 2016, an estimated 5.6 million children died before reaching their fifth birthday. About 80 per cent of under-five deaths occur in two regions, Sub Saharan Africa and Southern Asia region. Six countries account for half of the global under five deaths, namely, India, Nigeria, Pakistan, the Democratic Republic of the Congo, Ethiopia and China (UN Inter-agency Group for Child Mortality Estimation, 2017).

Unless sub-Saharan Africa countries are able to accelerate reductions in maternal and child deaths at an extraordinary pace, their achievement of proposed Sustainable Development Goal (SDG) target 3.1 of SDG 3 to reduce the global Maternal Mortality Ratio (MMR) to less than 70 per 100 000 live births and target 3.2 “end preventable deaths of newborns and under-five children” by 2030 is unlikely (United Nation, 2016 ;Chao *et al.*, 2018). Improving the evidence base and cost effective intervention, is vital in ending preventable maternal and child deaths by 2030(Wang *et al.*, 2016 ; United Nation, 2015).

Most maternal and under-five deaths are caused by diseases that are readily preventable or treatable with proven, cost-effective interventions. Infectious diseases and neonatal complications are responsible for the vast majority of under-five deaths globally (UN Inter-agency Group for Child Mortality Estimation, 2017, 2017). Accelerating the reduction in maternal and child mortality is possible by expanding effective preventive and curative interventions that target the main causes of maternal and child deaths (United Nations, 2018).

This dissertation deals with Group B Streptococcus (GBS), one of the leading preventable causes of newborn mortality. It directly relates to the SDG targets; target 3.1 to reduce the global MMR

to less than 70 per 100 000 live births by 2030, and target 3.2 “end preventable deaths of newborns and under-five children” by 2030.

1.2 Definition, maternal and neonatal colonization, vertical transmission and disease of group B streptococcus

Group B *streptococci* (GBS) are facultative, gram positive diplococci, with approximately 99% of strains showing (complete) hemolysis on blood agar plates (Gibbs *et al.*, 2004). These organisms are divided into ten serotypes (Ia, Ib, II, III, IV, V, VI, VII, VIII, IX) on the basis of capsular polysaccharides. The capsule represents the major virulence factor, which helps GBS evade host defense mechanisms by interfering with phagocytic clearance (Brigtsen *et al.*, 2015).

Puerperal sepsis has been associated with maternal morbidity and mortality for centuries (Noakes *et al.*, 2008). Group B *Streptococcus* was first identified as a cause of Puerperal sepsis in 1935 by Lancefield and Hare (Lancefield and Hare, 1935). Several cases of fatal puerperal sepsis related to GBS disease reported in 1937 (Fry, 1938). In the early 1960’s neonatal GBS infection association with maternal recto-vaginal colonization was observed. Group B *Streptococcal* infections have been well recognized since the 1970s in the scientific research communities, the medical professions and the public health agencies (Eickhoff *et al.*, 1964 ;Schuchat, 1998 ;McCracken Jr, 1973). By 1989, GBS was listed by Centers for Disease Control (CDC) as the leading infectious killer of newborn babies (Schuchat, 1998).

Group B *streptococci* are common inhabitants of the human gastrointestinal and genitourinary tracts (Hanson *et al.*, 2014 ;Doran and Nizet, 2004). Maternal GBS colonization is isolation of GBS from both the vagina and rectum swabs by culture and biochemical test during pregnancy (Baker and Barrett, 1973). Group B *Streptococcus* is an opportunistic microbial agent a major cause of perinatal bacterial infections, including bacteremia, endometritis, chorioamnionitis, and urinary tract infections in pregnant women and neonatal pneumonia, septicemia and meningitis in human newborns (Kalin *et al.*, 2015 ;Bae *et al.*, 2016 ;Pass *et al.*, 1982). GBS infection acquired from the colonized birth canal during pregnancy, labour or after membrane rupture can lead to

miscarriage, stillbirth, prematurity or invasive neonatal disease (Koenig and Keenan, 2009 ;Fiorella Bianchi-Jassir *et al.*, 2017).

Neonatal and young infant GBS disease can be classified into early-onset disease (EOD, onset during the first 6 days of life), and late-onset disease (LOD, onset between days 7–89 of life) (Joubrel *et al.*, 2015). Early-onset disease is caused by vertical transmission through colonized mothers during or just before birth (Seale *et al.*, 2016). Group B Streptococcus can ascend from the vagina to the amniotic fluid aspirated into the fetal lungs, leading to an invasive infection (Vanderhoeven *et al.*, 2014). It has been estimated that in the absence of any intervention, approximately 57.70% of newborn to colonized mothers become colonized (Le Doare *et al.*, 2016) and 1–2% of them progress to develop invasive disease (Russell *et al.*, 2017b).

There are three approach in prevention of GBS, universal microbiological screening and use of IAP, identification of clinical risk factors and use of IAP, and both universal microbiological screening and identification of clinical risk factors and use of IAP. Antibiotic prophylaxis based on clinical risk factors alone resulted in a 68% fall in the incidence of GBS neonatal sepsis; and when a culture-based protocol was used the incidence fall by 89%. World Health Organization (WHO) and CDC currently recommend screening, and use of IAP to prevent early neonatal GBS infection (Verani *et al.*, 2010 ;World Health Organization, 2016).

Vaccination may also promising strategy for preventing GBS infection (Heath *et al.*, 2017), Maternal GBS vaccines are in development. Study reported maternal vaccination with GBS elicited higher GBS serotype-specific antibody levels in infants and protect infant from GBS disease (Madhi *et al.*, 2017). Further, investigation needed to evaluate its efficacy for implementation (Lin *et al.*, 2018).

1.3 Statement of the problem

There were 33 000 cases of invasive GBS disease in pregnant or postpartum women, and 57 000 fetal infections/stillbirths worldwide. Up to 3.5 million preterm births may be attributable to GBS. Africa accounted for 54% of estimated cases and 65% of all fetal/infant deaths (Seale *et al.*, 2017). Furthermore, GBS is associated with approximately 15% of all infection related stillbirths (Madhi and Dangor, 2017). Maternal disease had an adverse effect on fetal or neonatal outcome,

about 28% of affected mothers had pregnancy loss due to miscarriage or stillbirth, or delivered an infant who developed GBS during early onset of sepsis (EOS) (Koenig and Keenan, 2009). Group B *Streptococcus* was responsible for 41.5% of all neonatal sepsis (Puopolo and Eichenwald, 2010). Treated cases of EOD had very high, 47% case fatality, especially within 24 hours of birth (Seale *et al.*, 2016).

While most babies recover from their GBS infection, some are stillborn, more die in the first weeks of life and others suffer lifelong disability. Survivors may suffer from hearing or visual loss, uncontrolled seizures, impaired psychomotor development and/or mental retardation (Dangor *et al.*, 2015). A prospective study with 5-year follow-up showed that GBS meningitis results in children with neurological deficiencies in 34.8% of cases ranging from partial sensory loss to profound mental retardation, blindness and deafness (Libster *et al.*, 2012).

Maternal colonization with GBS in either the genitourinary or gastrointestinal tract or the vertical transmission during labour and delivery causes early-onset invasive GBS disease (Vornhagen *et al.*, 2016). GBS-colonization in pregnancy poses a four-fold increased risk of early-onset infection (Seale *et al.*, 2016). Pregnant women who develop chorioamnionitis due to GBS give 62% EOND compared to 18% non GBS chorioamnionitis (Tudela *et al.*, 2012). Fetal loss or stillbirth occurs in 60% of cases of invasive GBS disease of pregnant women (Puopolo, 2014 ;Petersen *et al.*, 2014).

Group B streptococcus related maternal and neonatal diseases and deaths are preventable if early screening is done and colonized pregnant women are given IAP (Russell *et al.*, 2017a ;Verani *et al.*, 2010 ;Ohlsson *et al.*, 2014). The use of intrapartum antibiotics prophylaxis at least four hours before labor starts can reduce the risk of clinical sepsis by 65% in infants (Tumbaga and Philip, 2003; Petersen *et al.*, 2011). As a result of prevention efforts in the United States of America, the incidence of early-onset neonatal infections declined by 84% from 1993 to 2008 (Le Doare *et al.*, 2017). With advances made in neonatal care the case fatality rate of GBS has declined from 50 % to 4% (Rivera *et al.*, 2015). Despite availability of proven prevention strategies globally 75.8% of the low-income countries have no national policy (Creti *et al.*, 2017).

In Ethiopia, neonatal and stillbirths are high, 29/1000 live birth and 25/1000 birth respectively. Most of causes are preventable. GBS is preventable causes of stillbirth and neonatal deaths. There is no prevention policy of GBS related maternal and neonatal disease. Majority of GBS related studies conducted in Ethiopia focused on analyzing the prevalence of maternal colonization. The published information on the prevalence of maternal recto-vaginal GBS colonization was variable and limited. There is lack of information in eastern part of Ethiopia. Further, information on the extent of vertical transmission, associated risk factors and drug susceptibility pattern is grossly lacking. Therefore, the aim of this dissertation was to assess the prevalence, vertical transmission to their newborn, drug susceptibility pattern and risk factors of GBS among pregnant mothers and their newborns in eastern Ethiopia. The findings of the study will help, the policy makers, program implementers and other stakeholders for the establishment evidence based prevention strategy and tackle the problems and optimize maternal and perinatal outcomes related with GBS. Consequently, vulnerable pregnant mother and neonate will benefit by obtaining appropriate care and preventive service from the evidence based care.

1.4 Rationale of the study

Most maternal and newborn deaths are caused by diseases that are preventable. Health care services during pregnancy and after delivery are important to make progress toward reaching the sustainable development goal of avoiding maternal and newborn death due to preventable causes. Group B *Streptococcus* is a preventable cause of significant number of stillbirths and neonatal death. Despite this, there is little documented information on magnitude, vertical transmission and drug susceptibility pattern in Ethiopia. Indeed, maternal and neonatal health is now seen as a policy priority in the country. The level of concern was illustrated by the fact that national maternal and neonatal health strategy of (Health Sector Development Program IV 2010/11 – 2014/15) and revised maternal and neonatal program (Health Sector Transformation Plan 2015/16 - 2019/20) of Ethiopia have set up a strategy that can be implemented through maternal and child health services which clearly indicated the need to conduct a comprehensive and routine study that can address on the preventable cause of maternal and child morbidity and mortality. This study provides useful evidence to advance efforts to reduce still births and neonatal deaths.

1.5 Objectives

General objective

Maternal Group B *streptococcus* Recto-Vaginal colonization, vertical transmission to their newborn and antibiotic susceptibility pattern among pregnant women came to health facilities found in Harar town for delivery services, Eastern Ethiopia

Specific objective

1. To determine the magnitude of Group B *Streptococcus* recto-vaginal colonization among pregnant women came to health facilities found in Harar town for delivery services, Eastern Ethiopia
2. To determine Vertical Transmission of Group B *Streptococcus* and Associated Factors among pregnant women came to health facilities found in Harar town for delivery services, Eastern Ethiopian
3. To investigate the association of Maternal Group B *Streptococcus* Recto-Vaginal Colonization and Stillbirth among Pregnant Women came to health facilities found in Harar town for delivery services, Eastern Ethiopia
4. To determine Erythromycin and Clindamycin Resistance and associated factors of Group B *Streptococcus* isolates from pregnant women came to health facilities found in Harar town for delivery services, East Ethiopia

2. LITERATURE REVIEW

2.1 Magnitude of pregnant women GBS recto-vaginal colonization

The prevalence of maternal recto-vaginal GBS colonization varies across regions ranging from 5.1% in Denmark to 31.6% in Gambia (Table1). The burden of the disease is high in adolescent pregnancy (Anthony *et al.*, 1978 ;Petersen *et al.*, 2014). Pregnant women with no formal education and those dwelling in rural areas more frequently colonized by GBS (Ernest *et al.*, 2015), contact with domestic animals (Capan-Melser *et al.*, 2015), maternal obesity (Kleweis *et al.*, 2015), history of urinary tract infection during the pregnancy, history of premature childbirth or abortion, and HIV-positive serology were more frequently colonized by GBS (Mitima *et al.*, 2014).

Table 1: Magnitude of pregnant women GBS recto-vaginal colonization in varies countries

Country	Authors	Year	Prevalence	Study location	Study timing relative to the index pregnancy
Argentina	(Oviedo <i>et al.</i> , 2013)	2013	9.80%	Rural	<5 years
Bangladesh	(Samir K. Saha <i>et al.</i> , 2017)	2017	15%	Urban and rural	At birth
Thailand	(Turner <i>et al.</i> , 2012)	2012	12%	Rural	At delivery
Egypt	(Moussa <i>et al.</i> , 2012)	2012	29%	Urban	35-37
Saud Arabia	(Arain <i>et al.</i> , 2015)	2015	24%	Urban	35-37

Iran	(Darabi <i>et al.</i> , 2017)	2017	11.80%	Urban	35-37
Denmark	(Petersen <i>et al.</i> , 2014)	2014	5.10%	Urban and rural	35-37
UK	(Daniels <i>et al.</i> , 2011)	2011	21%	Urban and rural	At delivery
Italy	(Bianco <i>et al.</i> , 2016)	2016	9.80%	Urban and rural	35-37
Gambia	(Roca <i>et al.</i> , 2017)	2017	31.60%	Urban and rural	At delivery
Kenya	(Seale <i>et al.</i> , 2016)	2016	11.70%	Urban and rural	At delivery
South Africa	(Kwatra <i>et al.</i> , 2014)	2014	28.40%	Urban and rural	37+
W. Ethiopia	(Mengist <i>et al.</i> , 2016)	2016	19%	Urban and rural	35–37
N. Ethiopia	(Alemseged <i>et al.</i> , 2015b)	2015	13.70%	Urban and rural	35-37
S. Ethiopia	(Mohammed <i>et al.</i> , 2012)	2012	20.90%	Urban	35-37

2.2 Vertical transmission of GBS and associated factors

The extent of vertical transmission of GBS also shows considerable variation within and between geographic regions (Table 2). Vertical transmission of GBS can occur during pregnancy or the birth process from genitourinary or gastrointestinal tract of colonized pregnant women (Roca *et al.*, 2017).

Table 2: Proportion of vertical transmission in various countries

Country	Authors	Year	Colonized Mother(n)	colonized Neonate(n)	Vertical transmission
South Africa	(Madzivhandila <i>et al.</i> , 2011)	2011	551	289	52.50%
South Africa	(Clare L Cutland <i>et al.</i> , 2009)	2009	429	234	55.00%
Kenya	Seal(Seale <i>et al.</i> , 2016)	2016	104	30	28.84%
Zimbabwe	(Mavenyengwa <i>et al.</i> , 2010)	2010	120	74	61.70%
Gambia	(Le Doare <i>et al.</i> , 2016)	2016	253	146	57.70%
Iran	(Haghshenas Mojaveri <i>et al.</i> , 2014)	2014	61	30	49.20%
Germany	(Kunze <i>et al.</i> , 2011)	2016	152	17	11.20%
Italy	(Berardi <i>et al.</i> , 2014a)	2014	499	34	6.80%
Italy	(Berardi <i>et al.</i> , 2013)	2013	83	30	36.10%

Japan	(Katsuaki Kojima <i>et al.</i> , 2014)	2014	1910	273	14.00%
Sweden	(Hakansson <i>et al.</i> , 2008)	2008	266	181	68.00%

The risk factors for vertical transmissions include maternal fever during labour, prolonged rupture of membranes for ≥ 18 hours (PROM), preterm premature rupture of the membrane (PPROM), preterm delivery, and chorioamnionitis (Joachim *et al.*, 2009 ;Puopolo *et al.*, 2011 ;Hanan M al-Kadri *et al.*, 2013 ;Shah *et al.*, 2014 ;Berardi *et al.*, 2014b ;Berardi *et al.*, 2014a). Term pre-labour rupture of the membrane (TPROM) was also reported to have increased the risk of vertical transmission. Group B *streptococcus* causes membrane weakening and early rupture of the membrane. Early rupture of the membrane leaves the fetus and the amniotic fluid unprotected, which is a good medium for bacterial proliferation, thus increases the risk of infection (Vanderhoeven *et al.*, 2014).

Prevention of vertical transmission strategies includes identifying GBS colonized mother and use of IAP. Group B Streptococcus (GBS) colonized identification can be done through either universal screening of pregnant women or using the risk approach (Verani *et al.*, 2010). Intrapartum antimicrobial prophylaxis for women colonized with GBS is critical and can significantly decrease vertical transmission rate (Kunze *et al.*, 2011). Intrapartum antibiotic prophylaxis is highly effective if administered at least four hours before delivery. Early recognition and prompt treatment are crucial in preventing rapid invasive GBS disease progression (Fairlie *et al.*, 2013b).

2.3 Stillbirth association with GBS

Overall data necessary to quantify the contribution of GBS infection to the burden of stillbirth in developing countries is lacking. However, the high burden of GBS in developing regions suggests that the available data represent only the tip of the iceberg of the GBS-related stillbirths (Nan *et al.*, 2015). GBS is believed to be associated with approximately 15% of all infection related stillbirths (Madhi and Dangor, 2017). Africa accounts 73.78% of the estimated GBS stillbirth cases globally (Lawn *et al.*, 2017).

Intrapartum antibiotic prophylaxis (IAP) has been effective in reducing the early onset of neonate GBS disease if administered early. Antibiotics prophylaxis must be administered before the potential fetal exposure by screening for colonization, about 40% stillbirths occur before labour start (Arnold and Flint, 2017). Immunization of pregnant women with a GBS vaccine can be more effective in protecting fetus from GBS infection (Madhi and Dangor, 2017).

2.4 Erythromycin and clindamycin resistance and associated factors

Intrapartum antibiotic prophylaxis, administered to GBS-positive women, is effective in preventing vertical transmission (Verani *et al.*, 2010). Penicillin and ampicillin are the IAP of choice. Clindamycin and erythromycin are alternative antibiotics, recommended for about 11.5% penicillin-allergic pregnant women (Van Dyke *et al.*, 2009). For those at serious risk of anaphylaxis, clindamycin is recommended if the organism is susceptible, and vancomycin is recommended if there is clindamycin resistance or if susceptibility is unknown (CAGNO *et al.*, 2012).

In the past, GBS was generally susceptible to erythromycin and clindamycin. However, recent reports worldwide suggest that GBS resistance to clindamycin and erythromycin is increasing (Clifford *et al.*, 2011 ;Yook *et al.*, 2013). Factors significantly associated with resistance to both antibiotics include history of symptomatic sore throat in the two weeks before obtaining the specimen, and premature rupture of membranes (Yook *et al.*, 2013). Clindamycin resistance may also induced by erythromycin resistance through methylations (Bolukaoto *et al.*, 2015)

The following conceptual framework is designed based on the literature discussed above. For the purpose of this study, the factors are labeled as distal, intermediate, and proximal factors. The distal or underlined factors affect the intermediate, proximal and outcome variables. The intermediate factors affect the proximal and the outcome variables. Similarly the proximal factors are vulnerable for the outcome. This conceptual framework used for the analysis purpose (figure 1)

Figure 1: Conceptual framework in relation to the objective of the dissertation, Harar, Eastern Ethiopia, 2016



3. MATERIALS AND METHOD

3.1. Study setting and period

The study was conducted among health facilities in Harar town. The town is found in Harari Regional state. Harari regional state is one of the nine regional states of Ethiopia. The town is found 510 km far from Addis Ababa, the capital city of Ethiopia, to the east. The town is divided into six districts and sub divided into nineteen kebeles (the lowest administrative division in the country), named as kebel 01 to 19. The population of the town is estimated 131,297. Of this, 30.6% reproductive age group, 20% illiterate (14% women and 5.7% male). Oromo, Amhara and Harari are among the major ethnic group living in the town. Trade is the main livelihood of the people in Harar. In the town there were seven hospitals; two public hospitals, one police and one military hospital, two private hospitals, and one non-governmental hospital, four health centers, and 35 clinics. Health service coverage of the region is 100 %. Delivery services provided by Hiwot Fana Specialized University Hospital, Jugal Hospital, and Arategha Health Center were about 4600, 2700, and 300 deliveries per year respectively. All health facilities provided free of charge for delivery services including caesarean section. The study was conducted from June to October 2016. Figure-1 illustrates the map of the study setting in relation, Ethiopia, Harari region and Harar town (HRHB, 2017 ;CSA [Ethiopia], 2008 ;CSA, 2018).

