PREVALENCE OF BACTERIAL VAGINOSIS AMONG PREGNANT WOMEN ATTENDING ANTENATAL CARE IN TIKUR ANBESSA UNIVERSITY HOSPITAL, ADDIS ABABA, ETHIOPIA.

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ABBREVIATIONS

ANC	Antenatal Care
BASHH	British Association for Sexual Health and HIV
BV	Bacterial Vaginosis
CDC	Center for Disease Control and prevention
DNA	Deoxyribonucleic Acid
GTI	Genital Tract Infection
HIV1	Human Immunodeficiency Virus 1
HSV	Herpes Simplex Virus
IUD's	Intrauterine Device
IVF	Intravenous Fluid
KOH	Potassium Hydroxide
LBW	Low Birth Weight
NGO	Nongovernmental Organization
OPD	Outpatient Department
PCR	Polymerase Chain Reaction
PID	Pelvic Inflammatory Disease
PROM	Premature Rupture of Membrane
STD	Sexually Transmitted Disease
STI	Sexually Transmitted Infection
UTI	Urinary Tract Infections
WHO	World Health Organization

ABSTRACT

BACKGROUND: Bacterial vaginosis is one of the most common genital tract infections among reproductive age group. It is associated with different gynecologic and poor obstetric out come. The prevalence of bacterial vaginosis varies from country to country even in the same country it varies at population of interest. Different social and sexual factors can contribute for development of bacterial vaginosis. Bacterial vaginosis can be easily diagnosed by combination of two Amsel's criteria.

Objectives: This study was undertaken to determine the prevalence of bacterial vaginosis and to evaluate the accuracy of Amsel's criteria individually or in combination of two for the clinical diagnosis of bacterial vaginosis among pregnant women attending Antenatal care in Tikur Anbessa University Hospital, Addis Ababa, Ethiopia. An attempt has also been made to identify the possible risk factors associated with bacterial vaginosis.

Methods: In this observational study during the period from November 2011 to April 2012, 252 pregnant women were screened for bacterial vaginosis in Tikur Anbessa University Hospital. Vaginal swabs were collected for pH determination, saline wet mount microscopic examination to detect clue cells, KOH preparation for whiff test and Gram-stain evaluation of vaginal flora for diagnosis of bacterial vaginosis by Nugent scoring system. Accuracy of clinical diagnosis using individual and two of Amsel's criteria was evaluated.

Results: The prevalence of bacterial vaginosis was 18.3% by Amsel's two of three criteria and 19.4% using Gram stain Nugent scoring system. The prevalence of bacterial vaginosis was 31.6% and 15.9% among symptomatic and asymptomatic pregnant women respectively. A high percentage of bacterial vaginosis positive pregnant women were asymptomatic (63.3%) whereas the remaining 36.7% bacterial vaginosis positive pregnant women were complaining abnormal vaginal discharge with or without unpleasant smell. Comparing with Gram stain Nugent scoring methods the clinical diagnosis by Amsel's criteria had sensitivity of 85.7%, specificity of 98%, and positive predictive value of 91.3% and negative predictive value of 96.6%. The most sensitive and specific individual criterion was clue cells and with highest positive and negative predictive value. Amsel's criteria with the lowest sensitivity and specificity were whiff test and vaginal pH respectively. Combination of clue cells with

vaginal pH test were the highest in sensitive while whiff test with clue cells were the highest in specificity than the other combined two Amsel's criteria. After adjusted for other factors multiple life time sexual partner (OR: 8.6; 95% CI: 2.5, 29) and previous history of spontaneous abortion (OR: 5.9; 95% CI: 1.5, 23) had remained significantly associated with prevalence of bacterial vaginosis. They were the most likely to be a risk factors for bacterial vaginosis infection.

Conclusion and Recommendation: The prevalence of bacterial vaginosis among pregnant women was higher in asymptomatic pregnant women and associated with the factors multiple lifetime sexual partner and previous spontaneous abortion. Amsel's criteria diagnosis of bacterial vaginosis can be simplified using a combination of the two criteria, vaginal pH and clue cells, in settings where time or Gram staining is not available. So using these simplified diagnostic criteria is better to screen pregnant women as a routine activity during antenatal care.