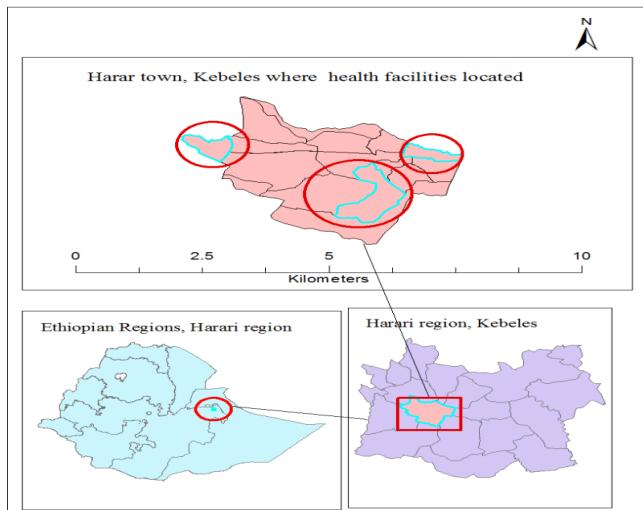


Figure 2: Map of Ethiopia, Harari Regional State and selected Kebele of Harar Town where study Health facilities located

3.2 Study design

The dissertation draws data from a cross-sectional facility-based study conducted by the investigators.

3.3 study population

The study participants were all pregnant women came for delivery services at labour and delivery room during the study period from June to October, 2016 were included. For all GBS colonized pregnant women their newborn both stillbirth and live birth also included to assess vertical transmission. All GBS isolated from pregnant women also used for antibiotic susceptibility test. Women who have used antibiotics in the two weeks during their pregnancy prior to specimen collection, pregnant women with antepartum hemorrhage due to contra indication for vaginal examination, health conditions that make the women physically or mentally incapable of responding, and women unwilling to consent were excluded.

3.4 Sample size determination and sampling procedure

The optimal sample size for the study as determined using a single population proportion formula

($n = \frac{Z^2 * p * (1-p)}{d^2}$), and 10% non-response rate were:

Z=percentile of the standard normal distribution

P= proportion of pregnant women GBS recto-vaginal colonization

d= desired precision of the estimate

n=total sample size

The following assumptions were made in calculating the sample size:

- 95% level of confidence
- Prevalence of pregnant women recto-vaginal GBS colonization at Hawasa 20.9%(Mohammed *et al.*, 2012)
- 2% margin of error (desired precision between sample and population parameter)
- 10% for non-response

Accordingly, the total sample size required for the study was 1731 pregnant women. The adequacy of the sample size for the various component of the study was checked. The study population was drawn from the participating health facilities proportional to their monthly client volume. Each component of the dissertation is summarized in Table 3 and brief description is presented below.

Table 3: Study objective, study population, and sample size, Harar town health facilities, eastern Ethiopia 2016

Study objective	Study population	Number of subject	Paper
Magnitude of pregnant women GBS recto-vaginal colonization	Pregnant women came for delivery services	1688	I
Vertical transmission and associated factors	Mother GBS recto-vaginal colonized with her new-born	231 mother-new born pair	II
Stillbirth association with GBS	Pregnant women	1688	III

	came for delivery services		
Erythromycin and clindamycin resistance and associated factors	Identified GBS strain from pregnant women	231 Isolates	IV

3.5 Data Collection

The data collection was undertaken at labour and delivery room. All pregnant women came to delivery services were interviewed by midwives who provided delivery services and fluent in the local language (Afan oromo and Amharic) and supervised by supervisors. Clinical record was collected by using checklist. Group B *streptococcus* bacteria isolated by swab culture, colony morphology and Gram staining, and biomedical test (sodium hippurate hydrolysis, catalase test, Christie-Atkins-Munch Petersen) and antibiotic susceptibility test was done by disk diffusion and D-test at Microbiology Laboratory of the Department of Medical Laboratory Sciences, College of Health and Medical Sciences, Haramaya University. For laboratory work, two medical microbiologists (to read, confirm, and interpret the test results) and three medical laboratory technologists (who assisted in media preparation and sterilization processes) were involved.

3.5.1 Data collection tools

Data were collected through interviewer-administered questionnaire, checklist for medical record, and laboratory test. The questionnaire and checklist were adapted from the Ethiopian Demographic and Health Survey and the World Health Organization indicators of health status of mother during labor, delivery, and of immediate newborn (Organization and UNICEF, 2009 ;Central Statistical Agency (CSA) [Ethiopia] and ICF, 2016). The questionnaire was initially prepared in English and then translated into the local languages (*Afaan Oromo*) and (*Amharic*) by language experts, and back into English to check its consistency. Training was given to data collectors, and their supervisors. The study questionnaire was pretested in Haramaya Hospital which is one of the nearby Hospitals. A research manual was prepared for use during training, data collection, and data management. It was conducted with the aim of clearing any ambiguity in

the questionnaire and culture and biomedical test procedure. Based on the finding of the pretest, minor modification made to the questionnaire before the actual commencement of data collection.

3.5.2 Data collection procedure

Laboratory procedure for isolation of group B *streptococcus* bacteria

For specimen collection, transportation, and processing, we followed the Centers for Disease Control and Prevention recommendations and the Standard Operating Procedures previously described elsewhere (Verani *et al.*, 2010 ;Wayne, 2015). In brief, maternal recto-vaginal colonization was confirmed when GBS was identified from mothers by swabs culture collected by the trained midwifery nurse from both vagina and rectum with a single swab applicator. Vertical transmission was confirmed within 6 hours after delivery, swab specimens were collected by the trained midwifery nurse at least from two sites (the ear canal, umbilicus, axilla, groin, or nose) of the stillborn and alive neonate using sterile cotton applicators. Soon after collection, the specimens were placed in sterile screw-capped test tubes containing Amie's transporting media with charcoal (Copan Diagnostics, Inc., New York, NY, USA) to sustain the viability of bacteria and transported to the Microbiology Laboratory of the Department of Medical Laboratory Sciences, College of Health and Medical Sciences, Haramaya University within 4 hours of collection in cold chain (+4°C). Subsequently, the specimens were inoculated in Todd-Hewitt broth (Oxoid, Hampshire, UK), an enrichment broth for GBS, supplemented with gentamycin (8mg/mL) and nalidixic acid (15 mg/mL; Biomerieux, France) to prevent the overgrowth of commensals, normal flora, and contaminants. The broth cultures were incubated aerobically at 35°C–37°C. After overnight incubation, the specimen was aseptically sub-cultured onto 5% sheep blood agar plates (Oxoid) and incubated at 35°C–37°C in an anaerobic condition. The cultures were examined by microbiologists on the following day, and all negative plates were re-incubated additionally for 18–24 hours and inspected again. Suspected isolates of GBS were identified by a combination of colony morphology, gram staining, and biochemical tests such as sodium hippurate hydrolysis (Oxoid), catalase test (Oxoid), and Christie-Atkins-MunchPetersen (CAMP) (Oxoid) reaction test. Finally, all β -hemolytic, Gram-positive cocci, hippurate hydrolysis-positive, catalase negative, and Christie-Atkins-Munch-Petersen test-positive isolates were reported as GBS (Cheesbrough, 2006).

We used Gram staining procedure to differentiate gram positive bacteria from with gram negative. Colonial morphology was used to identify GBS in its form of chain cocci, and in culture in its typically gray to whitish-gray in colour when grown on sheep blood agar.

The following Biochemical tests were done:

Christie-Atkins-MunchPetersen test: Using an inoculating loop, streak a beta-lysin-producing *Staphylococcus aureus* (ATCC25923) in a straight line across the center of a sheep blood agar plate. We were streaked test organism in a straight line perpendicular to the *S. aureus* leaving 1cm space between the two streaks. Incubate the plate at 37 degree Celsius in ambient air for 18-24 hours. Enhanced hemolysis is indicated by an arrow head-shaped zone of beta-hemolysis at the junction of the two organisms indicate positive.

Catalase test: we emulsify colony in 1 drop 3% hydrogen peroxide on glass slide. Immediate bubbling is positive catalase test.

Hippurate hydrolysis-positive: 1-3 colonies of a fresh isolate and emulsify in distilled water, hippurate disk drop into the suspension using forceps, incubate aerobically for 2 hours at 35-37°C, 2 drops of Ninhydrin reagent dropped, and re-incubate aerobically for 30 minutes at 35-37°C, blue to purple color indicate positive test.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was done by disk diffusion method. Purification of isolates was done before susceptibility testing. Detail procedures followed found on reference. Susceptibility testing was done on one isolate per patient. Antibiotics were placed onto a Muller-Hinton agar added with 5 % sheep blood following bacterial inoculation (0.5 McFarland of bacterial suspension). The plates were incubated at 37 °C in a CO₂ enriched environment for 20–24 hours. We measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. The zone margin was considered the area showing no obvious, visible growth that can be detected with the unaided eye. We measured the zones from the upper surface of the agar illuminated with reflected light, with the cover removed.

The D-test for inducible clindamycin resistance was performed on all isolates resistant to erythromycin but susceptible to clindamycin. Erythromycin and clindamycin disks were placed approximately 12 mm apart on the plate. Inducible clindamycin resistance by erythromycin was detected by a blunting of the clindamycin zone closest to the erythromycin disk, giving the appearance of a “D.” Blunting was defined as growth within the clindamycin zone of inhibition proximal to the erythromycin disk, indicating MLS_B-inducible methylation. Resistance to both erythromycin and clindamycin indicated MLS_B constitutive methylation. As a result the CLSI recommends that laboratories report D-test+ isolates as “resistant to clindamycin

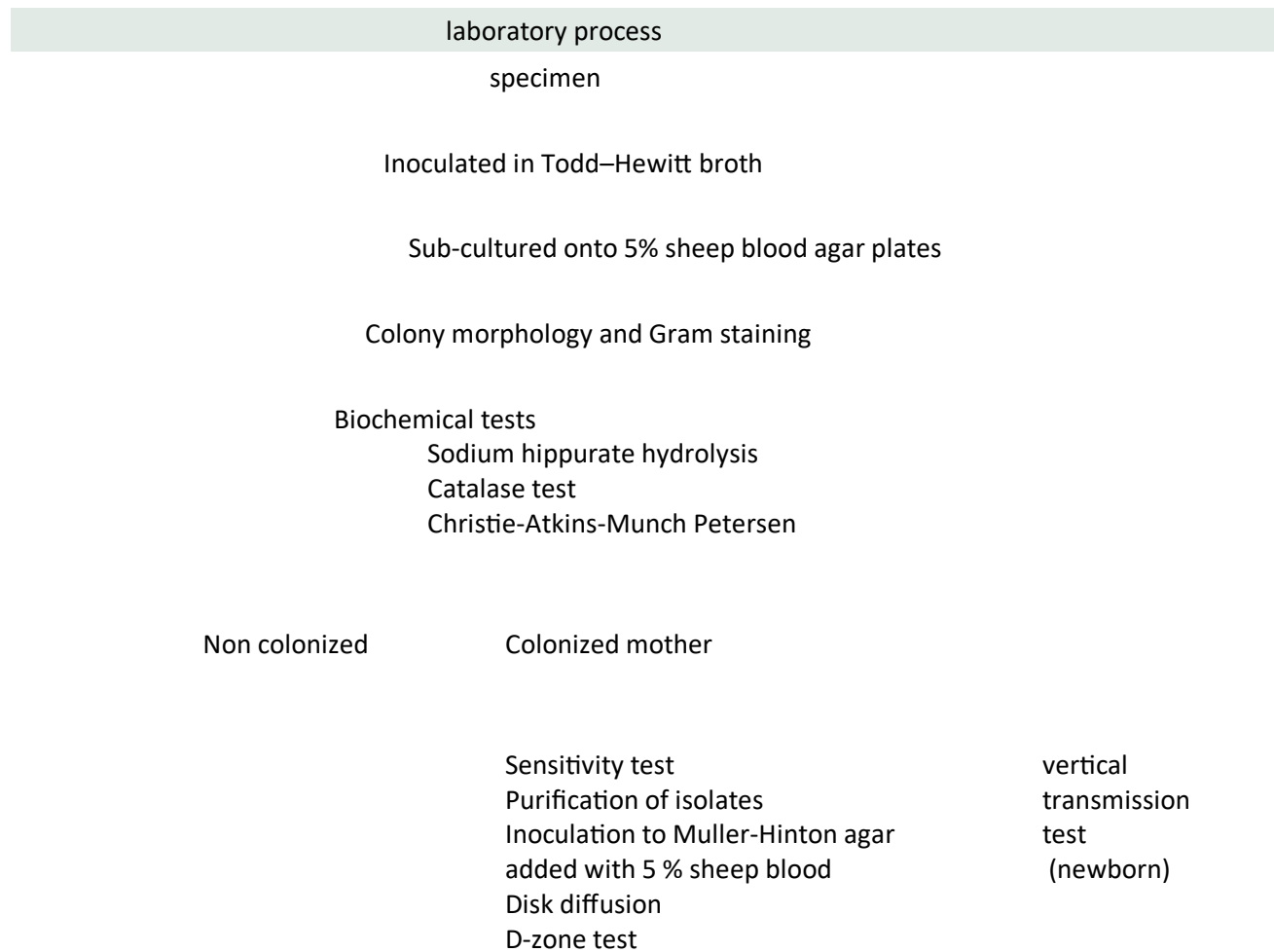


Figure 3: Schematic presentation of laboratory process Harar, Eastern Ethiopia, 2016

3.6 Study Variables

3.6.1 Dependent variable

Pregnant women GBS colonization, Vertical transmission, Stillbirth, and Erythromycin and clindamycin susceptibility were the main outcome variables

1. Pregnant women GBS colonization: it was confirmed when GBS was identified from both rectum and vagina without signs or symptoms of infection when the women arrived before vaginal examination. GBS positivity of pregnant women was confirmed by culture of recto-vaginal swab. Finally, all β -hemolytic, Gram-positive cocci, hippurate hydrolysis-positive, catalase negative, and Christie-Atkins-Munch-Petersen test–positive isolates were reported as GBS. Positive GBS was labeled as “yes” and negative GBS as “no” with a code of 1 and 0, respectively (CDC, 2010).
2. Vertical transmission (new born colonization): was the dependent variable in this study and understood as follows: it was confirmed when GBS was identified from both live born and stillbirths by swab culture, from the ear canal, umbilicus, axilla, groin, or nose without signs or symptoms of infection within 6 hours of delivery from colonized women (Seale, A. C. et, al, 2016). Finally, all β -hemolytic, Gram-positive cocci, hippurate hydrolysis-positive, catalase negative, and Christie-Atkins-Munch-Petersen test–positive isolates were reported as GBS. Positive GBS was labeled as “yes” and negative GBS as “no” with a code of 1 and 0, respectively (CDC, 2010).
3. Stillbirth: was the dependent variable in this study and obtained from medical records. It was label as “yes” and “no” with a code of 1 and 0, respectively.
4. Erythromycin and clindamycin susceptibility: was dependent variable, antibiotic susceptibility testing was done by disk diffusion method and the detection of inducible clindamycin resistance was done by using D-test method. The category of susceptibility include: **Sensitive (S)** category implies that isolates are inhibited by the usually achievable concentrations of antimicrobial agent when the dosage recommended to treat the site of infection is used; **Intermediate (I)** category includes isolates with antimicrobial agent minimum inhibition concentration (MICs) that approach usually attainable blood and tissue levels, and for which response rates may be lower than for susceptible isolates; **Resistance (R)** category implies that isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules, and/or that demonstrate MICs or zone diameters that fall in the

range where specific microbial resistance mechanisms are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies. We followed the CLSI recommendation cutoff point criteria for zone diameter and minimal inhibition concentration of disk content (Table 4) (CLSI, 2014).

Table 4: The cutoff point for zone diameter and minimal inhibition concentration of disk content interpretive criteria for Group B Streptococcus susceptibility test

Antimicrobial	Disk content	Zone Diameter Interpretive Criteria (nearest to whole mm)		
		Sensitive	Intermediate	Resistance
Erythromycin	15 µg	≥21	16-20	≤15
Clindamycin	2 µg	≥19	16-18	≤15

Source: CLSI performance standard, 2015, Pennsylvania, USA

3.6.2 Independent variables

Maternal age, education, pre labour rupture of membranes, fever, Hypertensive disease, ANC follow-up, and prolonged labour, birth weight, gestational age, were the independent variable

1. Age of the mother was recorded based on maternal response later and was grouped in to category for analysis.
2. Maternal education was grouped as “illiterate” for those who could not read and write and “literate” for all others, and was coded as 1 and 0, respectively (EDHS, 2016).
3. Wealth status: to determine the economic status of the pregnant women, a wealth index was generated using 33 variables. The wealth distribution was generated by applying principal components of the variables(Central Statistical Agency (CSA) [Ethiopia] and ICF, 2016 ;Vyas and Kumaranayake, 2006). The variables used include income, maternal occupation, ownership of durable assets, ownership of farm land, access to utility and infrastructure such as sanitation, source of water, housing character, and having bank saving/cooperative saving account (Krefis *et al.*, 2010 ;Vyas and Kumaranayake, 2006). Categorical variables were

made dummy before initiating analysis. Finally, the wealth status was determined by using three groups; rich as 1 middle as 2, and poor as 3.

4. Prolonged rupture of membranes (PROM) was obtained from medical records, rupture of membranes for ≥ 18 hours before delivery of the fetus.
5. pre-labor rupture of membranes at term (term PROM) were labeled as “yes” and “no” later for analysis with codes 1 and 0, respectively.
6. Maternal fever For the purpose of this study, a temperature of $\geq 37.5^{\circ}\text{C}$ was considered as maternal fever at admission and was obtained from the medical records during labor, and was labeled as “yes” and “no” with codes 1 and 0, respectively.
7. Intrapartum antibiotic prophylaxis: Data for IAP administration were also taken from the medical record and labeled as “yes” and “no” and coded with 1 and 0, respectively. Duration of IAP from the first time of administration to delivery was taken from the medical record and then estimated for ≥ 4 and < 4 hours.
8. Hypertensive disease was obtained from medical record and labeled as “yes” and “no” and coded with 1 and 0 respectively.
9. Antenatal care (ANC) follow-up: This classified as attend and not attend ANC in current pregnancy were labeled as “yes” and “no”
10. Prolonged labour was obtained from medical record and labeled as “yes” and “no” and coded with 1 and 0 respectively.
11. Birth weight was categorized as < 2500 gram and ≥ 2500 gram and labeled as “yes” and “no” and code with 1 and 0 respectively.
12. Gestational age was categorized as < 37 weeks and ≥ 37 weeks and labeled as “yes” and “no” and code with 1 and 0 respectively.

3.7 Data quality assurance

Data collectors were thoroughly trained. Specimen collection and interviews were conducted in private places to ensure privacy. Supervisor and principal investigators rechecked for the completeness of data before the mother and newborn were discharged and their card was returned back to the main record office. Laboratory processing, we followed the Centers for Disease Control and Prevention recommendations and the Standard Operating Procedures (Cheesbrough, 2006 ;Verani *et al.*, 2010). Two professionals were involved in measuring and reading the results. Moreover, in case of disagreement, investigators were also involved in the decision. All negative plates were re-incubated additionally for 18–24 hours and inspected again. The quality of the culture media and antimicrobial disks was checked using standard American Type Culture Collection (ATCC) reference strain of *Staphylococcus aureus* ATCC 25923, *Escherichia coli* (ATCC 25922), *S. agalactiae* isolates (ATCC 12386) (CDC, 2010).

3.8 Data processing and analysis

Data that passed all the field work and checks of the supervisor were entered Epi-data software. The data was double entered in to the computer using trained data clerks. The two data set were checked one another and validated. Stata Version 14 software was used to analyze the data. The relationships between variables were explored using bivariable analysis. Chi-square tests were used to compare proportions of categorical variables. The proportion of maternal and newborn colonization was determined using descriptive analysis.

Log binomial regression analysis was used to identify the risk factors of vertical transmission. Association was expressed as prevalence ratios (PR) and their 95% confidence interval. Log binomial regression directly models the prevalence ratio (PR) in binary and common outcome variables. The log binomial estimate is found to be the “best” estimate since it maximizes the correct binomial likelihood (Williamson *et al.*, 2013).

Logistic regression analysis was used to identify the association of maternal recto-vaginal group B streptococcus colonization and stillbirth. Association was expressed as Odds ratios (OR) and their 95% confidence interval.

The Robust Poisson regression directly estimates the prevalence ratio (PR) in binary and common outcome variables when there is a convergence problem in Log binomial regression analysis (Deddens and Petersen, 2008). Association of erythromycin and clindamycin resistance and reported previous drug use was expressed as prevalence ratios (PR) and their 95% confidence interval.

The explanatory variables were tested for multicollinearity before entering them into the multivariable model using the Variance Inflation Factor (VIF) test, the Tolerance test, and values of the standard error. No multicollinearity problem was found. The Hosmer-Lemeshow goodness-of-fit tests were used to test for model fitness (Blizzard and Hosmer, 2006).

3.9 Ethical considerations

The study was reviewed and approved by the Institutional Health Research Ethics Review Committee of the Colleges of Health and Medical Sciences, Haramaya University. Consent was obtained from regional administration, regional health department, and hospital and health center administration. Written consent was also obtained from each woman before commencement of data collection. Participants under the age of 18 provided their assent in addition to written consent of their parent or legal guardian. The study participants provided with immediate feedback on their health problem. Colonized women were provided antibiotic prophylaxis, and neonates with health problems were treated and immediate referral was also facilitated. Counseling was given for mother with stillbirth.

4. RESULTS

4.1 Brief profile of the respondents (Paper I-IV)

To examine maternal group B streptococcus recto-vaginal colonization and stillbirth association with GBS (Paper I and III), a total of 1688 pregnant women participated in the study which resulted in a 97.51 % response rate. Of these, about half (47.04%) had no formal education and (44.14%) of them were from rural area. To investigate vertical transmission (Paper II) a total of 231 mother-newborn pair were included. In paper IV which examines antibiotic susceptibility, a total of 231 GBS isolates from colonized pregnant women were included.

Table 5: The distribution of colonized pregnant women by socio economic characteristics Harar town, Eastern Ethiopia, 2016

Variables/characteristics		Paper I and III Total (1688) n(%)	Paper II and IV Total (231) n(%)
Health facilities	Hiwot Fana Hospital	1,023(60.6)	135(58.44)
	Jugal Hospital	611(36.2)	87(37.66)

	Arategna Health Center	54(3.2)	9(3.90)
Residence of pregnant women	Urban	943(55.86)	123(53.25)
			108(46.75)
Age in year	Rural	745(44.14)	
	≤19	301(17.83)	62(26.84)
	20-29	971(57.52)	96(41.56)
	30-34	416(24.64)	73(31.60)
Ethnicity	Oromo	1,214(71.92)	163(70.56)
	Amhara	267(15.82)	39 (16.88)
	Harari	95(5.63)	15(6.49)
	Other	112(6.64)	14(6.06)
Religion	Muslim	1,286(76.18)	183(79.22)
	Orthodox	308(18.25)	40(17.32)
	Others	94(5.57)	8(3.46)
		894(52.96)	128(55.41)
Educational status	Literate		
	Illiterate	794(47.04)	103(44.59)

4.2 Summary of the major findings

The details of the findings from each study are presented in the papers attached at the end of this dissertation. In this section the overview of main finding from the four papers are presented.

Table 6: Summary of the major findings, Harar town health facilities, eastern Ethiopia 2016

Paper	Objective / research question	Main finding
I	Magnitude of pregnant women GBS recto-vaginal colonization	The magnitude of pregnant women GBS colonization was 13.68% (95% CI: 12.04-15.32). Magnitude was more frequent in age level adolescent pregnancy, illiterate and low wealth index pregnant women.
II	Proportion of Vertical transmission of group B Streptococcus	The Proportion of vertical transmission was 45.02% (95% CI: 38.49, 51.68) Pre-labor rupture of membranes at term,

	Factors associated with vertical transmission	prolonged rupture of the membrane ≥ 18 hours, intrapartum maternal fever, and IAP received ≥ 4 hours were significantly associated with vertical transmission of GBS.
III	Stillbirth association with GBS	The odds of having a stillbirth was 8.93 times higher among recto-vaginal GBS colonized pregnant women
IV	Erythromycin and Clindamycin Resistance Factors associated with erythromycin and clindamycin resistance	Resistance to erythromycin and clindamycin were 56(24.24%) and 53(22.94%) respectively. Antibiotics used in current pregnancy before two weeks of data collection was significantly associated erythromycin and clindamycin resistance

4.3 Magnitude of pregnant women GBS recto-vaginal colonization

Health facility based screening conducted from June to October 2016 investigated the proportion of prevalence of pregnant women GBS recto-vaginal colonization and associated risk factors in pregnant women came for delivery services from the town and urban and rural population. A total of 1688 pregnant women participated in this study. Proportion of pregnant women identified as having GBS colonization was 13.68% (95% CI: 12.04-15.32).

4.4 Vertical transmission and associated factors

A health facility based cross-sectional study was conducted from June to October 2016 to identify the determinants of Vertical transmission of Group B *Streptococcus* from colonized mother to her new born. The study participants were 231 mother-newborn pair at delivery. All mothers were confirmed GBS recto-vaginal colonized.

The mean (\pm SD) gestational age and the mean (\pm SD) birth weight of the newborn were 37.89 weeks (± 2.21) and 3,022.14 g (± 581.69), respectively. Vertical transmission rate of 45.02% (95% CI: 38.49, 51.68). Of 104 vertical transmission cases, 65 (62.50%) received no intrapartum antibiotic prophylaxis (IAP), 28 (26.92%) received it < 4 hours before delivery, and 11 (10.58%)

received it ≥ 4 hours before delivery. Log binomial regression analysis identified Pre-labor rupture of membranes at term, prolonged rupture of the membrane ≥ 18 hours, intrapartum maternal fever, and IAP received ≥ 4 hours were significantly associated with vertical transmission of GBS ($P < 0.05$).



Figure 4: Risk factors of associated Group B Streptococcus vertical transmission, Harar, Eastern Ethiopia, 2016

4.5 Stillbirth association with GBS

The proportion of stillbirth in this study was 8.53% (95% CI: 7.19, 9.86). Of these 40.97% were from colonized mothers, 59.03% were from non-colonized mothers. After controlling for potential confounders, the odds of having a stillbirth was 8.93 times higher among recto-vaginal GBS colonized pregnant women [AOR= 8.93; 95% CI; (5.47, 14.56)].



Figure 5: Risk factors of stillbirth, Harar, Eastern Ethiopia, 2016

4.6 Erythromycin and clindamycin resistance and associated factors

A cross-sectional study was conducted to determine the proportion of erythromycin and clindamycin strain susceptibility and associated factors from pregnant women came to health facilities for delivery services.

A total of 231 GBS purified strain from recto-vaginal colonized pregnant women involved in this study. Of these 231 identified strains, resistant to erythromycin and clindamycin were observed in 24.2% and 22.94% respectively. Nineteen isolates 8.2% were resistant to both erythromycin and clindamycin and 16.01% were resistant to erythromycin but susceptible to clindamycin. Resistance to erythromycin and clindamycin was significantly associated with antibiotics used in current pregnancy before two weeks of data collection.



Figure 6: Erythromycin and Clindamycin Resistance and associated factors of *Streptococcus agalactiae* isolate, Harar, 2016

5. DISCUSSION

5.1 Summary of major findings

In this dissertation, magnitude of maternal GBS recto-vaginal colonization was found to be 13.68% (95% CI: 12.04-15.32). The magnitude of maternal GBS recto-vaginal colonization was higher among adolescent pregnancy, illiterate compared to literate, lower wealth index. Intrapartum antibiotic prophylaxis was significantly reduced vertical transmission of GBS in the health facilities. Vertical transmission is significantly associated with PROM, term PROM, maternal fever, and IAP use. GBS colonization was mainly associated with stillbirth. Resistance of GBS isolates to erythromycin and clindamycin were observed in 24.2% and 22.94% respectively. Resistance to erythromycin and clindamycin was significantly associated with antibiotics used in current pregnancy before two weeks of data collection.

5.2 Magnitude of pregnant women GBS recto-vaginal colonization

The overall proportion of GBS colonization in this study was 13.68%, although it is similar to some of the previously reported prevalence the result indicates the occurrence of GBS colonization significantly vary from context to context (Mohammed *et al.*, 2012 ;Woldu *et al.*, 2014 ;Alemseged *et al.*, 2015a)

In this study, recto-vaginal colonization of GBS was more prevalent in adolescent pregnant women compared to other age groups. It has a big implication; since adolescent pregnancy by itself is high risk (Conde-Agudelo *et al.*, 2005), recto-vaginal GBS colonization complicates the health status of adolescent women and pregnancy outcome.

Report from Kenya revealed maternal GBS colonization was less common in women with low socio-economic status (Seale *et al.*, 2016). However, in this study maternal GBS colonization was less common in women with higher wealth index. The reason might be need further investigation. Wealth index is a relative measure of wealth. In poor communities, the higher wealth index does not show the person is wealthy. However, pregnant women of higher economic status more seek health care and use antibiotics (Amin *et al.*, 2010).

5.3 Vertical transmission and associated factors

In this study, vertical transmission of GBS from colonized mothers at birth was high 45.02%. The vertical transmission rate reported in this study was consistent with those reported in studies from other low income countries (Madzivhandila *et al.*, 2011 ;Le Doare *et al.*, 2016). The reasons for high vertical transmission can be explained by lack of screening, diagnose the case, treatment and prevention strategy (Berardi *et al.*, 2014a).

The membrane rupture, of recto-vaginal GBS colonized pregnant women was revealed increased risk to occur before labour began in this study. Normally, the membrane rupture after labour began. This can be explained the fact that, the cytolysis effect of GBS causes early rupture of the membrane (Vanderhoeven *et al.*, 2014). After rupture, the amniotic fluid leaks out of the uterus, through the vagina put the life of the fetus at risk (Emechebe, 2015 ;Ibishi and Isjanovska, 2015).

Furthermore, in prolonged rupture of the membrane, the fetus exposed to maternal flora without any protective membrane barriers, enhances the transmission, which can result in colonization of the fetus, contamination of amniotic fluid, which is a good medium for bacterial proliferation, in the absence of timely preventive measures. The fetus aspirate the contaminated amniotic fluid in the uterus, the fetus exposed to pneumonia, sepsis and meningitis (Joubrel *et al.*, 2015 ;Dangor *et al.*, 2015).

In this study, we found that mothers who received adequate IAP was significantly associated with decreased risk of vertical transmission, which is consistent with the findings of studies conducted elsewhere (Fairlie *et al.*, 2013a). IAP helps to prevent vertical transmission of GBS by reducing the bacterial load of rectum and vagina and, thus, preventing GBS from ascending and proliferating in the fetus and amniotic fluid compartment (McNanley *et al.*, 2007).

In countries where there is low utilization of institutional delivery rate is low and in resource-poor settings the feasibility of introducing IAP is challenging. Antisepsis at delivery has been revealed to be ineffective (Nishihara *et al.*, 2016). However, maternal vaccination may provide a feasible strategy to reduce GBS disease in countries where there is low utilization of maternal care and in countries with resource-poor settings (Heyderman *et al.*, 2016 ;Kwatra *et al.*, 2015 ;Edwards *et al.*, 2015).

5.4 Stillbirth association with GBS

This study showed a strong association between stillbirths and maternal GBS colonization, even after controlling for other potential confounders. Other studies have also reported similar findings (Nan *et al.*, 2015 ;Seale *et al.*, 2016). This can be explained the fact that, group B *streptococcus* causes cytolysis breaches maternal-fetal barriers to cause intrauterine fetal infection, and fetal death [8].

There was a case report of intrauterine fetal death associated with acute placental villitis and acute necrotizing chorioamnionitis by early-onset group B *streptococcal* infection. After delivery, the placenta revealed multifocal neutrophilic infiltration in chorionic villi, which was also accompanied by acute necrotizing chorioamnionitis. Group B *streptococcal* were detected in villous vessels as well as in the major organs of the fetus, and cultured from maternal blood (Bae *et al.*, 2016).

The effectiveness of the clinical benefit of IAP in reducing stillbirth is limited. Similarly in this study, exposure of colonized pregnant women to IAP had no significant association with stillbirth. The reason could be, antibiotics for prophylaxis must be administered before the potential exposure, but stillbirth may occur before labour started (Nan *et al.*, 2015). In this study, 45.77% of stillbirths occurred before labour began, 50.69% did not exposed to IAP.

To achieve the sustainable development goal plan, in reduction of GBS related stillbirth the initiation taken by world health organization for viability of vaccine against GBS need strengthen (World Health Organization, 2017).

5.5 Erythromycin and clindamycin resistance and associated factors

The high rate of erythromycin and clindamycin resistance in GBS strongly indicates the need to monitor antibiotic susceptibility periodically to ensure that β -lactam allergic mothers receive appropriate and effective IAP (Fairlie *et al.*, 2013a). That is also in line with the world health assembly recommendation regarding antibiotic resistance due to overuse and misuse and urged (World Health Organization, 2014 ;Jasovský *et al.*, 2016). Our data also shows antibiotics use in the index pregnancy increased the odds of resistant to both erythromycin and clindamycin, which is consistent to previous studies (Yook *et al.*, 2013). Widespread antibiotic prophylaxis use may

be contributing to the development of erythromycin and clindamycin resistance in GBS (Pikkemaat *et al.*, 2016)

The availability of routine antimicrobial susceptibility testing to provide information on resistance trends, including emerging resistance is very essential for routine clinical practice and for the development of effective policies against antimicrobial resistance (AMR). Antimicrobial susceptibility testing is not often performed in most rural laboratories due to lack of capacity. In the absence of patient-specific antimicrobial susceptibility testing, community-based antimicrobial surveillance data may be very useful to health care professionals in particular communities or regions to treat infections with specific susceptible antimicrobials. Antimicrobial surveillance needs to be conducted regularly as resistance level can vary even in the same geographic area over time (Ayukekbong *et al.*, 2017).

6. METHODOLOGICAL DISCUSSION

Validity and strength

This study is one of the largest laboratory-based studies on Group B *streptococcus* colonization, vertical transmission and drug susceptibility of the identified isolates among pregnant women in sub-Saharan Africa. The large sample size allows us assessing stillbirth associated to group B streptococcus colonization, mother-newborn pair vertical transmission status and associated factors, antimicrobial susceptibility and associated factors.

We used a structured and well pre-tested questionnaire for data collection. In addition, uniform checklist was used to extract data for medical record. The data collection tools were developed by reviewing data collection instruments previously used by WHO and EDHS (Central Statistical Agency (CSA) [Ethiopia] and ICF, 2016 ;World Health Organization and UNICEF, 2009). The information obtained from the medical records was verified by the study team. Hence, we minimized in as much as possible data errors, missing records and under-reporting those are potential sources of bias in health facility based studies (Talley *et al.*, 1994). Hence, missed record and under-reporting may not be a problem for this study. Furthermore, to minimize measurement bias, most of the variables checked by data collectors and supervisor before the mother and newborn discharged.

Specimens were collected by midwives who received extensive training for six days and sample collection procedures were prepared on a chart and were posted in places in the triage room where they can be easily visible. We also took well designed and implemented procedures for transporting specimens to the lab. Transport time, storage temperature and concentration had significant impacts on the recovery of GBS (Stoner *et al.*, 2004). The use of appropriate transport media can help sustain the viability of GBS in settings where immediate laboratory processing is

not possible (Teese *et al.*, 2003 ;Verani *et al.*, 2010). GBS isolates can remain viable in transport media for several days at room temperature; however, the recovery of isolates declines during 1–4 days, particularly at high temperatures. Even when appropriate transport media are used, the sensitivity of culture is greatest when the specimen is stored at 4°C before culture and processed within 24 hours of collection (Ostroff and Steaffens, 1995 ;Stoner *et al.*, 2004). We used Amie transport media and cold box and transported to the laboratory and inoculated to the broth media within 4 hours, this improve the viability of the strains.

Well trained and experienced laboratory technologists and microbiologists were involved in the laboratory processing following standard operating procedures (Cockerill and Patel, 2015 ;Verani *et al.*, 2010). To minimize measurement and diagnostic bias two professionals were involved in measuring and reading the results. Moreover, in case of disagreement, investigators were also involved in the decision. The quality of each new batch culture medium was also checked by testing *E. coli* (ATCC® 25922), *S. aureus* (ATCC® 25923), and *P. aeruginosa* (ATCC® 27853) reference strains (Cockerill and Patel, 2015).

Generating GBS vertical transmission data is among the major strength of the study. To identify vertical transmission we collected specimen within 6 hours (Seale *et al.*, 2016). Collection of specimen immediately after delivery exposes the fetus to maternal genital secretions (contamination) that may affect the detection of GBS. Some studies collected specimen from babies at 24 to 48 hours after delivery expecting active replication of the GBS would make GBS vertical transmission detection easier (Berardi *et al.*, 2014a). We used selective media that contain nalditic acid and gentamycin to reduce the effect of contaminant because delaying specimen collection in health facilities increases the risk of contamination by hospital pathogens in low-income settings, immediate specimen collection may preferred to avoid cross contamination (Seale *et al.*, 2016). Generating evidence on GBS related stillbirth was also other strength of the study.

The adoption of standard laboratory procedures was critical in ensuring the quality of the laboratory works. In this study the quality of the culture media and the antimicrobial disks was assessed as per CLSI recommendations. Instruments used for measurements in the lab were calibrated before each procedure (Cockerill and Patel, 2015). The quality of antimicrobial disks

were checked using *Streptococcus pneumoniae* (ATCC® 49619) and *Streptococcus agalactiae* (ATCC*12403) reference strains. The zone of inhibition of erythromycin and clindamycin was within the established quality control limit (25-30 mm for erythromycin and 19-25 mm in diameter for clindamycin)(Cockerill and Patel, 2015).

Limitation and Generalizability

There are some limitations of the study. First, this was a health facility study and in one of the major towns of Ethiopia. Thus, may not represent the rural population where deliveries happen mostly at home. It was not possible to include rural sites and home deliveries because of resources limitations. Second, we followed routine diagnostic and recording practices in health facilities, which may have some limitations as the level of training and experience of health care providers may not be the same at all times in low income-settings because standard operation procedures and health care guidelines are not fully implemented in all facilities. Thus, there may be some diagnostic accuracy issues leading to information bias. Furthermore, Inter and intra laboratory validation was not done due to resource limitation. We have used standard strains.

7.