Keywords Bacterial vaginosis, Pregnancy, Amsel's criteria, Nugent Scoring System, Ethiopia

CHAPTER I: INTRODUCTION

1.1. Introduction

Vaginal discharge is one of the most common complaints in gynecology and is often encountered in the primary care setting (Geller and Nelson, 2004). Vaginal infections or episodes of vaginitis often frustrate affected women and challenge their clinicians. Each year millions of dollars are spent for over-the-counter therapies to help eradicate this annoying problem. Among the common types of vaginitis, bacterial vaginosis (BV) is the one most frequently encountered. In fact, it is the most common cause of vaginitis in women of child bearing age. It is characterized by off-white, thin, foul smelling discharge, which is more noticeable after unprotected intercourse (CDC, 2008; Sobel, 2000). Despite these characteristic symptoms, many women who have BV are asymptomatic. This has been recognized as a significant problem in women's health care and many questions are unanswered regarding this condition (Klebanoff *et al*., 2004). The organism causing BV is normally present in the female genital tract; however, at times it can overgrow and cause infection (Hillier, 1998; Vogel *et al*., 2006). There have been several risk factors identified that predispose women to BV and there are only a few treatment options presently available. Additionally, recurrent bacterial vaginosis is increasing in incidence (Smart *et al*., 2004).

Bacterial vaginosis is not an infection in the classic sense. Homogenous terms for the same condition are nonspecific vaginosis or *Haemophilus*, *Corynebacterium*, or *Gardnerella* vaginalis (Spiegel, 2002b). In essence, it is best described as a shift in the vaginal ecosystem characterized by an overgrowth of anaerobes, and a decrease in *Lactobacillus*. This shift then causes degradation of the natural flora that helps keep the vaginal tissue healthy (Joesoef and Schmid, 2005). Loss of this normal flora depletes the vaginal defense system and the problem can be further exacerbated if other challenges to the defense system are present such as concurrent infections, menstruation, and stress (Carr *et al*., 1998). Changes as described above are thought to increase a women's vulnerability to any type of vaginitis, including bacterial vaginosis, although there is little scientific evidence to support this claim. It is also recognized that BV is a very common infection in women, and there is a lack of

understanding regarding the triggers and factors for the onset and resolution of it (Gellar and Nelson, 2004).

Bacterial vaginosis is a sexually associated condition but it is not considered a sexually transmitted disease. However, the role sex plays in the transmission has not been completely resolved (CDC, 2008). A large number of sexual and social risk factors for BV have been identified. Risk factors, include, but are not limited to: alteration in vaginal mucosa, elevated vaginal pH, reduction of H_2O_2 producing *Lactobacilli*, frequent sexual intercourse, new or multiple sex partners, uncircumcised partners, concomitant vaginal infections/STD, nonwhite race, and prior pregnancy just to name a few (Smart *et al*., 2004; Cherpes *et al*., 2008). Certain health and hygiene practices of women also put them at risk for this type of vaginosis and its recurrence. Among these are: hormonal changes, menses, use of tampons (left in longer than advised), poor hygiene, use of IUD's, immunological status, and douching (Eschenbach *et al*.,2000; Gellar and Nelson, 2004). Even though studies have demonstrated the above risk factors and health habits may encourage normal flora to become abnormal (a more alkaline change and decrease in *Lactobacilli*) in the vagina and lead to BV, there are women who have this problem when no risk factors can be identified. At present, due to incidence of BV related preterm labor and delivery, there is much debate regarding screening and treatment of this condition in pregnancy. Recently, evidenced based practice guidelines have been proposed for bacterial vaginosis infection in pregnant women, but currently there is no consensus as to whether to screen for or treat bacterial vaginosis in the general pregnant population (Yudin and Money, 2008).

Prevalence of BV varies depending on the population of interest. It is almost exclusively found in sexually active women of reproductive age. The prevalence rates of bacterial vaginosis among pregnant vary from 6.4% to 38% (Kirakoya-Samadoulougou *et al*., 2008; Dadhwal *et al*., 2010; Goyal *et al*., 2005; Vogel *et al*., 2006; Mitchell *et al*., 2009a; Marx *et al*., 2010; Romoren *et al*., 2007). It has been found that the lower the socioeconomic statuses of the population, the higher the incidences of bacterial vaginosis, which may indicate health and hygiene factors, play an even bigger role than anticipated (Allsworth and Peipert, 2007).