CONCLUSION

In this study about one in seven pregnant women were identified to having recto-vaginal GBS colonization. The magnitude of vertical transmission of GBS was very high (45.02%). IAP provision was very low though IAP is proven to be effective to reduce vertical transmission. Pre-labor rupture of membranes at term, prolonged rupture of the membrane (≥ 18 hours), intrapartum maternal fever, significantly associated with vertical transmission of GBS. Maternal recto-vaginal GBS colonization was strongly associated with stillbirth. The prevalence of erythromycin and clindamycin resistance was high and significantly associated with the use of antibiotics. Antenatal care (ANC) screening, treatment and prevention for Group B streptococcus can prevent neonatal death due to GBS. Overall, GBS colonization is highly prevalent and requires immediate attention.

8.

RECOMMENDATION

Programme implication

In this study about one in seven pregnant women were recto vaginal GBS colonized. Our result makes clear that vertical transmission of GBS from pregnant women to her offspring is high. Adequate use of IAP reduced the rate of vertical transmission. It appears to be feasible to implement IAP by health professionals in all health facilities where skilled health professionals available. Penicillin G 5 MU intravenously (IV), then 2.5 MU IV every 4 hours or ampicillin administered at 2 g IV, then 1 g every 4 hours until delivery are the preferred and recommended IAP agent. It is affordable for health facilities because maternal services in Ethiopia are free of charge. Beta-lactam allergic patients receive erythromycin or clindamycin IV in an equivalent dosage. Women with reported beta-lactam allergy, but at low risk for anaphylaxis, should receive cefazolin 2 g IV, then 1 g every 8 hours, while those at high risk of anaphylaxis (prior history of anaphylaxis, angioedema, respiratory distress, or urticarial following administration of a penicillin or cephalosporin) should receive clindamycin 900 mg IV every 8 hours (if the GBS is susceptible) or vancomycin 1 g IV every 12 hours (CDC, 2010).

Awareness of health professionals on the risk factors of vertical transmission; term prelabour rupture of the membrane, preterm labor, preterm premature rupture of membranes, PROM exceeding 18 hours, infant with prior GBS disease, maternal intrapartum fever ($\geq 38^{\circ}\text{C}$), and women positive for GBS isolated from the urine at any time are important risk factors easily identified by health professionals and need intervention.

Vertical transmission prevention guidelines recommend administration of IAP 4 or more hours before delivery. Delay health seeking and the low utilization of health services together with low awareness of neonatal danger signs are problematic and highlight the need for major action to generate demand for health services and improve community knowledge to reduce neonatal death. Several health promotion strategies could be used to strength efforts at community level to improve community recognition of neonatal danger signs and help-seeking behaviors. Innovative approaches noted in the literature include a mobile video show designed to improve community knowledge, attitudes, and beliefs regarding maternal and newborn health, especially care-seeking behavior and the use of a skilled birth attendant and postnatal care. In India a 24 hour obstetric help line available to community members and health workers was found to contribute to reducing delays associated with deciding when to seek medical care, and identifying health facilities for appropriate services, and care delivery (Desta, B., et al. 2014; Sibley, L.M., et al. 2017).

Despite the possibilities shown by such strategies, alongside improved service delivery and health worker training, further efforts will be required. Further emphasize the important role that health extension workers must continue to play in relaying information health care seeking, as well as working to actively engage community members to encourage women and their families to seek help (Sibley, L.M., et al, 2014)

The medical literature demonstrates that inadequate hospital protocols or the lack of consistent protocols for diagnosis, management, consultation, and/or referral can lead to confusion and unnecessary variation in patient care. Incongruities in clinical settings have been repeatedly shown to compromise quality of patient outcomes. Accordingly, the development and adoption of standardized protocols as the best practice for addressing for the prevention of GBS related disease at Ethiopian health facilities are recommended (Burgansky, A., D, 2016).

Antimicrobial resistance poses a threat to the future of human and animal health, compromising the treatment of basic infections and the capacity for routine medical procedures. Addressing prescribing and dispensing practice has been a focal point of strategies to decrease antimicrobial usage for many years, but implementers lack data on the current status of health care professionals' understanding and experience of antimicrobial resistance that could guide interventions. An increasing number of resistant microbial strains reported each year across both

human and animal populations in developed and developing countries (World Health Organization, 2014). Health professionals are expected to work on teaching of drug allergy, antibiotic susceptibility monitoring, and monitoring of prescription needs attention. Antimicrobial resistance requires a multi-sectorial approach involving experts in human, animal, and environmental health (Van Puyvelde, S., S., 2017) because, antibiotic residues in soil, waste water, and manure might have a greater impact on the spread of resistance than currently estimated (Pikkemaat, M., et al., 2016). The health facilities should be empowered to conduct regular screening for erythromycin and clindamycin sensitivity test along with IAP administration

Policy implications

GBS disease is a major public health problem and need to get a priority in the national policy to achieve sustainable development goal 3.1. Prevention guidelines need at national level on prevention of GBS vertical transmission and treatment of cases that could be translated in to action at health facilities and the community level. A clear policy is need on the strategies and working relationship between ministries (Health, agriculture and environmental), NGOs, private sector and local organizations to scale up prevention of drug resistance in the health facilities and in the community

Research implications

A large scale national representative study is needed to fully understand the scale of the problem and its sequelae. Such study would give stronger evidence to spearhead national policy formulation and in developing operational guidelines.

The cost effectiveness for all pregnant women screened at 35±37 weeks of gestation for vaginal and rectal GBS colonization or clinical risk based approach, and use of IAP, at the time of labor or rupture of membranes, need further investigation.

Further long term studies are needed on effectiveness of prevention of GBS disease in reducing maternal and neonatal mortality. Comparing the effectiveness of universal screenings method and risk based approach in different setting will helps to increase the uptake of the intervention.

Well-designed prospective studies are needed to determine how much of the GBS attributable to stillbirth national is. Identification of the case and estimation of the burden of disease at community level need further investigation. Follow up studies that aim at observing the burden of the disease in the community and the impact of the GBS on long term complication are recommended. Serological grouping and molecular characterization of resistant GBS gene, which would provide further description on the distribution of strain, need further investigation. Effort needed to realize the licensed vaccine against GBS.

9.

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ANNEX I Paper I-V

Magnitude of Group B *Streptococcus* Recto-Vaginal Colonization among pregnant Women:
Harar, Eastern Ethiopia

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Abstract

Background: Maternal recto vaginal colonization of Group B *Streptococcus* (GBS) is the main risk factors for GBS related stillbirths, neonatal and maternal infection. However, there is limited data on recto-vaginal colonization prevalence in Ethiopia. This study was, therefore, aimed to identify the magnitude of GBS recto vaginal colonization in Eastern Ethiopia.

Methods: Cross sectional study was conducted among 1688 pregnant women who came to health facilities found in Harar town for delivery services, Eastern Ethiopia from June to October, 2016. Data were collected through face –to- face interview using a pre-tested structured questionnaire and check list for clinical record. GBS positivity of the pregnant women was confirmed by recto-vaginal swabs cultured on to Todd-Hewitt broth medium supplemented with Gentamicin and Nalidixic acid and subsequently sub-cultured on 5 % sheep blood agar followed

by identification of isolates based on colonial morphology, Gram stain, catalase reaction, hippurate hydrolysis and Christie, Atkins, Munch-Petersen (CAMP) test. The magnitude of pregnant women GBS recto vaginal colonization is presented along with the 95% Confidence Intervals (CI).

Results: Out of the 1688 pregnant women, 231, 13.68% [95% confidence interval (CI) 12.04-15.32%] women were recto-vaginal GBS-colonized at delivery. Proportion was highest in age level of ≤ 19 years (62/301, 20.59% (16.17–25.61)), highest in illiterate (128/794, 16.12 % (13.62-18.86%)) and lowest in higher level of education (28/296(9.46%) (6.37–13.38%)) and highest in low wealth index 91/563(16.16%)(13.21-19.46%)) women.

Conclusions: the magnitude of maternal recto vaginal colonization of GBS was high and highest prevalent among adolescent pregnant women, illiterate and low wealth index. Therefore, maternal screening and use of IAP is recommended. For further, adolescent, illiterate and poor pregnant women need special, targeted interventions GBS prevention.

Key words: prevalence, intrapartum, GBS, recto vaginal colonization, Harar, Ethiopia

Background

Group B *Streptococcus* (GBS) also known as *Streptococcus agalactiae* is a β -hemolytic encapsulated gram-positive bacterium [1] causes perinatal bacterial infections, including bacteremia, endometritis, chorioamnionitis, and urinary tract infections in pregnant women and neonatal pneumonia, septicemia and meningitis in newborns and stillbirth [2]. Maternal colonization is the principal risk factor [3]. GBS colonized the recto vagina ascend in to the uterus and can be leading to maternal and fetal infection [4, 5].

Chorioaminionitis due to Group B *Streptococcus* is associated with 62% of Early-Onset neonatal Group B *Streptococcus* disease (EONGD), and 60% of fetal loss or stillbirth. Severe maternal

GBS sepsis was associated with higher infant sepsis [6, 7], treated case fatality of neonatal infection is 47.2% [6]. Survival develops lifelong neurological complication[8].

There were variations in prevalence of GBS pregnant women recto vaginal colonization across and within regions ranging from 6.8 to 26.7 percent [9]. The variation was based on ethnic groups, maternal age, educational status, parity, and economic status, the specimen sampling site, the timing of collection and the microbiological methods used [3, 10].

The current recommendation to prevent EONGD is to screen pregnant women by culture of recto vaginal swab at 35 to 37 weeks gestation and use of IAP [10, 11]. IAP reduce bacterial load of rectum and vagina and thus preventing GBS from ascending and proliferating in the fetus and amniotic fluid compartment [12]. Currently vaccine against GBS is also under development [13]. GBS was identified by World Health Organization (WHO) as a high priority for the development of a vaccine for maternal immunization because of the high technical feasibility for successful development [14].

Countries, where there is good capture of cases and routine laboratory surveillance, GBS is now well-recognized as one of the leading cause of neonatal death. Strategies of IAP have been applied to address this burden. However, there remains uncertainty regarding the GBS in low- and middle-income countries. This study was, therefore, aimed to identify the magnitude of GBS recto vaginal colonization in Eastern Ethiopia.

Materials and Method

Study area

This study performed at three health facilities (Hiwot Fana Specialized University Hospital, Jugal Hospital and Arategna Health Centers) of Harar town. The town is found 510 km far from Addis Ababa, the capital city of Ethiopia, to the east. Ethiopia is a low-income country with a total fertility rate of 4.6, maternal mortality 421/10000, neonatal mortality rate of 29/1000 live births [15], maternal mortality due to infection 13% [16]and neonatal mortality due to infections about

34.3%[17]. No screening for GBS or IAP protocol was in place for prevention of GBS disease at the time of the study.

Study design and sampling

Health facility based cross-sectional study was conducted between June and October 2016. The study subjects were pregnant women admitted to labor and delivery room at selected health facilities for delivery. The sample size was determined with the assumption previous proportion 20.9% [18] 95% CI, 2% margin of error, and 10% non-response rate, total sample size of 1731. Sample was taken proportional to the client volume of the health facilities. Women who took antibiotics in the last two weeks during their pregnancy prior to specimen collection, pregnant women with antepartum hemorrhage, those women who were physically or mentally incapable of responding, and women with multiple pregnancies were excluded from the study [19].

Data collection

Data were collected through interview administered pretested structured questionnaire; check list for medical record; and laboratory test results. The questionnaires and checklist were adapted from the Ethiopian Demographic and Health Survey and World Health Organization indicators of health status of mother during labor, delivery and immediate newborn[15, 20]. The questionnaire was initially prepared in English and then translated into the local language, “Afaan Oromo” and “Amharic”, by language experts, and back into English to check its consistency.

A six-day training was given to data collectors and the supervisors. For laboratory work, two medical microbiologists (for read, confirm and interpret test results), and three medical laboratory technologists (assists in media preparation and sterilization processes) were involved. A research manual was prepared for use during training, data collection, and data management.

Measurement

Maternal recto vaginal colonization was confirmed when GBS bacteria identified from pregnant women by swabs culture from both vagina and rectum. Positive GBS labeled as “yes” and negative labeled as “no”. Age of the mother was recorded as reported in medical records and later grouped as 15-19, 20-24, 25-29 and 30-34 and 35+ with code 1, 2, 3, 4 and 5 respectively for

analysis. Maternal education was grouped as “illiterate” for those who could not read and write and all others as “literate” and was coded 1 and 0, respectively.

To determine the economic status of the women, a wealth index was generated using 33 variables. The wealth distribution was generated by applying principal components these variables [15, 21]. The variables used included income, maternal occupation, ownership of durable assets, ownership of farm land, access to utilities and infrastructure such as sanitation, source of water, housing characteristics and the ownership of a bank saving/cooperative savings account [21, 22]. Categorical variables were made dummy before initiating analysis. Finally, the wealth status was determined by using three groups, rich as 1 middle as 2, and poor as 3.

Laboratory test

For specimen collection, transportation and processing, we followed CDC recommended and Standard Operating Procedures (SOPs) previously described elsewhere [23, 24]. In brief, all specimens were collected using sterile cotton applicators. Samples were collected from both vagina and rectum. Vaginal samples were collected by inserting a swab into the vagina. The swab was rolled round through 360 degrees against the vaginal wall at the mid portion of the vault. For rectal specimens, a swab was carefully inserted approximately 1.5 - 2 cm beyond the anal sphincter and then gently rotated to touch anal crypts. All study samples were collected by midwives and as soon as after collection, the specimens were placed in a sterile screw capped test tubes containing Amie’s transporting media with charcoal (Copan Diagnostics, Inc., New York) to sustain the viabilities of bacteria and transported to the Microbiology Laboratory of the Department of Medical Laboratory Sciences, College of Health and Medical Sciences, Haramaya University within 4 hours of collection in cold chain (+4⁰C). Subsequently, specimens were inoculated in Todd–Hewitt broth (Oxoid, UK), an enrichment broth for GBS, supplemented with gentamycin (8 mg/ml) and nalidixic acid (15 mg/ml) (Biomérieux, France) to prevent the overgrowth of commensals, normal flora and contaminants. The broth cultures were incubated aerobically at 35-37⁰C. After overnight incubation, specimen was aseptically sub cultured onto 5% sheep blood agar plates (Oxoid, UK) and incubated at 35-37⁰C in an anaerobic condition. The cultures were examined by Microbiologists on the following day, and all negative plates were re-incubated additionally for 18–24 hours and inspected again. Suspected isolates of GBS were

identified by a combination of colony morphology, gram's staining and biochemical tests such as sodium Hippurate hydrolysis (Oxoid, UK), catalase test (Oxoid, UK) and Christie, Atkins, Munch-Petersen (CAMP) (Oxoid, UK) reaction test. Finally, all β -hemolysis, Gram positive cocci, Hippurate hydrolysis positive, catalase negative and CAMP test positive were reported as GBS.

Statistical analysis

The data were double entered and cleaned using Epi-Data Version 3.1 and analyzed using STATA version 14. The magnitude of each pregnant women GBS recto-vaginal colonization was calculated by dividing the number of pregnant women who test positive the total number of pregnant women in the study and was reported with 95% Confidence Intervals (CI).

Ethical considerations

The study was reviewed and approved by the Institutional Health Research Ethics Review Committee of the Colleges of Health and Medical Sciences, Haramaya University. Written consent was obtained from each woman before commencement of data collection. Participants under the age of 18 provide their assent in addition to written consent of their parent or legal guardians. To maintain confidentiality, interviews and specimen collection were conducted in a separate room and all information obtained in this study was handled with anonymity. Colonized women provided antibiotic prophylaxis and neonate with health problems were treated and immediate referral was also facilitated.

Result

A total of 1688 pregnant women participated in the study with a response rate of 97.5%. Almost all of the respondents were married (95.73%), most of them Muslim (76.18%) and Oromo (71.92%) in ethnicity about half had no formal education (47.04%) and were from rural area (44.14%). Their age ranged from 15 to 46 years with the mean (standard deviation (SD)) of 26.59 \pm 5.63 years.

Five hundred sixty one (33.23%) of the women had never attend care given during pregnancy at a health facility. The mean number of pregnancies per woman was 2.9 (with a range from 1 to 15)

and the mean number of deliveries was 2.6 (with a range from 1 to 14). About two-in-third (66.8%) of the women received antenatal care (ANC).

Table 1: The distribution of colonized pregnant women by socio economic characteristics Harar town, Eastern Ethiopia, 2016

Characteristics	Frequency (n)	Percent (%)
Health facilities		
Hiwotfana	1,023	60.6
Jugal	611	36.2
Arategna	54	3.2
Residence of pregnant women		
Urban	943	55.86
Rural	745	44.14
Age in year		
≤19	301	17.83
20-24	510	30.21
25-29	461	27.31
30-34	275	16.29
≥35	141	8.35
Ethnicity		
Oromo	1,214	71.92
Amhara	267	15.82
Harari	95	5.63
Somali	15	0.89
Other	97	5.75
Religion		
Muslim	1,286	76.18
Orthodox	308	18.25
Protestant	89	5.27
Catholic	5	0.3
Marital status		
Married	1,616	95.73
others	72	4.27
Educational status		
Literate	894	52.96
Illiterate	794	47.04
Gravida		
1-2	989	58.59
3-5	423	25.06
>5	276	16.35
Para		

0	722	42.77
1-2	560	33.18
3-5	286	16.94
>5	120	7.11
ANC follow up		
No	561	33.23
Yes	1,127	66.77

Magnitude of GBS by characteristics

Out of the 1688 pregnant women, 231, 13.68% [95% confidence interval (CI) 12.04-15.32%] women were GBS-colonized at delivery. Proportion was highest in age level of ≤ 19 years (62/301, 20.59% (16.17–25.61)), almost similar at rural and urban sites (108/745, 14.5% (12.05–17.23%)), (123/943, 13.04% (10.95–15.36%) respectively; highest among illiterate (128/794, 16.12 % (13.62-18.86%)); lowest in higher level of education (28/296(9.46%) (6.37–13.38%)) and highest in low wealth index 91/563(16.16%)(13.21-19.46%).

Table 2: Characteristics of the study participants by GBS recto vaginal colonization Harar town, Eastern Ethiopia, 2016

Characteristics	Total (n)	Pregnant women GBS test result	
		Negative, n (%)	Positive, n (%)
Age of pregnant women in year			
≤ 19	301	239(79.4)	62(20.6)
20-24	510	471(92.35)	39(7.65)
25-29	461	404(87.64)	57(12.36)
30-34	275	226(82.18)	49(17.82)
≥ 35	141	117(82.98)	24(17.02)
Residence of pregnant women			
Town	943	820(86.96)	123(13.04)
Rural	745	637(85.5)	108(14.50)
Health Facility			
Hiwot Fana	1,023	888(86.8)	135(13.20)
Jugal	611	524(85.76)	87(14.24)
Aratenha HC	54	45(83.33)	9(16.69)
Education status			
Illiterate	794	666(83.88)	128(16.12)
1-10 grade	598	523(87.46)	75(12.54)
>10 grade	296	268(90.54)	28(9.46)

Marital status	Married	1,616	1393(86.2)	223(13.8)
	Others	72	64(88.89)	8(11.11)
Gravida	<3	989	876(88.57)	113(11.43)
	3-5	423	371(87.71)	52(12.29)
	>5	276	210(76.09)	66(23.91)
Wealth Index	Low	563	472(83.84)	91(16.16)
	Middle	586	514(87.71)	72(12.29)
	Better	539	471(87.38)	68(12.62)

Discussion

This study identified the magnitude of GBS recto vaginal colonization among pregnant women in Eastern Ethiopia using a CDC and CLSI recommended approach of specimen collection, transportation, and processing. Although prior studies in Ethiopia have tried to document the magnitude of GBS colonization among the pregnant women, studies that follow the recommended CDC procedure was minimal and no study conducted in the eastern Ethiopia. In this study maternal recto vaginal GBS colonization was highest prevalent among women of younger age groups, uneducated, lower wealth index.

Prevalence of GBS recto vaginal colonization in pregnant women is variable the reason may be due to difference in socio-demographic, clinical risk factor, and population difference in natural immunity [9, 25]. The GBS colonization in our study is comparable with studies conducted in Kenya[3]. GBS adhere to vaginal epithelium, ascend by vaginal epithelium exfoliation, and disseminates infection [26, 27]. IAP reduce the bacterial load of rectum and vagina and, thus, preventing GBS from ascending and proliferating in the fetus and amniotic fluid compartment [12]. To achieve sustainable development goal of their perinatal mortality and morbidity reduction program, health facilities found in the country need to include GBS related maternal and neonatal mortality prevention in to their strategies

Pregnant women of various ages were found to be at risk of GBS colonization. This study found out GBS colonization was highest prevalent in adolescent pregnant women, consistent with study reported at United State of America and Denmark[28, 29]. The reason needs further

investigation. Pregnancy during adolescence could have negative consequences for the health and well-being of mothers and their newborns [30]. GBS synergize the over burden for adolescent pregnancy. Health facilities in the countries need to give targeted interventions that focus on the adolescent pregnancy and GBS prevention.

In this study the highest prevalence was among illiterate and low wealth index. Again, this finding is supported by previous reports from other geographical settings [31]. Poor is facing higher risks of malnutrition and lower receiving in key health interventions [32]. It is unclear whether differences in access to healthcare, poor nutritional status, differences in antimicrobial drug usage, population differences in natural immunity, and possibly differences in serotypes and strain

virulence of colonizing isolates needs further investigation.

WHO developed strategic plan for vaccine development against GBS disease. The administration of GBS vaccine to women during pregnancy could be a feasible and more practical approach for challenges of a GBS screening and IAP use in low and middle income countries (LMIC) [14]. However, a recent study in South Africa estimates that a joint vaccination/risk-based screening program would be more effective in reducing GBS disease compared with vaccine alone while remaining cost effective. Thus Screen and IAP use is remain GBS prevention strategy even after the vaccine becomes available [33].

However, most of LMIC have no guideline for screening and IAP use. The medical literature demonstrates that lack, inadequate or inconsistent hospital protocols for screening can lead to confusion, unnecessary variation in patient care, and compromise quality of patient outcomes [34]. Screening is very essential for routine clinical practice and for the development of effective policies against GBS disease prevention [35]. In Ethiopian also there is no clear guideline, this may be due to lack of knowledge and capacity. The results of this study throw light on the current initiatives that are focused on improving maternal and newborn health care delivery system, the

magnitude of the problem, and call for the necessary actions to be taken in order to strengthen effort in prevention of GBS disease

We collect the specimen from vagina and rectum, used Todd-Hewitt broth followed by sub culture with 5% sheep blood agar, and specimen collected at delivery. Detection of GBS improved when rectal and vaginal swabs used, vaginal sampling yield only about 50 % [10]. Use of Todd-Hewitt broth followed by sub culture with 5% sheep blood agar also improved the yield of group B streptococcus. In direct agar plating 54.7% of woman who are GBS carriers have false-negative culture results [36]. Furthermore, maternal colonization is persistent (transient), interment, or chronic; screening at 35 to 37 weeks of gestation may not reveal the actual colonization status at labour and delivery [11]. Studies have reported that some women who are negative at their initial screening may be colonized at delivery. False-negative rates ranging from 4-8% have been reported [37]. To preserve better viability and stability of GBS, specimens were transported to the laboratory, inoculated into broth media, and incubated within 4 hours of specimen collection.

This study was not without limitations. Interviewer and recorder bias might have occurred during the interviews and recording. However, supervisor and principal investigators rechecked for the completeness of data before the mother and newborn were discharged and their card was returned back to the main record office. Sample collection was done by the midwife prenatal care providers and there may have been differences in swab techniques despite specimen collection training [38]. Specimen collection procedure were prepared and posted in the room where easily visible. Measurement and diagnostic bias might also have occurred during measurement and diagnoses; however, two professionals were involved in measuring and reading the results. Moreover, in case of disagreement, investigators were also involved in the decision. Phenotypic and genotypic that further characterizes *Streptococcus agalactiae* by serotype and gene was not performed. As the study was done in health facilities, the findings may not able to be generalized to the entire community.

In conclusions, the magnitude of maternal recto vaginal colonization of GBS was high and highest prevalent among adolescent pregnant women, illiterate and low wealth index. Therefore, maternal

screening and use of IAP is recommended. For further, adolescent, illiterate and poor pregnant women need special, targeted interventions GBS prevention

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Authors' contribution

TA, AW, YB, GE, and BS conceived and designed the paper, involved in data collection, performed the statistical analysis, interpret the results wrote and reviewed the manuscript. DM performed laboratory work and interpret the laboratory results. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests

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Vertical Transmission of Group B *Streptococcus* and Associated Factors among Pregnant Women: A cross-sectional study, Eastern Ethiopia

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Abstract

Background: A Fetus and neonate infection which is caused by Group B Streptococcus (GBS) is one of the vertically transmitted diseases. However, there is limited information on vertical transmission of GBS in low-income countries. This study, therefore, aimed to determine the rate of vertical transmission of GBS and associated factors among pregnant women in Eastern Ethiopia.

Methods: A cross sectional facility based study was conducted among pregnant women in Harar town, Eastern Ethiopia from June to October, 2016. GBS-positivity of the pregnant women was confirmed by culture of recto vaginal swab. Vertical transmission at birth was confirmed by culture on swabs taken from the ear canal, umbilical, axilla, groin or nose within six hours after birth. Prevalence ratio along with 95% CI was estimated to examine factors associated with vertical transmission using Log-binomial regression analysis.

Results: Out of 231 GBS colonized pregnant women at delivery, 104 births were identified as GBS colonized with vertical transmission rate of [(PR), 45.02%; 95% CI: (38.49, 51.68)]. Of 104 vertical transmission 65(62.50%) received no intrapartum antibiotic prophylaxis (IAP), 28(26.92%) < 4 hours before delivery, and 11(10.58%) received ≥ 4 hours before delivery. Pre labor rupture of membranes at term (Term PROM) [PR= 1.93; 95% CI; (1.04,3.57)], prolonged rupture of the membrane (PROM) ≥ 18 hours [PR= 1.76;95% CI;(1.13, 2.74)], intrapartum maternal fever[(PR)= 1.40; 95% CI; (1.13, 1.75)] , and IAP received ≥ 4 hours [(PR)= 0.17 ; 95% CI:(0.09, 0.30)] were significantly associated with vertical transmission of GBS.

Conclusion: The magnitude of vertical transmission of GBS was very high. However, adequate received IAP was very low. Efforts need to be strengthened to screen pregnant women during antenatal care and use of IAP as necessary. Furthermore, maternal vaccination may provide a feasible strategy to reduce vertical transmission.

Key words: GBS, vertical transmission, newborn, log- binomial, Ethiopia

Introduction

Group B streptococci (GBS) are gram positive bacteria [1]. It causes invasive newborn and fetal infection. Early-onset of neonatal disease (EOND) occurs in the first weeks of life (0–6 days), mostly associated with sepsis [2]. Vertical transmission of GBS can occur during pregnancy or the birth process from genitourinary or gastrointestinal tract of colonized pregnant women [3]. GBS colonizing the rectum and vagina of pregnant women ascends and causes chorioamnionitis, leading to fetal and neonatal colonization [4]. Mortality risk among newborns with GBS colonization was 6.6 fold higher than for those without GBS [5]. EOND occur 1.4 per 1,000 neonates in the general population and 7.8 per 1,000 among GBS colonized women.[6]

Neonates with early onset of GBS (EOGBS) disease are more likely to have respiratory distress disease and convulsions, and require longer hospital stays [7]. Case fatality is highest in EOND when it occurs within 24 hours of birth.[4] Survivors suffered from uncontrolled seizures, impaired psychomotor development, profound mental retardation, blindness, and deafness.[8]

Worldwide, vertical transmission of GBS shows considerable variation within and between geographic regions ranging from 11.2% to 57.7% [9, 10]. The identified risk factors for vertical transmissions were maternal fever during labour, prolonged rupture of the membrane, preterm delivery, chorioamnionitis, maternal GBS sepsis and intrapartum antibiotic prophylaxis.[11, 12]

Intrapartum antibiotic prophylaxes for women colonized with GBS can significantly decrease vertical transmission [13]. IAP can also significantly reduce the rates of EOD and case fatality rate [6, 14]. The effectiveness of IAP was high if IAP administered at least four hours before delivery. Early recognition and treatment promptly are crucial in preventing rapid invasive GBS disease progression[15].

Countries that introduced prevention strategy including universal routine antenatal GBS screening, identification of risk factors, and offering antibiotic prophylaxis at delivery were able to significantly reduced the disease [16, 17]. However, most of Sub-Saharan African (SSA) countries do not have clear guideline for prevention despite the high rate of vertical transmission [10, 18, 19]. That could be due to lack of information on vertical transmission of GBS. Thus, this

study aimed to investigate the magnitude of vertical transmission of Group B *streptococcus* among colonized women at birth and factors that are associated with vertical transmission.

Materials and Methods

Study setting

The study was conducted in three selected health facilities in Harar town, eastern Ethiopia. Harar town is found in Harari Regional State, which is one of the nine Regional States of Ethiopia. The town is located 510 km far from Addis Ababa, the capital city of Ethiopia. The total population of the town is about 101,934. In the town, there are six Hospitals (four governments and two private) and four health centers providing delivery services. For this study, Hiwot Fana Specialized Haramaya University Hospital, Jugal Hospital and Arategna Health Centers were selected because they had the largest client load in their delivery services [20].

Study design and period

Health facility based cross-sectional study was conducted between June and October 2016. The study participants were pregnant women admitted to labor and delivery room at selected health facilities for delivery. A total of 1688 pregnant women during active labor were examined for recto-vaginal GBS colonization. In this study, sample size was determined with the assumption of 95% CI, power of the study 80%, control to case ratio 2:1, percent of control (newborn did not colonized) exposed (fever) 2%, percent of case (newborn colonized) exposed 26%.[11] Accordingly, a sample size of 81 mother–newborn pair was calculated. However, during assessment of GBS colonization study, which was designed for a different study, the total number of colonized pregnant women was found to be 231 (127 non-colonized newborn and 104 colonized newborn). To address other exposure variables and increase the power of the study, we included all the colonized mothers that were identified beyond the minimum requirement in the final analysis. Women who took antibiotics in the last two weeks during their pregnancy prior to specimen collection, pregnant women with antepartum hemorrhage, those women who were physically or mentally incapable of responding, and women with multiple pregnancies were excluded from the study[9].

Data collection:

Data were collected through interview administered questionnaire; check list for medical record; and laboratory test results. The questionnaires and checklist were adapted from the Ethiopian Demographic and Health Survey (EDHS) and WHO indicators of health status of mother during labor, delivery and immediate newborn [21, 22]. The questionnaire was initially prepared in English and then translated into the local language, “Afaan Oromo” and “Amharic”, by language experts, and back into English to check its consistency.

A Six day training was given to data collectors midwives and their supervisors. For laboratory work, two medical microbiologists (for read, confirm and interpret test results), and three medical laboratory technologists (assists in media preparation and sterilization processes) were involved. A research manual was prepared for use during training, data collection, and data management.

Vertical transmission was the dependent variable in this study and understood as follows: it was confirmed when GBS identified from both live-born and still birth by swabs culture from ear canal, umbilical, axilla, groin or nose without signs or symptoms of infection within 6 hours of delivery from colonized women [4, 9]. Positive GBS labeled as “yes” and negative labeled as “no” with a code of 1 and 0, respectively. Age, educational status, fever in current pregnancy, rupture of the membrane, gestational age, still birth and interpartum antibiotic administration, were independent variables in this study.

Age of the mother recorded based on maternal response later grouped as 15-25 and 26-46 with code 0 and 1 respectively for analysis. Maternal education was grouped as “illiterate” for those who could not read and write and all others as “literate” and was coded 1 and 0, respectively.

Rupture of membranes was obtained from medical records. Prolonged rupture of the membrane that took ≥ 18 hours to delivery of the fetus; Preterm premature rupture of membranes (PPROM); and Pre labour rupture of membranes at term (Term PROM) was label as “yes” and “no” later for analysis code 1 and 0 respectively. For the purpose of this study, a temperature of $\geq 37.5^{\circ}\text{C}$ was considered as the threshold score for maternal fever at admission and then 3 times at standard intervals during labor labeled as “yes” and “no” with code 1 and 0 respectively. Still birth was

obtained from medical record. It was labeled as “yes” and “no” and coded with 1 and 0 respectively.

The current vertical transmission prevention guideline, recommend administer of intrapartum antibiotics prophylaxis 4 or more hours before delivery. According to revised CDC guidelines for the prevention of neonatal GBS disease, all pregnant women should be screened at 35±37 weeks' gestation for vaginal and rectal GBS colonization. IAP administration, at the time of labor or rupture of membranes, should be performed in several cases: known maternal GBS colonization, preterm labor, preterm premature rupture of membranes, prolonged rupture of membranes exceeding 18 hours, prior infant with group B *streptococcal* disease, maternal intrapartum fever ($\geq 38.0^{\circ}\text{C}$), and women positive for GBS isolated from the urine at any time [23].

Penicillin G is preferred the recommended IAP agent 5 MU intravenously (IV), then 2.5 MU IV every 4 hours or ampicillin 2 grams IV, then 1 gram every 4 hours until delivery. Beta-lactam allergic patients receive erythromycin or clindamycin intravenously in equivalent dosage. Women with reported beta-lactam allergy, but at low risk for anaphylaxis should receive cefazolin 2 grams IV, then 1 gram every 8 hours, while those at high risk of anaphylaxis (prior history of anaphylaxis, angioedema, respiratory distress or urticaria following administration of a penicillin or cephalosporin) should receive clindamycin 900 mg IV every 8 hours (if the GBS is susceptible) or vancomycin 1g IV every 12 hours [23].

Pregnant women who received IAP less than 4 hours before delivery have inadequate protection [15]. Data for IAP administration was also taken from the medical record and labeled as “yes” and “no” and code with 1 and 0 respectively. Duration of IAP from first time of administration to delivery was taken from medical record then estimated for “ ≥ 4 hours” and “ < 4 hours”. Later, for the regression analysis, “women not received” and “received inadequate” were grouped as “no and inadequate” and recorded as “0” and those “received adequate” were recorded as “1”.

For specimen collection, transportation and processing, we followed CDC recommended and Standard Operating Procedures (SOPs) previously described elsewhere [23, 24]. In brief, within 6 hours after delivery, swab specimens were collected by the trained midwifery nurse at least from 2 sites (the ear canal, umbilical, axilla, groin or nose) of the still births and live births using sterile

cotton applicators. Soon after collection, the specimens were placed in a sterile screw capped test tubes containing Amie's transporting media with charcoal (Copan Diagnostics, Inc., New York) to sustain the viabilities of bacteria and transported to the Microbiology Laboratory of the Department of Medical Laboratory Sciences, College of Health and Medical Sciences, Haramaya University within 4 hours of collection in cold chain (+4°C). Subsequently, specimens were inoculated in Todd–Hewitt broth (Oxoid, UK), an enrichment broth for GBS, supplemented with gentamycin (8 mg/ml) and nalidixic acid (15 mg/ml) (Biomérieux, France) to prevent the overgrowth of commensals, normal flora and contaminants. The broth cultures were incubated aerobically at 35-37°C. After overnight incubation, specimen was aseptically subcultured onto 5% sheep blood agar plates (Oxoid, UK) and incubated at 35-37°C in an anaerobic condition. The cultures were examined by Microbiologists on the following day, and all negative plates were re-incubated additionally for 18–24 hours and inspected again. Suspected isolates of GBS were identified by a combination of colony morphology, gram's staining and biochemical tests such as sodium Hippurate hydrolysis (Oxoid, UK), catalase test (Oxoid, UK) and Christie, Atkins, Munch-Petersen (CAMP) (Oxoid, UK) reaction test. Finally, all β-hemolysis, Gram positive cocci, Hippurate hydrolysis positive, catalase negative and CAMP test positive were reported as GBS.

Statistical analysis

The data were double entered and cleaned using Epi-Data Version 3.1 and analyzed using STATA version 13. Log binomial regression model was used to assess the association between independent variables and the outcome variable (vertical transmission). Log binomial regression directly models the prevalence ratio (PR) in binary and common outcome variables. The log binomial estimate is found to be the “best” estimate since it maximizes the correct binomial likelihood. [25] Possible interactions between covariates were tested. The explanatory variables were tested for multicollinearity before entering them into the multivariable model using the Variance Inflation Factor (VIF) test, the Tolerance test, and values of the standard error. No multicollinearity problem was found. Correlation matrix and covariate matrix was tested for the final model. Log likelihood ratio test and Akaike's information criterion and Bayesian information

criterion was used to select final model. The Hosmer-Lemeshow goodness-of-fit tests were used to test for model fitness [26].

Crude prevalence ratios (CPR) with 95% confidence intervals were estimated to assess the association between each independent variable and the outcome variable, and a p-value was determined. Variables with p-value ≤ 0.2 in the bivariate analysis were considered in the multivariable analysis by assuming that the outcome varies according to risk factors, along with maternal age, well known predictors of vertical transmission, regardless of the cut-off point for p-value. Adjusted Prevalence Ratio (APR) along with 95% confidence intervals were estimated to assess the strength of the association, and a p-value < 0.05 was used to declare the level of statistical significance in the multivariable analysis.

Ethical considerations

The study was reviewed and approved by the Institutional Health Research Ethics Review Committee of the Colleges of Health and Medical Sciences, Haramaya University. Written consent was obtained from each woman before commencement of data collection. Illiterate mothers consented by their thumbprint. Participants under the age of 18 provide their assent in addition to written or thumbprint consent of their parent or legal guardians. To maintain confidentiality, interviews and specimen collection were conducted in a separate room and all information obtained in this study was handled with anonymity. Colonized women provided antibiotic prophylaxis and neonate with health problems were treated and immediate referral was also facilitated.

Results

A total of 1688 pregnant women were screened for recto-vaginal GBS colonization at labour and delivery room. Of this 231 (13.68%) pregnant women were culture positive for GBS. In this study, these 231 GBS colonized pregnant women were used to identify vertical transmission and its associated factors. The mean (\pm SD) age of women was 25.31 (\pm 6.70) years. More than half of the mothers 128 (55.41%) had no formal education; 108 (46.75%) of them were from rural area; and 50 (21.65%) parity 5 and more times. Vertical transmission proportion at delivery was, 104/231 (45.02%; 95% CI; 38.49, 51.68). Vertically transmitted at birth from a total of still birth and live birth were 31/59(52.54%) and 73/172 (42.44%) respectively. The mean (\pm SD)

gestational age and the mean (\pm SD) birth weight of the newborn was 37.89 week (\pm 2.21) and 3022.14 gm (\pm 581.69), respectively. Among 231 newborns 30 (12.99 %) of them were born preterm ($<$ 37 weeks' gestation). Of these 104 colonized birth, 39(37.50%) had been exposed to IAP and 65 (62.50%) had no exposure to IAP. Of these, 39 IAP exposed, 28(71.80%) were exposed $<$ 4 hours and 11(28.20%) were exposed \geq 4 hours. Of all newborns, those exposed to IAP \geq 4 hours were not colonized. However, all newborns those exposed to IAP $<$ 4 hours were colonized (Table 1).