1.2. Literature Review

1.2.1. Etiologies and Microbiology of Bacterial Vaginosis

Normal vaginal flora consists of both aerobic and anaerobic bacteria, with *Lactobacillus* species being the predominant microorganisms and accounting for greater than 95% of all bacteria present. *Lactobacilli,* facultative aerobic gram-positive bacteria that are normally present in the gut, mouth, and vagina, are a key component in maintaining an acidic pH of the vagina by producing lactic acid from glucose are believed to provide defense against infection (Hillier, 1998). PCR amplification has demonstrated that the dominant *Lactobacillus* species in normal vaginal environment are *L. crispatus* and *L. jensenii*. However, *L. iners* abundance was high in all categories including BV. Hydrogen peroxide producing *Lactobacilli* are present in lower concentrations and are less prevalent in women with bacterial vaginosis than in women with a normal vaginal flora (Hillier *et al*., 1993, Zozaya-Hinchliffe *et al*., 2010).

In contrast, bacterial vaginosis is a polymicrobial syndrome resulted from a decreased concentration of *Lactobacilli* and an increase in pathogenic bacteria, mainly anaerobic or microaerophiles. The major bacteria detected in bacterial vaginosis are *Gardnerella vaginalis*, *Prevotella* species, *Porphyromonas* species, *Bacteroides* species, *Peptostreptococcus* species, *Bifidobacterium*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, and *Mobiluncus* species. *Fusobacterium* species and *Atopobium vaginae* are also common (Hill, 1993; Pepien *et al*., 2011).

G. vaginalis taxonomy has changed repeatedly in recent decades. It was designated as *Corynebacterium vaginalis* and *Haemophilus vaginalis* (Spiegel, 2002b). Based on DNA hybridization now classified as a gram variable, non motile, non encapsulated non spore forming rod bacterium. The natural habitat of this organism is the vagina of sexually mature women. It can also cause vulvovaginitis (vaginosis). *G. vaginalis* is found in over 90% of women showing the symptoms of this infection, usually together with other bacteria including in particular obligate anaerobes (*Mobiluncus, Bacteroides, Peptostreptococcus*) (Hillier, 1993).

Mobiluncus species (*M. mulieri and M. curtisii*) are gram variable, curved rod shaped and obligate anaerobic colonize the vagina and frequently isolated in cases of bacterial vaginosis together with *Gardnerella vaginalis* and other bacteria. It is isolated mostly from women having bacterial vaginosis and sometimes in women without BV infection. *M. curtisii* was rarely detected in the latter group. The significant difference in the prevalence of *M. curtisii* between women with bacterial vaginosis and uninfected women suggests that this species could be involved in the pathogenesis of bacterial vaginosis (Schwebke and Lawing, 2001).

Atopobium vaginae are gram positive anaerobic bacteria isolated from women who have abnormal vaginal flora that categorized under either intermediate or BV by Nugent score. Eventhough this organism are susceptible to clindamycin, cephalosporins, carbapenems, ampicillin/sulbactam and linezolid, they are highly resistant to Metronidazole (Ferris *et al*., 2004). *Fusobacterium neucleatum* are a small spindle-shaped rod, gram negative anaerobic bacteria belonging to the family *Bacteroidaceae.* They are non motile, non spore forming and unlike other anaerobic non spore forming bacteria produce butyric acid as a major product of the fermentation of glucose and peptone (Bolstad *et al*., 1996).

The predominant anaerobic bacteria in BV includes Gram negative rods (*Prevotella* species, *Porphyromonas* species, *Fusobacterium nucleatum* and *Bactriodes ureolyticus*), Gram positive cocci (*Peptostreptococcus* species). Other BV-associated organisms include the *Viridans streptococci*, *Streptococcus acidominimus* and *S. morbillorum*, and *Mycoplasma hominis* (Zhou *et al*., 2004). The newly proposed BV related bacteria including Bacterial vaginosis associated bacteria 2 (BVAB2), *Megasphaera*, *Leptotrichia* and *Eggerthella* like bacteria are also associated with BV (Tamrakar *et al*., 2007).

1.2.2. Epidemiology of Bacterial Vaginosis

Estimates of incidence and prevalence of BV are complicated by the fact that BV is asymptomatic in approximately 50% of women (BASHH, 2012), i.e. the vaginal floras are disturbed, but no abnormal discharge or odour is present. Therefore reported prevalence will depend on whether the diagnostic method used is based on symptoms alone, or includes microscopy.

Bacterial vaginosis found worldwide among women of reproductive age. It is the most common infectious cause of vaginitis, being about twice as common as candidiasis (Carr *et al*., 1998; CDC, 2010). The prevalence of BV varies widely among the different populations studies and it has been most widely studied among women attending publically supported sexually transmitted infection clinics, family planning clinics, and obstetrical clinics (Jones *et al.,* 2007; Yudin and Money, 2008). Higher prevalence of BV is commonly reported from developing country than developed country (35% vs. 24.8%) (Gillet *et al.,* 2011).