Table 1: Characteristics of the study participants by vertical transmission at birth Harar, Eastern Ethiopia, 2016 (n= 231)

Characteristics	GBS test results at delivery		
	Total	Negative (n, %)	Positive (n, %)
Age of mothers (in year)			
15-26	124(53.68)	71(57.26)	53(42.74)
26-46	107(46.32)	56(52.34)	51(47.66)
Residence			
Urban	123(53.25)	65(52.85)	58 (47.15)
Rural	108(46.75)	62(57.41)	46 (42.59)
Educational level of mothers			
Illiterate	128(55.41)	73(57.03)	55 (42.97)
Literate	103(44.59)	54(52.43)	49 (47.57)
Parity			
Primi	78 (33.77)	43(55.13)	35(44.87)
1-4	103(44.59)	54(52.43)	49(47.57)
\geq 5	50 (21.65)	30(60.00)	20(40.00)
Term Pre-labour Rupture of the membrane			
No	64 (27.71)	53(82.81)	11(17.91)
Yes	167(72.29)	74(44.58)	93(55.69)
Rupture of membrane \geq 18 hs			
No	64 (27.71)	50(78.13)	14(21.88)
Yes	167(72.29)	77(46.11)	90(53.89)

Fever during labour				
	No	136(58.87)	94(69.12)	42(30.88)
	Yes	95(41.13)	33(34.74)	62(65.26)
Preterm Pre-labour Rupture of the membrane				
	No	207(89.61)	116(56.04)	91(43.96)
	Yes	24 (10.39)	11(45.83)	13(54.17)
Gestational age				
	≥37 weeks	201(87.01)	112(55.72)	89(44.28)
	<37 weeks	30 (12.99)	15(50.00)	15(50.00)
APGAR score at fifth minute				
	≥7	136 (57.14)	78(57.35)	58(42.65)
	<7	95 (42.86)	49(51.58)	49(51.58)
IAP				
	Yes	131 (56.71)	92(70.23)	39(29.77)
	No	100 (43.29)	35(35.00)	65(65.00)
Time at which IAP before delivery				
	< 4hour	28 (100.00)	0	28 (21.37)
	≥ 4 hours	103 (88.63)	92 (89.32)	11(10.68)
Women received IAP				
	No/ inadequate	128 (100.00)	35(27.34)	93 (72.66)
	Adequate	103 (88.63)	92 (89.32)	11(10.68)
Still birth				
	No	172 (74.46)	99 (57.56)	73(42.44)
	Yes	59 (25.54)	28(47.46)	31(52.54)
Neonatal death within 6 hours				
	No	225(97.40)	124(55.11)	101(44.89)
	Yes	6 (2.60)	3(50.00)	3(50.00)

Predictors of vertical transmission

In univariable regression analysis, the following variables were associated with vertical transmission: pre labour rupture of membranes at term (Term PROM), prolonged ruptures of the

membranes (PROM) \geq 18 hours, birth from mothers who were febrile during labour and delivery, birth from mothers who received adequate IAP (Table 2)

To estimate the relative contribution of each factor to vertical transmission of GBS, adjusted prevalence ratio (APR) was derived from Log binomial regression models. Term PROM [APR= 1.93; 95% CI; (1.04,3.57)] , PROM [APR =1.76;95% CI;(1.13, 2.74)] , intrapartum maternal fever [APR= 1.40; 95% CI; (1.13, 1.75)] were significantly associated with vertical transmission. Adequate IAP received (\geq 4 hours) mother compared to received no and received inadequate ($<$ 4 hours) decreases the prevalence ratio of vertical transmission by 83% [APR=0.17 ; 95% CI:(0.09, 0.30)] (Table 2).

Table 2: Results of Bivariable and Multivariable log binomial model on factors associated with vertical transmission of GBS at birth in Harar, Eastern Ethiopia, 2016

Characteristics	Total	Crude PR		Adjusted PR	
		PR	[95% CI]	PR	[95% CI]
age of mothers in year					
15-25	124(53.68)	1	1		
26-49	107(46.32)	0.89	0.67, 1.19	0.99	0.81, 1.12
Term Pre-labour Rupture of the membrane					
No	64 (27.71)	1		1	
Yes	167(72.29)	3.24	1.86, 5.64*	1.93	1.04, 3.57*
Rupture of membrane \geq 18 hs					
No	64 (27.71)	1		1	
Yes	167(72.29)	2.46	1.51, 3.99*	1.76	1.13, 2.74*
Fever during labour					
No	136(58.87)	1		1	
Yes	95(41.13)	2.11	1.57, 2.82*	1.40	1.13, 1.75*
Still birth					
No	172 (74.46)	1		1	
Yes	59 (25.54)	1.23	0.91, 1.66	1.12	0.86, 1.38
Women received IAP					
No/ inadequate	128 (55.41)	1		1	
Adequate	103 (44.59)	0.14	0.08, 0.25*	0.17	0.09, 0.30*
Preterm Pre-labour Rupture of the membrane					
No	207(89.61)	1			
Yes	24 (10.39)	1.23	0.82, 1.83		
Gestational age					

≥37 weeks	201(87.01)	1	
<37 weeks	30 (12.99)	1.12	0.76, 1.66
Educational level of mothers			
Illiterate	128(55.41)	1	
Literate	103(44.59)	0.9	0.67, 1.20
APGAR score at fifth minute			
≥7	136 (57.14)	1	
<7	95 (42.86)	1.13	0.85, 1.50

*≤ p, 0.05, PR= Prevalence Ratio IAP= Intrapartum Antibiotic prophylaxis

Discussion

In this study, vertical transmission of GBS from colonized mothers at birth was 45.02%. Factors that were significantly associated with vertical transmission were term pre-labour rupture of the membrane, prolonged rupture of the membrane ≥ 18 hours before delivery; mothers had fever during labour and mother received adequate IAP.

The vertical transmission rate reported in this study was consistent with studies from other low income countries [4, 10, 18]. However, it was clearly higher than those reported from high income countries where a guideline for GBS prevention is commonly available [9, 11]. These discrepancies might be also due to vary in demographic characteristics, geographic location, and service availability [4]. High rate of vertical transmission contribute for high neonatal and maternal morbidity and mortality due to GBS. Vertical transmission of GBS is preventable, health care providers and policy makers need to give consideration in their maternal and neonatal mortality reduction strategies [5].

Pre labour rupture of membranes at term (Term PROM) was also reported to have increased the risk of vertical transmission.[12] Group B *Streptococcus* infection of the choriodecidua induces dysfunction of the cytokeratin network in amniotic epithelium resulting in membrane weakening cause early rupture of the membrane [27]. Rupture of the membrane leaves the fetus and the amniotic fluid, which is a good media for bacterial proliferation, unprotected and thus increases the risk of infection.

Prolonged rupture of membranes (≥ 18 hours) was also reported to have increased the risk of vertical transmission in GBS colonized women [28]. Prolonged exposures of fetus to maternal

bacterial flora without any protective membrane barrier enhance the transmission which can result in colonization of the fetus in the uterus in the absence of timely preventive measures [29].

Women with both microbial invasion of the amniotic cavity and acute histologic chorioamnionitis had the highest intra-amniotic inflammatory response, which was mediated by interleukin-6 concentrations. Fever is one of inflammatory response manifestation and increases the risk of neonatal colonization [11, 12, 30]. During the delivery process once the fetus is colonized by GBS it is highly likely to develop pneumonia and/or sepsis [31]. GBS can be isolated from cord blood and lung aspirates and the GBS bacterial pigment/ lipid toxin cause hemolysis or pyroptosis that exacerbates fetal damage and increases the risk of fetal loss [32].

This study found out that mother received adequate IAP was significantly associated with decreased risk of vertical transmission, which is consistent with studies conducted in elsewhere [33]. IAP help to prevent GBS vertical transmission by reducing bacterial load of rectum and vagina and thus preventing GBS from ascending and proliferating in the fetus and amniotic fluid compartment [34]. In low utilization of maternal care and resource-poor settings countries, feasibility of adequate IAP is challenging [19]. Antisepsis at delivery has been revealed to be ineffective [35]. However, maternal vaccination may provide a feasible strategy to reduce GBS disease in low utilization of maternal care and resource-poor countries [36].

All newborns, those exposed to IAP \geq 4 hours in this study found out that not colonized. However, all newborns those exposed to IAP $<$ 4 hours are colonized. Sufficient antibiotic levels in the fetal bloodstream during labor are required to prevent fetal and neonatal infection. Four hours of IAP was needed to reduce the number of women with positive GBS cultures [15]. GBS vaginal colony counts decrease by 5-fold within administering 2 hours of intravenous penicillin G and by 50-fold within 4 hours [34]. Delay in administering IAP due to factors such as late presentation to the labour ward, precipitous delivery or misidentification of risk factors can result in inadequate IAP cover [19].

In Ethiopia 34.3% neonatal mortality was due to infections [37]. The proportion of GBS related neonatal death is not investigated. High proportion, 72% of women was giving birth at home [21]. Maternal knowledge on the risk factors was low [38]. Furthermore, there is no clear guideline for prevention strategies to specific GBS vertical transmission. The results of this study

can inform current initiatives that are focused on improving maternal and newborn health care delivery system, the magnitude of the problem and calls for the necessary actions in order to strengthen efforts to reduce neonatal mortality.

To our knowledge, this is the first study to present vertical transmission of GBS. We used questionnaire that was adapted from WHO and EDHS pregnancy and pregnancy outcome indicators. Data collectors were thoroughly trained. Specimen collection and interviews were conducted in private places to ensure the privacy. Although some studies collected swabs from vagina only to identify GBS colonized mothers, we have collected from both rectum and vagina that increase the detection rate [11, 39]. Moreover, some studies collect swabs from a single anatomic site of newborn to identify vertical transmission, we collected from ear canal, umbilical area, axilla, groin or nose to identify vertical transmission [9]. To preserve better viability and stability of GBS, specimens transported to the laboratory, inoculated into broth media and incubated within 4 hours of specimen collection. Log binomial regression was used to estimate prevalence ratio of vertical transmission. It is appropriate for such common prevalent binary outcome. However, odds ratios (ORs) that over-estimates when the outcome is not rare rather than PRs were the most frequently reported [11].

This study was not without limitations. Interviewer and recorder bias might occur during the interviews and recording. However, supervisor and principal investigators rechecked for the completeness of data before the mother and newborn discharged and their card returned back to the main record office. Measurement and diagnostic bias might have also occurred during measurement and diagnoses however, two professional involved in measuring and reading of the results. Moreover, in case of disagreement investigators also involved for decision. Serotyping and PCR test were not done that characterize the accordance of GBS serotypes form the mother and newborn. Because the study was done in health facilities, the findings may not be inferred to the entire community

In conclusion, this study the prevalence of vertical transmission of GBS was found to be high. However, pregnant women received adequate IAP was very low. Initiating and strengthening GBS screening and introducing timely IAP are necessary. Furthermore, maternal vaccination may provide a feasible strategy to reduce vertical transmission.

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Authors' contribution

TA, AW, YB, GE, and BS conceived and designed the paper, involved in data collection, performed the statistical analysis, interpret the results wrote and reviewed the manuscript. DM performed laboratory work and interpret the laboratory results. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests

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Paper III

Maternal Group B *Streptococcus* Recto Vaginal Colonization increases the Odds of Stillbirth: Evidence from eastern Ethiopia

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Abstract

Background: Group B Streptococcus (GBS) causes a significant number of stillbirths. Despite this, there is little documented information on the association between stillbirth and pregnant women's GBS recto vaginal colonization in Sub Saharan Africa. As such, this study was aimed at identifying the association between stillbirth and pregnant women's GBS recto vaginal colonization in Eastern Ethiopia.

Methods: A health facility-based cross sectional study was conducted among 1688 pregnant women who came for delivery services in Harar town, Eastern Ethiopia from June to October in 2016. Data were collected using a pre-tested structured questionnaire and check list for clinical record. Group B *streptococcus* (GBS) positivity of the pregnant women was confirmed by culture of recto vaginal swab using selective media. The association between GB streptococcus colonization and stillbirth was examined using multivariable logistic regression analysis. Level of statistical significance was declared at P value < 0.05.

Results: Of the 1688 pregnant women who participated in the study, 144 had stillbirths, representing a proportion of 8.53% [(95% CI: (7.19, 9.86)]. GBS colonization at birth was detected in 231 (13.68%; 95% CI 12.04, 15.32). Of these 144 stillbirths 59 (40.97%) were from colonized mothers, 72(59.03%) were from non-colonized mothers. Of these 59 stillbirth from colonized mothers, 32(54.23%) were intrapartum stillbirth, 27(45.77%) were antepartum stillbirth occur before exposed to intrapartum antibiotic prophylaxis (IAP). After controlling for potential confounders, the odds of having a stillbirth was 9.08 times higher among recto vaginal GBS colonized pregnant women [AOR= 8.93; 95% CI; (5.47, 14.56)].

Conclusions: This study demonstrated significant association between maternal recto vaginal GBS colonization and stillbirth. Efforts to reduce stillbirth need to consider prevention of GBS colonization among pregnant women.

Key words: stillbirth, GBS, maternal recto vaginal colonization, Harar, Ethiopia

Background

The prevention of stillbirth remains a major global challenge. Of the estimated 2.6 million stillbirths that occur globally every year, 98% occur in low-income and middle-income countries [1]. Efforts to reduce stillbirths in low-income countries have not shown much progress. Sub-Saharan Africa harbors the highest stillbirth rates with Ethiopia ranking fifth among the ten countries with the highest stillbirth rates in the world [1, 2].

Stillbirth is a major cause of psycho-social distress, grief and guilt to families[3]. In sub-Saharan Africa, mothers who had stillbirth are often subjected to severe psychosocial pressure; especially women who experience repeated stillbirth can be outcasted, dishonored and subjected to divorce [4, 5].

The causes of stillbirth varies greatly from country to country, in low income countries in general about one-third of all stillbirths are attributed to maternal infection during pregnancy[6]. Group B *Streptococcus* colonization and vertical transmission can cause fetal infection and stillbirth as a direct result of toxin-induced cytolysis [7, 8]. GBS is estimated to account for 15% of all infection related stillbirths globally [9] and Africa harbor 73.78% of the estimated GBS caused stillbirth[10]. Other factors associated with stillbirth occurrence include higher maternal age, residence, lower economic status, lower education level, poor antenatal care uptake, antepartum hemorrhage, essential hypertension, pre-eclampsia, obstetric complication during labor, preterm delivery, low birth weight, and fetal mal-presentation[11, 12].

Group B *Streptococcus*-caused stillbirths are preventable [9, 13]. However, most of Sub-Saharan African (SSA) countries do not have guidelines for prevention of GBS infection and the subsequent associated health risks to the mother and fetus [14]. The inaction could be related to a lack of evidence from the region. As such, this study assessed the association between GBS colonization and stillbirth in Eastern Ethiopia.

Materials and Method

Study area: This study was performed in three health facilities (Hiwot Fana Specialized University Hospital, Jugal Hospital and Arategna Health Centers) in Harar town, Eastern Ethiopia. Harar is located 510 km east of the capital city of Ethiopia, Addis Ababa. Ethiopia is a low-income country with a total fertility rate of 4.6/woman[15], stillbirth rates of 25.5/1000 birth[11], has a neonatal mortality rate of 29/1000 live births[15], with infections accounting for 34.3% of neonatal deaths [16].

Study design and sampling: A health facility based cross-sectional study was conducted between June and October 2016. The study subjects were pregnant women admitted to labor and delivery rooms at selected health facilities. This study is part of a larger study conducted to assess GBS colonization among a total 1688 pregnant women. Women who took antibiotics in the last two weeks during their pregnancy prior to specimen collection, pregnant women with antepartum hemorrhage, women who were physically or mentally incapable of responding, and women with multiple pregnancies were excluded from the study [17]. Samples were taken proportional to the client volume of the health facilities.

Data collection:

Data were collected using a pretested and structured questionnaire. In addition, checklist was used to extract data for medical record, and laboratory test was done to detect GBS colonization from recto-vaginal swab. The data collection tools were developed by reviewing previous related data collection instruments[15, 18]. The questionnaire was initially prepared in English, translated into the local language, “Afaan Oromo” and “Amharic”, by language experts, back translated into English to check for consistency.

A six-day training was given to midwives and the supervisors who served as data collectors. The training for data collectors and supervisors addressed issues related to data collection procedures and tools, interviewing techniques, medical record review, and specimen collection procedures. For the laboratory work, two medical microbiologists (to read, confirm and interpret test results), and three medical laboratory technologists (to assist in media preparation and sterilization processes) were involved. A research manual was prepared and implemented during training, data collection, and data management.

Measurement

Stillbirth was the dependent variable in this study and obtained from medical records. It was labeled as “yes” and “no” with a code of 1 and 0, respectively. ANC follow-up, hypertensive disease, women’s GBS test results, prolonged labour, birth weight, and gestational age were independent variables in this study.

Age of the mother was recorded as reported in medical records and later grouped as 15-19, 20-24, 25-29 and 30-34 and 35+ with code 1, 2, 3, 4 and 5 respectively for analysis. Maternal education was grouped as “illiterate” for those who could not read and write and all others as “literate” and was coded 1 and 0, respectively. Number of birth (parity) was grouped as one primiparity (one delivery), multiparous (2-4 delivery), and grand multiparous (≥ 5 delivery) and code with 1, 2 and 3 respectively[19]. To determine the economic status of the families, wealth index was used. The wealth distribution was generated by applying principal components analysis to 33 household variables. The wealth status was determined by using three groups, poor as 1, middle as 2, and better as 3. [20].

Hypertensive disease and prolonged labour was obtained from medical record and labeled as “yes” and “no” and coded with 1 and 0 respectively. Birth weight was categorized as < 2500 gram and ≥ 2500 gram and labeled as “yes” and “no” and code with 1 and 0 respectively. Similarly gestational age was categorized as < 37 weeks and ≥ 37 weeks and labeled as “yes” and “no” and code with 1 and 0 respectively.

The specimen collection, transportation and processing was done following the Center of Disease Control (CDC) and Standard Operating Procedures [21, 22]. This study is part of our larger study conducted to assess recto-vaginal GBS colonization among women and detailed laboratory procedures are described in our previously published article [23]. In brief, all specimens were collected by midwives using sterile cotton applicators from both vagina and rectum at admission to the labour and delivery room of the study health facilities. Soon after collection, the specimens were placed in sterile screw-capped test tubes and transported to the Microbiology Laboratory of the Department of Medical Laboratory Sciences, College of Health and Medical Sciences,

Haramaya University within 4 hours of collection in cold chain (+4°C). A positive GBS reading was labeled as “yes” and a negative reading was labeled as “no”.

Statistical analysis

Initially crude odds ratios (CORs) along with 95% confidence intervals were estimated to assess the association between each independent variable and the outcome variable. Variables with p-value ≤ 0.20 in the bivariable analyses were considered in the multivariable logistic regression model. Important variables deemed to be considered were also included, though they did not reach a p-value less than 0.20. The explanatory variables were tested for multicollinearity before entering them into the multivariable model using the Variance Inflation Factor (VIF) test, the tolerance test, and values of the standard error. No multicollinearity problem was found. Correlation matrix and covariate matrix was tested for the final model. Log likelihood ratio test and Akaike's information criterion and Bayesian information criterion was used to select final model. The Hosmer-Lemeshow goodness-of-fit tests were used to test for model fitness[24]. The logistic regression model was used to assess the association between women's GBS recto vaginal colonization and the outcome variable (stillbirth) by controlling for other potential confounding variables. Adjusted Odds Ratio (AOR) along with 95% confidence interval was estimated to assess the strength of the association, and a p-value < 0.05 was used to declare the level of statistical significance.

Ethics approval and consent to participate

The study was reviewed and approved by the Institutional Health Research Ethics Review Committee of the College of Health and Medical Sciences at Haramaya University. Written consent was obtained from each participating woman before commencement of data collection. Participants under the age of 18 provide their assent in addition to written consent of their parent or legal guardians. To maintain confidentiality, interviews and specimen collection were conducted in a separate room and all information obtained in this study was handled with anonymity. Women detected to have GBS colonization were provided antibiotic prophylaxis, women who had stillbirth received counseling.

Result

The study enrolled a total of 1688 pregnant women. Almost all of were married (95.73%), 47.04% had no formal education, 76.18% were Muslim in religion, and 44.14% were rural residents. The mean age and standard deviation of the women was 26.59 ± 5.63 years. GBS colonization at birth was detected in 231 (13.68%; 95% CI 12.04, 15.32). Of the 1688 pregnant women who participated in the study, 144 had stillbirths, representing a proportion of 8.53% [(95% CI: (7.19, 9.86)]. Of these 144 stillbirths 59 (40.97%) were from colonized mothers, 72(59.03%) were from non-colonized mothers. Of these 59 stillbirth from colonized mothers, 32(54.23%) were intrapartum stillbirth, 27(45.77%) were antepartum stillbirth occur before exposed to intrapartum antibiotic prophylaxis (IAP) (Table 1).

Table 1: Characteristics of the study participants Harar town, Eastern Ethiopia, 2016

Characteristics	Frequency (n)	Percent (%)
Residence of pregnant women		
Urban	943	55.86
Rural	745	44.14
Age in year		
≤19	301	17.83
20-24	510	30.21
25-29	461	27.31
30-34	275	16.29
≥35	141	8.35
Ethnicity		
Oromo	1,214	71.92
Amhara	267	15.82
Harari	95	5.63
Somali	15	0.89
Other*	97	5.75
Religion		
Muslim	1,286	76.18
Orthodox	308	18.25
Protestant	89	5.27
Catholic	5	0.3
Marital status		
Married	1,616	95.73
others **	72	4.27
Educational status		
Literate	894	52.96
Illiterate	794	47.04
Para		
1	722	42.77

2-4	755	44.73
≥5	211	12.50
ANC follow up		
No	561	33.23
Yes	1,127	66.77
Gestational age		
≥37 weeks	1,540	91.23
<37 weeks	148	8.77
GBS test result		
Negative	231	13.68
Positive	1457	86.32
IAP received		
No	964	57.11
Yes	724	42.89

Others* Gurage, Tigre, Silte,...; others** single, separated, divorced, widowed; IAP intrapartum antibiotic prophylaxis

The multivariable logistic regression analysis showed a strong association between stillbirths and recto vaginal GBS colonization [AOR= 8.93; 95% CI; (5.47, 14.56)]. Other factors that were significantly associated with stillbirth included having ANC follow-up [AOR =0.53; 95% CI ;(0.34, 0.82)], having hypertensive disorders [AOR= 4.66; 95% CI; (2.77, 7.84)], having prolonged labor [AOR=3.65; 95% CI :(2.38, 5.60)], low birth weight [AOR=1.81; 95% CI :(1.09, 3.03)], and preterm delivery [AOR=4.24; 95% CI :(2.41, 7.45)] (Table 2)

Table 2: - Factors associated with stillbirth among pregnant women, Harar, Eastern Ethiopia, 2016

Characteristics	Stillbirth n=144(%)	Live birth n=1544(%)	Crude OR			Adjusted OR		
			OR	95% CI		OR	95% CI	
Women GBS test results								
Negative	5.83	94.17	1			1		
		74.46		3.8			14.5	
Positive	25.54		5.53	3	7.99	8.93***	5.47	6
ANC follow up								
No	15.15	84.85	1			1		
		94.76		0.2				
Yes	5.24		0.3	1	0.43	0.53**	0.34	0.82
Anemia								
No	7.88	92.12	1			1		
		84.97		1.2				
Yes	15.03		2.06	7	3.34	0.79	0.42	1.45
Hypertensive disorder								
No	6.32	93.68	1			1		
		73.66		3.5				
Yes	26.34		5.29	9	7.79	4.66***	2.77	7.84
Prolonged labour								

No	6.10	93.90	1			1		
		83.42		2.1				
Yes	16.58		3.06	5	4.34	3.65***	2.38	5.60
Para								
1	8.17	91.83	1			1		
		91.79		0.6				
2-4	8.21		1.00	9	1.45	1.46	0.88	2.42
		89.10		0.8				
≥5	10.90		1.31	2	2.28	1.29	0.58	2.92
Age of women								
≤19	10.63	89.37	1			1		
	6.86	93.14		0.3				
20-24			0.6	7	1.02	1.11	0.60	2.05
	9.33	90.67		0.5				
25-29			0.86	3	1.4	1.10	0.58	2.11
	5.82	94.18		0.2				
30-34			0.51	7	0.96	0.42	0.19	0.95
	12.77	87.23		0.6				
≥35			1.23	6	2.27	0.76	0.30	1.92
Resident								
Urban	5.20	94.80	1			1		
		87.25		1.8				
Rural	12.75		2.66	6	3.81	1.27	0.69	2.33
Educational status								
literate	6.26	93.74	1			1		
		88.92		1.3				
illiterate	11.08		1.86	1	2.64	1.13	0.69	1.83
Wealth status								
Poor	14.03	85.97	1			1		
Middle	7.00	93.00	0.46	.30	0.68	1.06	0.58	1.91
		95.55		0.1				
Better	4.45		0.28	7	0.45	0.65	0.29	1.44
Birth weight								
≥2500 g	5.98	94.02	1			1		
		77.82		3.1				
<2500 g	22.18		4.48	1	6.44	1.81*	1.09	3.03
Gestational age								
≥37 weeks	5.97	94.03	1			1		
		64.86		5.7				
<37 weeks	35.14		8.52	2	12.69	4.24***	2.41	7.45
IAP received								
No	7.37	92.63	1			1		
		91.47		1.0				
Yes	8.53		1.41	0	1.98	1.01	0.67	1.51

IAP, intrapartum antibiotic prophylaxis; OR, odds ratio; CI confidence intervals; * = p, 0.05; ** = p, 0.01; *** = p, 0.001

Discussion

This study showed a strong association between stillbirths and maternal GBS colonization, even after controlling for other potential confounders. Other studies have also reported similar findings [25, 26]. It is evident that Group B streptococcus cytolysis breaches maternal-fetal barriers to

cause intrauterine fetal death[7]. The stillbirths due to recto vaginal colonization of pregnant women are due to ascending infection. The genome sequencing studies have demonstrated the GBS isolated at birth from the skin of newborns were genetically identical to maternal GBS colonizing isolates[25].

Intrapartum antibiotic prophylaxis (IAP) is highly effective in preventing newborn's GBS colonization[27]. However, the effectiveness of IAP in reducing stillbirth is not highly effective indicating any antibiotic prophylaxis need to be administered before a potential exposure as stillbirth may occur before labour starts [28]. In this study, 50.69% of stillbirths occurred from GBS colonized women not exposed to IAP.

Furthermore, identifying pregnant women who need IAP has been challenging for most low- and middle-income countries (LMICs) countries since most pregnant women did not attend antenatal care and most deliveries occur at home[29]. However, recent achievements in increasing coverage for antenatal care and institutional/facility delivery could improve the possibility of introducing screening, prevention and treatment interventions in routine ANC and delivery services.

Maternal vaccination could also be cost-effective prevention strategy since it can induce population-level herd immunity that is not achievable through IAP [30, 31]. Maternal vaccination could protect the fetus from invasive disease in utero. A trivalent GBS polysaccharide-protein conjugate vaccine was recently evaluated in phase 2 clinical trials among pregnant women[30]. The vaccine was well tolerated and induced capsular-specific antibody responses, in non-pregnant and pregnant women. Maternal vaccination led to higher GBS serotype-specific antibody concentrations in infants than did placebo, with both interventions resulting in similar safety profiles [32].

The burden of stillbirth on families, especially women, is severe and long lasting. The associated stigma and taboo hinder mothers from seeking care timely even in high-income countries. Mothers are unlikely to seek medical care for stillbirth and thus the condition could go unreported.[33]. Since women coming to health facilities for delivery could be those with better access to health services and perhaps more aware of the benefits of seeking health services for improved newborn health, the occurrence of stillbirth is likely to be an underestimation. One could

also argue those women who opted for institutional delivery could be those with health problems the reported stillbirth represents an overestimation.

Antenatal care (ANC) follow up was one of the predictors' of stillbirth. Similar findings were reported in other studies conducted in different part of the world [11, 34]. Although, early diagnose and treatment of risk factors, counseling on maternal danger sign and advantage of health facilities delivery by skilled health professionals important to reduce fetal, maternal and child morbidity and mortality, recommended four times ANC coverage (32%) in Ethiopia is very low [15].

In this study preterm delivery is significantly associated with stillbirth, and this is consistent with previous study[34]. Furthermore, GBS ascending infection, resulting in infection of fetal membranes, decidua and fetus causing premature rupture of the membrane. Prolonged exposure of fetus to maternal bacterial flora without any protective membrane barrier enhances the transmission. Host inflammatory response stimulation of prostaglandin and protease synthesis are increases uterine contractility and results in preterm delivery[35]. Vascular insufficiency in pre-eclampsia reduces blood flow to the placenta, leading to hypoxia of the fetus may be associated with stillbirth [36, 37]. In prolonged long labor, there is also an increased chance that a fetus will suffer from asphyxia may be due to umbilical cord compression, placental abruption, maternal low blood pressure and birth trauma. Problems with the placenta pose risks to the fetus, including lack of oxygen and nutrients, which can impair fetal growth could lead to low birth weight and preterm delivery [12, 38].

The strength of our study derives from the inclusion of a relatively high number of participants, and the study's practice of obtaining swab samples from both the rectum and vagina rather than only one in order to improve the yield [39]. All study samples were transported to the Laboratory of our University Bacteriology Research unit within 4 hours [40]. We chose selective media standard recommended by CDC [22].

We tried to minimize measurement and diagnostic biases by assigning two professionals to measure and read the results. Thus, we believe the procedures used have helped to reasonably

estimate the association. One of our limitations is we did not collect microbiological evidence of invasive GBS disease from a normally sterile site such as fetal blood from the umbilical cord or from the heart, lung aspirate, cerebrospinal fluid, or fetal tissues as recommended [41] due to resource constraints. In addition, unaccounted and residual confounding could have effect on the association [42, 43].

In conclusion, this study identified strong association between maternal recto vaginal colonization of GBS and stillbirth. Efforts to reduce stillbirth need to consider prevention of colonization by GBS among pregnant women. Further stillbirth reduction efforts need to consider introducing effective prevention strategies and improving the quality ANC and intrapartum care.

Abbreviations

GBS: Group B streptococcus; ANC: antenatal care; CDC: center for disease control; SOPS: Standard Operating Procedures; SDG: sustainable development goal; IAP: Intrapartum antibiotic prophylaxis; LMICS: low-and-middle income countries

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contribution

TA, AW, GE, BS and YB conceived and designed the paper, involved in data collection, performed the statistical analysis, interpret the results, wrote and reviewed the manuscript. DM

interprets the result, wrote and reviewed the manuscript. All authors read and approved the final version of manuscript before submission.

Ethics approval and consent to participate

The study was reviewed and approved by the Institutional Health Research Ethics Review Committee of the Colleges of Health and Medical Sciences, Haramaya University. Written consent was obtained from each woman before commencement of data collection. Participants under the age of 18 provide their assent in addition to written consent of their parent or legal guardians. To maintain confidentiality, interviews and specimen collection were conducted in a separate room and all information obtained in this study was handled with anonymity. Colonized women provided antibiotic prophylaxis and neonate with health problems were treated and immediate referral was also facilitated.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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Erythromycin and clindamycin susceptibility pattern and associated factors of *Streptococcus agalactiae* among pregnant women in Harar, eastern Ethiopia

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Abstract

Background: Erythromycin and clindamycin use as alternative intrapartum prophylaxis for pregnant women colonized with Group B *Streptococcus* and allergic to β -lactam antibiotics. However, the emergence of erythromycin and clindamycin resistant Group B *Streptococcus* is a growing problem. There is a limited evidence of antibiotic susceptibility patterns in low-income countries. Therefore, this study aimed to determine erythromycin and clindamycin susceptibility pattern and associated factors of Group B *Streptococcus* isolates from pregnant women in Eastern Ethiopia.

Methods: A cross sectional study among Group B *Streptococcus* recto vaginal colonized pregnant women was conducted in eastern Ethiopia. Antibiotic susceptibility testing was performed using disk diffusion method, and then by erythromycin inducible resistance to clindamycin by D-test method. The prevalence ratio along with 95% confidence level was estimated to determine factors associated with erythromycin and clindamycin resistance of Group B *Streptococcus* using Robust Poisson regression analysis. The level of statistical significance was declared as p value < 0.05 .

Results: Group B *Streptococcus* isolates retrieved from 231 recto-vaginal colonized pregnant women were used in this study. Of these, 56 Group B *Streptococcus* (24.24%) [95% CI: 18.86, 30.29] and 53 isolates (22.94%) [95% CI: 17.68, 28.91] were resistant of erythromycin and clindamycin, respectively. Nineteen isolates (8.2%) were resistance to both erythromycin and clindamycin. While, 37 (16.01%) were resistant to erythromycin but susceptible to clindamycin. The use of antibiotics in current pregnancy before two weeks of data collection showed significant association with erythromycin and clindamycin resistance [APR= 1.89; 95% CI: 1.21, 2.94] and [APR= 2.62; 95% CI: 1.66, 4.14], respectively.

Conclusion: In conclusion, the prevalence of erythromycin and clindamycin resistance was high and significantly associated with the use of antibiotics. Routine antibiotic susceptibility testing needed to be done before using clindamycin and erythromycin prophylaxis and treatment.

Keywords: GBS, Resistance, Disk diffusion, D-test, Harar, eastern Ethiopia

Introduction

Group B *Streptococcus* (GBS) (also called *Streptococcus agalactiae*) recto vaginal colonization in pregnant women is responsible for invasive GBS diseases in newborns. The transmission occurs vertically during pregnancy or childbirth and result in preterm delivery or stillbirth [1, 2]. Intrapartum antibiotic prophylaxis, administered to GBS colonized women, was 83.7% effective in prevention of vertical transmission [3]. Penicillin and ampicillin are the intrapartum antibiotic prophylaxis (IAP) of choice, but not recommended for penicillin allergic patients. The extreme rarity of non-susceptible of GBS has been reported, so that routine test is not necessary [4, 5].

Clindamycin and erythromycin are alternative antibiotics recommended for about 10.2% penicillin-allergic pregnant women [6, 7]. However, the prevalence of GBS isolates with resistance to erythromycin and clindamycin have increased over the last decade [8]. The distribution of erythromycin and clindamycin resistance GBS across the world is due to difference in socio-demographic and health care delivery systems. Furthermore, the increasing trend of erythromycin and clindamycin resistance reported in the last 20 years [9, 10]

More than fifteen percent of neonates exposed to IAP develop early onset of GBS diseases [11]. The effectiveness of clindamycin was about 22% [5, 12]. GBS resistance may have contributed to the limited effectiveness [12]. An infant who developed early-onset clindamycin-resistant GBS disease after his mother had received prophylactic clindamycin was reported [13]. However, almost all patients (91%) who received clindamycin and erythromycin did not have antibiotic sensitivity testing [14]. The identified risk factors were symptomatic sore throat, illness and exposure to the antibiotics, ribosomal modification, and erythromycin resistance induced clindamycin resistance [15, 16].

Screening to identify isolates and monitoring of antibiotic susceptibility pattern is important [17]. The reduction of neonatal infections in countries where there is clear guidelines for GBS screening, use IAP, monitoring of the emergence of erythromycin and clindamycin resistance, apply the appropriate antibiotic for penicillin-allergic pregnant women are reported [14, 18]. There is little information documented on resistance and associated factors to erythromycin and

clindamycin in Sub Saharan Africa. Therefore, this study was designed to identify GBS resistance to erythromycin and clindamycin in eastern Ethiopia.

Materials and Methods

Study setting

The study was conducted in Harar town, eastern Ethiopia. Harar town is located at 510 km from Addis Ababa, Ethiopia. The total population of the town is about 101,934. The town consist of six health facilities providing delivery services (four governments and two private hospitals) and four health centers. For this study, Hiwot Fana Specialized University Hospital, Jugal Hospital and Arategna Health Center were selected because they had the largest client load in their delivery services [19].

Study design and period

Health facility based cross-sectional study was conducted between June 2016 and October 2016. This study is part of a large study that was conducted to assess maternal GBS recto-vaginal colonization and vertical transmission. A total of 231 GBS bacteria isolated from recto-vaginal GBS colonized pregnant women were included in this study.

Data collection

Data were collected through interview administered questionnaire, checklist to extract from a medical record, and laboratory tests. The questionnaire and checklist were adapted from the Ethiopian Demographic and Health Survey (EDHS) and World Health Organization (WHO) indicators of health status of mother during labor, delivery and immediate newborn [20, 21]. The questionnaire was initially prepared in English and then translated into the local language, “Afaan Oromo” and “Amharic”, by language experts, and back into English to check its consistency.

Six day training was given to data collectors and their supervisors. For laboratory work, two medical microbiologists (to read, verify and interpret test results), and three medical laboratory technologists (assists in media preparation, inoculation and sterilization processes) were involved. A research manual was prepared for training, data collection, and data management.

Measurement

Erythromycin and clindamycin antibiotic susceptibility patterns were the dependent variables. Susceptibility testing was done using disk diffusion method and D-test. Clinical and laboratory standards institute (CLSI) criteria was used to determine the sensitivity, intermediate sensitivity and resistance pattern of antibiotics in the testing panel (Table 1) [4]. Sensitive, intermediate and resistance coded as 1, 2, and 3, respectively. For analysis purposes sensitive and intermediate recoded as 0 and resistances recoded as 1.

The main independent variables were age, educational status, resident, and history of abortion, anemia of the current pregnancy, and antibiotics use in the current pregnancy before two weeks of specimen collection. Age of the mother, recorded based on maternal response, later grouped as ≤ 20 , 21-30 and >30 with code 0, 1 and 2, respectively for analysis. Maternal education was grouped as “illiterate” for those who are unable to read and write and all others as “literate” and was coded 1 and 0, respectively. Antibiotics use in current pregnancy two weeks before data collection recorded as no and yes and was coded 1 and 0, respectively.

Specimen collection, isolation and antimicrobial susceptibility test

Specimen collection, transportation, and methods of GBS isolation were described in our previously published article [22]. Antibiotic susceptibility testing was done using disk diffusion method based on CLSI guideline [4]. In brief, bacterial suspension equivalent to 0.5 McFarland standards was swabbed onto Muller Hinton agar plates prepared with 5% (v/v) sheep blood. McFarland standards was prepared by mixing 2 ml of 1% sulfuric acid and 1.175% barium chloride to obtain a barium sulfate solution with 0.5 optical density, which provides a standard bacterial suspension. Then, erythromycin (15 μg) and clindamycin (2 μg) (Oxoid, Ltd, UK) were placed and incubated at 37 °C in a 5% CO₂ enriched environment for 20–24 hours. The zone inhibition was taken as the area showing no obvious, visible growth that can be detected with the unaided eye. The diameter of the zone of inhibition was measured using digital caliper to the nearest millimeters and interpreted as sensitive to erythromycin if zone of inhibition was ≥ 21 or intermediate if 16–20 or resistance if ≤ 15 , whereas sensitive to clindamycin if the zone of inhibition was ≥ 19 or intermediate if 16–18 or resistance if ≤ 15 mm in diameter [4]. The

measurement of the diameter was made from the back of the plate illuminated by reflected light, against a dark background.