Bacterial vaginosis is the commonest vaginal infection among women of reproductive age in the United States. A report from unselected women indicates that 29% BV prevalence and the prevalence varied with age, race or ethnicity, education, and poverty (Allsworth and Peipert, 2007). Generally the prevalence can be reached from 3%- 47.2% among sexually active women of university attendant and a primary healthcare attendance respectively (Gillet *et al.,* 2011). In Canada, the prevalence of bacterial vaginosis reached 9-23% in pregnant women (Einarson and Koren, 2002).

According to the study which was done in Peru to determine the prevalence of BV among low income young women using BVBlue test shows that 27% of the women were positive for BV and bacterial vaginosis had association with age, history of sex work, marital status and current trichomonas or bacterial STD (Jones *et al*., 2007).

On the other hands 20.5% incidence of BV among pregnant women had been documented in India and most common incidence was seen in age group 18-27, primipara between gestational weeks 11-20, and low socioeconomic status. In addition poor pregnancy outcomes such as preterm labor, PROM and fetal complications were found more in pregnant women who had bacterial vaginosis, bacterial vaginosis with UTI as compared to those without bacterial vaginosis (Lata *et al*., 2010).

Besides this, a study done in Philadelphia to develop screening tool to predict BV status shows forty percent prevalence of BV using Nugent criteria among pregnant women in their first trimester (Nelson *et al.*, 2007a). A prospective cohort study conducted in Birmingham to determine the association between personal hygiene and BV also indicates 40.4% prevalence of bacterial vaginosis among non pregnant women (Klebanoff *et al*., 2010).

According to a study done among Japanese pregnant women indicates out of 132 enrolled women 98 (74.2%) have normal vaginal flora, 21(15.9%) have intermediate and 13(9.8%) were BV. This literature also indicates the existence of different *Lactobacilli* species between women having BV and normal flora (Tamrakar *et al*., 2007).

The prevalence of bacterial vaginosis in Africa setting varies greatly at different country. A study done in Nairobi among pregnant women with HIV1, the prevalence of BV was 37% (Marx *et al.*, 2010). Six point four percent BV prevalence were reported from Burkina Faso with the high prevalence was seen in the age group of 25-29 and BV was strongly associated with history of previous spontaneous abortion (Kirakoya-Samadoulougou *et al*., 2008). The prevalence of BV among pregnant women in Tanzania is 20.9%. This study also shows the prevalence difference by women HIV status and indicates high BV prevalence among *HIV* positive women than *HIV* negative (37.2% vs. 19.6%) (Msuya *et al*., 2009). In other study that was undertaken in Botswana to determine the prevalence of *Trichomonas vaginalis* (TV) infection and bacterial vaginosis (BV) among pregnant women showed that the prevalence of BV was 268 (38%) and among this 205 (76%) is asymptomatic (Romoren *et al*., 2007).

A study done by Aynalem *et al* to assess the frequency of bacterial vaginosis in women visiting a gynecological clinic in Addis Ababa and to characterize the most common lactic acid bacteria isolated from vaginal fluid indicates 32% prevalence of BV. But due to small sample size the finding was not enough to make reasonable comparisons and to associate different variables with BV (Aynalem *et al*., 2010).

1.2.3. Risk Factors Associated with Bacterial Vaginosis

Although the cause(s) of BV are not well known, and onset and remission can be spontaneous, there are a number of clinical, behavioral and demographic characteristics that can predispose a woman to BV. Factors include:

a. Ethnicity

BV is more common in black women than in white (BASHH, 2006; Allsworth and peipert, 2007). The reasons for this are unknown but some studies have shown black women have a lower number and narrower range of *Lactobacilli* (Antonio *et al*., 1999; Zhou *et al*., 2007). This may be due to genetic predisposition and/or cultural behaviors, e.g. douching, which is more commonly practiced by black women than white, although it is not known if douching is causal, or a response to symptomatic BV.