All GBS isolates resistant to erythromycin but susceptible to clindamycin was also tested for inducible clindamycin resistance using double disk diffusion (D-test). Disks of erythromycin (15 µg) and clindamycin (2 µg) were placed approximately 12 mm apart on Muller-Hinton agar plates following inoculation with bacterial suspension. Over or under dilutions of bacterial suspension was maintained comparing with a 0.5 McFarland standard. After overnight incubation at 37 °C in a 5% CO₂ enriched environment, inducible clindamycin resistance by erythromycin was detected by a blunting of the clindamycin zone closest to the erythromycin disk, giving the appearance of a “D” shape. Blunting was defined as growth within the clindamycin zone of inhibition proximal to the erythromycin disk, indicating inducible clindamycin resistance [4, 16] (Table 1).

Quality Control

New batches of Mueller Hinton medium were tested to ensure that the batch is satisfactory for growth of organisms before use. Stock antibiotic disks were stored at –20 °C. Disks were allowed to reach room temperature before opening for use to prevent condensation and possible loss of potency. The performance quality of erythromycin and clindamycin disks were checked using *Streptococcus pneumoniae* (ATCC® 49619) and *Streptococcus agalactiae* (ATCC® 12403) reference strains. The zone of inhibition of erythromycin and clindamycin was within the established quality control limit (25-30 mm for erythromycin and 19-25 mm in diameter for clindamycin) (Table 1) [4]. All used reference strains were obtained from the Ethiopian Public Health Institute. A manufacturer’s instruction and a standard procedure developed by CLSI were strictly followed in order to optimize bacterial growth conditions so that the inhibition of growth is attributed to the antibiotic agent and the effects of nutrient limitations, temperature differences or other environmental conditions are eliminated.

Table 1: Antimicrobial zone diameter interpretive criteria and inducible clindamycin resistance test of Group B Streptococcus

Antimicrobial agent	Disk content	Zone Diameter Interpretive Criteria (nearest to whole mm)		
		S	I	R

Erythromycin	15 µg	≥21	16-20	≤15
Clindamycin	2 µg	≥19	16-18	≤15
Penicillin G	10µg	≥24	-	-
Ampicillin	10µg	≥24	-	-
Ceftriaxone	30 µg	≥24	-	-
Vancomycin	30 µg	≥17	-	-
Ciprofloxacin	5 µg	≥21	16-20	≤15
Norfloxacin	10 µg	≥17	13-16	≤12
Test Method	Inducible Clindamycin Resistance			
Medium	Muller Hinton agar supplemented with sheep blood (5% v/v)			
Antimicrobial Concentration	15-µg erythromycin disk 2-µg clindamycin disk spaced 12 mm apart			
Inoculum	Direct colony suspension, equivalent to a 0.5 McFarland standard, using colonies from an overnight (18 to 20 hours) sheep blood agar plate			
Incubation Conditions	35°C ± 2°C; 5% CO ₂			
Incubation Length	20–24 hours			
Results	Flattening of the zone of inhibition adjacent to the erythromycin disk (referred to as a D-zone) = inducible clindamycin resistance Hazy growth within the zone of inhibition around clindamycin = clindamycin resistance, even if no D-zone is apparent			
Quality Control (QC) recommendations	<i>S. pneumoniae</i> ATCC* 49619 for routine QC of erythromycin and clindamycin disks			

Source: CLSI performance standard, 2015, Pennsylvania, USA

Note: *S. aureus* ATCC* American Type Culture Collection; MHA, Mueller-Hinton agar; QC, quality control, mm = millimeter

Statistical analysis

Data were double entered and cleaned using Epi-Data Version 3.1 and analyzed using STATA version 14. Summary and proportions of the independent variables were computed against erythromycin and clindamycin resistant. Robust Poisson regression model was used to assess the

association between independent variables and the outcome variable (erythromycin and clindamycin susceptibility pattern). The Robust Poisson regression directly estimates the prevalence ratio (PR) in binary and common outcome variables when there is a convergence problem in Log binomial regression analysis [23]. Possible interactions between covariates were tested. The explanatory variables were tested for multicollinearity before entering them into the multivariable model using the Variance Inflation Factor (VIF) test, and values of the standard error. No multicollinearity problem was found. Correlation matrix and covariate matrix were tested for the final model.

Crude PRs with 95% confidence intervals (CIs) were estimated to assess the association between each independent variable and the outcome variable, and a p value was determined. Variables with a p-value less than 0.25 were considered for multivariable analysis. Important variables deemed to be considered were also included, though they did not reach a p-value less than 0.25. Adjusted prevalence ratio (APR) along with 95% CIs was estimated to assess the strength of the association, and a p-value < 0.05 was used to declare the level of statistical significance in the multivariable analysis.

Ethical considerations

The study was reviewed and approved by the Institutional Health Research Ethics Review Committee of the College of Health and Medical Sciences, Haramaya University. Written consent was obtained from each woman before commencement of data collection. Participants under the age of 18 provided their assent in addition to written consent of their parent or legal guardians. To maintain confidentiality, interviews and specimen collection were conducted in a separate room and all information obtained in this study was handled with anonymity. Colonized women provided antibiotic prophylaxis and neonate with health problems were treated and immediate referral was also facilitated.

Results

In this study, Group B Streptococcus bacteria retrieved from 231 recto- vaginal colonized pregnant women were used. Of these, resistant to erythromycin and clindamycin were observed in 56 isolates, (24.24%) [95% CI: 18.86, 30.29] and 53 isolates (22.94%) [95% CI: 17.68, 28.91], respectively. Nineteen isolates (8.2%) were resistant to both erythromycin and clindamycin. Of

the 231 isolates, 37 (16.01%) were resistant to erythromycin but susceptible to clindamycin. A clear, D-shaped zone of inhibition around the clindamycin disk was observed in 18 isolates while a D-shaped zone containing inner colonies growing up to the clindamycin disk was observed for 9 isolates. In addition, 7 isolates were clindamycin resistant and erythromycin susceptible, but were not inducible and showed no blunting of the clindamycin zone (Tables 2).

Table 2: Characteristics of the study participants' by erythromycin and clindamycin antibiotic susceptibility against GBS, Harar, Eastern Ethiopia, 2016

Characteristics	Total(n)	Erythromycin		clindamycin	
		Sensitive n (%)	Resistant n (%)	Sensitive n (%)	Resistant n (%)
Age of women (in years)					
≤20	89	68(76.4)	21(23.6)	66(74.16)	23(25.84)
21-30	101	72(71.29)	29(28.71)	79(78.22)	22(21.78)
>30	41	35(85.37)	6(14.63)	33(80.49)	8(19.51)
Residence					
Urban	123	96(78.05)	27(21.95)	89(72.36)	34(27.64)
Rural	108	79(73.15)	29(26.85)	89(82.41)	19(17.59)
Educational status					
Literate	103	82(79.61)	21(20.39)	76(73.79)	27(26.21)
Illiterate	128	93(72.66)	35(27.34)	102(79.69)	26(20.31)
History of abortion					
No	172	129(75)	43(25)	133(77.33)	39(22.67)
Yes	59	46(77.97)	13(22.03)	45(76.27)	14(23.73)
Anemia in current pregnancy					
No	174	128(73.56)	46(26.44)	137(78.74)	37(21.26)
Yes	57	47(82.46)	10(17.54)	41(71.93)	16(28.07)
Current pregnancy antibiotics use before two weeks					
No	160	129(80.63)	31(19.38)	135(84.38)	25(15.63)
Yes	71	46(64.79)	25(35.21)	43(60.56)	28(39.44)

Factors associated with erythromycin and clindamycin resistance

To evaluate the associated risk factors of erythromycin and clindamycin susceptibility against GBS, adjusted prevalence ratio (APR) was derived from Robust Poisson regression model. Antibiotics used in the current pregnancy before two weeks of data collection showed significant

association with erythromycin and clindamycin resistance [APR= 1.89; 95% CI: 1.21, 2.94] and [APR= 2.62; 95% CI: 1.66, 4.14], respectively (Table 3).

Table 3: Robust Poisson regression output of factors associated with erythromycin and clindamycin antibiotic susceptibility against GBS, Harar, Eastern Ethiopia, 2016.

Characteristics	Erythromycin		Clindamycin	
	APR	95% CI	APR	95% CI
Age of the women (in years)				
≤20	1		1	
21-30	1.24	0.77, 2.02	0.90	0.54, 1.49
31+	0.54	0.23, 1.24	0.64	0.22, 1.83
Residence				
Urban	1		1	
Rural	1.24	0.77, 2.02	0.64	0.38, 1.05
Educational status				
Literate	1		1	
illiterate	1.26	0.76, 2.07	0.84	0.51, 1.39
History of abortion				
No	1		1	
Yes	0.91	0.52, 1.57	1.05	0.61, 1.79
Anemia in current pregnancy				
No	1		1	
Yes	0.66	0.34, 1.27	1.42	0.86, 2.3
Use of antibiotics in the current pregnancy before two weeks of data collection				
No	1		1	
Yes	1.89	1.21, 2.94*	2.62	1.66, 4.14*

Note: * $p \leq 0.05$; GBS, group B Streptococcus; CI, confidence intervals; APR, Adjusted prevalence ratio.

Antimicrobial susceptibility pattern of GBS isolates

The susceptibility patterns of GBS isolated from pregnant women against eight antimicrobial agents are presented in Table 4. All GBS isolates were uniformly sensitive to penicillin, ampicillin, and vancomycin. Relatively, GBS showed low resistance to ceftriaxone and high resistance to erythromycin and clindamycin with 24.24% and 22.94 %, respectively.

Table 4: Antimicrobial susceptibility patterns against GBS, eastern Ethiopia, 2016

Antimicrobials	Sensitivity pattern		
	Susceptible n(%)	Intermediate n(%)	Resistant n(%)
Penicillin	231(100)	-	-
Ampicillin	231(100)	-	-
Vancomycin	231(100)	-	-
Ceftriaxone	226(97.84)	-	5(2.16)
Erythromycin	152(65.8)	23(9.96)	56(24.24)
Clindamycin	174(75.32)	4(1.73)	53(22.94)
Ciprofloxacin	152(65.8)	11(4.76)	68(29.44)
Norfloxacin	193(83.55)	4(1.73)	34(14.72)

Discussion

In this study, resistance of GBS isolates to erythromycin and clindamycin from colonized pregnant women was 24.24% and 22.94% respectively. Factors that significantly associated to erythromycin and clindamycin resistance GBS isolates were antibiotics used in current pregnancy before two weeks of data collection. All isolates were sensitive to penicillin, ampicillin, and vancomycin.

One-fourth isolates of GBS resistance to erythromycin in this study was almost consistent with reported from South Africa [24]. Erythromycin resistance mechanism in GBS is mostly due to ribosomal modification encoded by erm genes or through an efflux pump (moving antibiotics out of the cell) mediated by mefA gene [25]. The high rate of erythromycin resistance in GBS strongly supports the current CDC recommendation that antibiotic susceptibility testing performed before the use of erythromycin and clindamycin; to ensure that β -lactam allergic mothers receive appropriate and effective intrapartum chemoprophylaxis [16].

The prevalence of resistance clindamycin among GBS isolates in this study was consistent with reported from South Africa [24]. Clindamycin resistance may evolve alteration of the binding site by enzymatic ribosomal modification encoded erm gene [25]. This study also, found out that 11.68% clindamycin resistance induced by erythromycin resistant. This mefA gene cause

cross-resistance to all constitutive macrolide, and lincosamide antibiotics [25]. Although macrolide (erythromycin) and lincosamide (clindamycin) antibiotics are chemically distinct, they have a similar mode of action. This, the high rate of erythromycin resistance suggests that laboratories should consider using the D-zone test for all GBS strains erythromycin resistant to identify induced clindamycin resistance [16].

The uses of antibiotics in current pregnancy before two weeks of data collection were more likely to be resistant to both erythromycin and clindamycin. This found to be consistent with study in Korea [15]. Widespread antibiotic prophylaxis use may be contributing to the development of erythromycin and clindamycin resistance in GBS [10]. Pregnant women are at high risk for drug resistance bacteria due to physiological change. However, during pregnancy, antibiotics are prescribed in many contexts. Health care providers need to give consideration for appropriate use of antibiotics during pregnancy and sensitivity testing of erythromycin and clindamycin before use helps to achieve national and global strategies to combat serious concern of antibiotic resistance [26, 27]

In Ethiopia, inappropriate use of antibiotic is 35.9% [28]. Furthermore, no clear specific guideline for monitoring erythromycin and clindamycin resistance GBS. The results of this study throw light on the current initiatives that are focused on improving maternal and newborn health care delivery system, the magnitude of the problem, and call for the necessary actions to be taken in order to strengthen efforts in prevention of antibiotic resistance.

Clinical laboratory standard institute recommendations were followed. Quality control and induction test controls were performed on each testing day, reference strains for checking the quality and performance of the media was done. We performed double diffusion test for erythromycin resistant and clindamycin susceptible isolates to identify potential inducible clindamycin resistance.

As a limitation, the serological grouping and molecular characterization of resistant GBS genes, which would provide further description on the distribution of strains, was not done. The minimum inhibitory concentration test, which may generate a quantitative erythromycin and

clindamycin susceptibility profile, was also not performed. These need further investigation in the study area.

In conclusion, the prevalence of erythromycin and clindamycin resistance to GBS was high and associated with the use of antibiotics. Efforts needed to be strengthened to conduct routine susceptibility testing of clindamycin and erythromycin before use as GBS intrapartum prophylaxis. Further investigation needed to identify antibiotic use in the community

Abstract

APR Adjusted Prevalence Ratio

CDC Centers for Disease Control

CLSI Clinical and Laboratory Standards Institute

EDHS Ethiopian Demographic and Health Survey

GBS Group B *Streptococcus*

IAP Intrapartum Antibiotic Prophylaxis

PR Prevalence Ratio

VIF Variance Inflation Factor

WHO World Health Organization

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Authors' contribution

TA, AW, YB, GE, and BS conceived and designed the paper, involved in data collection, performed the statistical analysis, interpret the results wrote and reviewed the manuscript. DM performed laboratory work and interpret the laboratory results. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests

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ANNEX II: Curriculum Vitae

Tesfaye Assebe Yadeta



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Haramaya University College of Health and Medical Science, P.O.Box 235, Harar, Ethiopia, EastAfrica

Personal information

Name	Tesfaye Assebe
Sex	Male
Date of birth	April, 1978 GC
Place of birth	Horro Guduru Wollega Zone Horro
Country	Ethiopia

Educational background and qualification

PhD Student in Public Health	Haramaya University, Ethiopia	2015 onwards
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MSC in Reproductive & Maternity Health nurse	Addis Ababa University , Ethiopia	2009
BSc in Nursing	Jimma University, Ethiopia	2005
Advance Diploma in psychiatry Nurse	Ammanuel School of psychiatry nurse, Ethiopia	20001
Diploma in Nursing	Nekemte school of nurse, Ethiopia	1998

Employment

October 2015 in Public Health PhD student Haramaya University

July 2012-2015 lecturer at Haramaya University school of nursing and midwifery with a responsibilities of advising student in research, teaching and clinical and community practice

July 2009- 2011 psychiatry nursing department head at Haramaya University

January 2001-September 2003 Head of psychiatry unit and HIV counselor in shambo Hospital

November 1999-January 2000 Nurse director (Matron) in shambo Hospital

November 1998 head nurse and care provider in shambo Hospital

National and community engagement extra activities

Professional Assessors in assisting Higher Education Relevance and Quality Agency (HERQA) for quality assurance and accreditation of higher education.

Professional contribution in capacity building by providing training for health care provider and junior university staff

- With ICAP Ethiopia and CDC course directors and facilitators in training Basic emergency obstetrics (BEmONC) and Helping baby breath since 2011 Oromia region, Harari region, Dire Dawa Administrative and Haramaya University instructors

- With AIDSTAR-One Ethiopia facilitator of training on effective teaching skills and infection prevention and patient safety for Haramaya University instructors.
- With CDC Ethiopia Facilitators in Providing training on provider initiative counselling and testing (PICT) and National comprehensive PMTCT/MNCH/RH training package for staff and students of the college and health care providers working with collaboration of CDC and Haramaya University.
- With JHPIEGO as core team member for (standard based management and recognitions) SBM-R at Haramaya University college of health science to initiate and progress the approach in the University.

Special training and certificate

- Scientific writing June 18-21 2014 Haramaya University
- Application of Statistical software STATA June 4-7 2014 Haramaya University
- Basic statistical analysis system, Regression analysis, Analysis of count data, Survival analysis, Longitudinal data analysis May 3 to 17, 2015 Haramaya University
- Qualitative research method as part of PhD Study
- Basic and TOT National Comprehensive PMTCT/MNCH/RH January 26- February 7, 2015 Haramaya University
- Infection prevention and control March 7- March 11, 2011 The AIDSTAR-One project
- Integrated management of childhood illness (IMNCI) 28 August -3 September 2005
- Basic emergency obstetric and new born care (BEmONC) June 01- 21, 2010, World Health Organization and Ministry of Health
- Comprehensive family planning May 29- June 8, 2012 Federal Minister of Health
- TOT on Essential Nutrition Action in the context of HIV and AIDS
- curriculum development and staff strengthening Ethiopian Health Training Initiative Curriculum Development 28 May- 7 June, 2007
- effective teaching skill and student performance assessment and evaluation based on the foundation of educating health care providers September 16 to 24 2011, CDC, Ethiopia

- mid-level management on EPI 22 March to 26 March, 2010

Research presentation

Factors Affecting Parent-Adolescent Discussion on Reproductive Health Issues in Harar, Eastern Ethiopia: A Cross-Sectional Study. Oral presentation on 22nd Ethiopian Public Health Association Annual Conference Addis Ababa Ethiopia

Awareness and Health Care Practice of Mothers' About Obstetric Danger Signs at Haramaya District, Eastern Ethiopia. Oral presentation on Ethiopian Society of Obstetricians and Gynaecologists Association Annual Conference Addis Ababa Ethiopia

Vertical transmission of group B Streptococcus and associated factors among pregnant women: a cross-sectional study, Eastern Ethiopia. Poster presentation, International symposium on streptococcus agalactae, South Africa Cape town, 2018

Professional association membership

Ethiopian Public Health Association, Addis Ababa, Ethiopia

Ethiopian Nurse Association, Addis Ababa, Ethiopia

Ethiopian Midwifery Association, Addis Ababa, Ethiopia

Grant (main)

Tesfaye Assebe Yadeta^{1*}, Haji Kedir Bedane², and Abera Kenay Tura¹. Factors Affecting Parent-Adolescent Discussion on Reproductive Health Issues in Harar, Eastern Ethiopia: A Cross-Sectional Study

Tesfaye Assebe Yadeta^{1*}, Fekede Asefa Kumsa². Awareness and Health Care Practice of Mothers' About Obstetric Danger Signs at Haramaya District, Eastern Ethiopia

Publication (peer reviewed)

Tesfaye Assebe Yadeta^{1*}, Haji Kedir Bedane², and Abera Kenay Tura¹. Factors Affecting Parent-Adolescent Discussion on Reproductive Health Issues in Harar, Eastern Ethiopia: A Cross-Sectional Study

Tesfaye Assebe Yadeta^{1*}, Fekede Asefa Kumsa². Awareness and Health Care Practice of Mothers' About Obstetric Danger Signs at Haramaya District, Eastern Ethiopia

Kedir Teji^{1*}, Yadeta Dessie¹, Tesfaye Assebe¹, Meyrema Abdo². Anaemia and nutritional status of adolescent girls in Babile District, Eastern Ethiopia

References

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I certify the best of my knowledge and belief that these data correctly describe my qualification, my expert and me. I can submit these supporting documents any time up on request.

Tesfaye Assebe