b. Douching

Douching alters vaginal pH, removes the protective *Lactobacilli*, and can introduce perfumes, preservatives and other materials that may inhibit *Lactobacilli* and/or encourage pathogens. It is therefore not surprising that douching is associated with an increased risk of BV (CDC, 2008; Ness *et al*., 2002). Recent studies showed up to a 2 fold relative risk for developing BV among those who douched regularly, and a reduced risk on cessation of douching (Allsworth and peipert, 2007; Brotman, 2008a and 2008b). Vaginal douching and sexual activity in black women were shown to be associated with an increased risk of BV in the second trimester of pregnancy (Trabert and Misra, 2007). BV has variable association with non douching hygiene behavior with positive association with bathing frequency. This study also states that subsequent douching is a predisposing to BV because after and before adjustment of the other hygiene confounder it has strong association with BV (Klebanoff *et al*., 2010).

c. Menstrual cycle and hormonal changes

Incidence of BV is found to be common around the time of menstruation (Eschenbach *et al*., 2000; Joesoef & Schimd , 2005). Menses and hormonal changes alter vaginal pH throughout the menstrual cycle. It is not clear how much the changes observed in vaginal flora around this time are related to hormones or to the products of menstruation (which have a higher pH); however some authors suggest that hormonal changes may trigger the floral imbalance. BV is less common in women on hormonal contraception (Smart *et al*., 2004; Holzman *et al*., 2001).

d. Sexual activity

BV is not regarded as a sexually transmitted infection (STI), but prevalence is generally higher amongst sexually active women, and in women with multiple partners (BASHH 2006; Allsworth and Peipert, 2007) and those attending STI clinics (Schwebke, 2005; Sobel, 2000). BV is also more common among women with uncircumcised male partners (Cherpes *et al*., 2008). It is generally accepted to be associated with sexual activity (Schwebke, 2003) and has been described as 'sexually enhanced' (Verstraelen *et al*., 2010), but no causative microorganism has been found to be transmitted between partners, and it can arise and disappear spontaneously in sexually active and non-sexually active women, including virgins (BASHH, 2006; Joesoef and Schmid, 2005; Allsworth and Peipert, 2007). Women who have sex with women have a 2-2.5- fold greater likelihood of developing BV compared with heterosexual women (Smart *et al*., 2004). The reasons for this are unclear, but may be at least in part due to the introduction of many bacterial species, which overwhelm the vaginal ecosystem. As for heterosexual women, the risk increases with increasing numbers of sexual partners (Bailey *et al*., 2004).

Due to the epidemiology some authors argue that BV is an STI (Schwebke, 2003; Bradshaw *et al.,* 2006), and the US Centre for Disease Control and Prevention (CDC) include BV within their Sexually Transmitted Diseases Guidelines (CDC, 2010). Many women are embarrassed about consulting their doctors for treatment and results an increase in a number of self-medicated women with vaginitis symptoms by spending up to thousands of dollar for such treatments. Categorizing BV as sexually transmitted is likely to enhance the stigma that surrounds BV, and further inhibit women seeking advice from their health care provider. The concept of an unbalanced versus a diseased vaginal ecosystem is important for both patient and doctor to understand, and will help break the taboo that surrounds this condition (BASHH, 2006; Cherpes *et al*., 2008).

e. Other factors

Other factors linked to an increased risk of developing BV include concomitant infections – particularly STIs (Smart *et al.*, 2004), early first coitus (Allsworth and Peipert, 2007),

women using an IUD (BASHH, 2006), low socioeconomic status (Allsworth and Peipert, 2007), cigarette smoking (BASHH, 2006; Cherpes *et al.*, 2008) and smoking during pregnancy (Vogel *et al.*, 2006). Use of perfumed products or antiseptics in the bath may contribute to the risk of BV, and are advised against (BASHH, 2006).

1.2.4. Pathogenesis and Pathology of Bacterial Vaginosis

Like other pathogenic microorganism bacterial vaginosis is developed from interaction of different microbial product. These microbial products disturb the normal defense mechanism. The virulence factor for bacteria's that causes bacterial vaginosis includes (Spiegel, 2002a):

- Succinic acid produced by *Prevotella, Bacteroides* and *Mobiluncus,*
- Sialidase (neuramindase) enzymes produced by *P.bivia,* some strains of *P.disiens* and *Gardnerella,*
- Hemolysin produced by *G.vaginalis*, synergetic relationship between *G.vaginalis* and *P.bivia.*
- Biofilms production by *Gardnerella* helps other anaerobic bacteria to adhere and prevented from toxic oxygen and H_2O_2.

Normal vaginal ecosystem is hostile for the growth of abnormal microorganism causing bacterial vaginosis. Because the predominant normal flora, *Lactobacilli* produce several antimicrobial compounds, including lactic acid, H_2O_2, lactacin, and acidolicin. Of these: H_2O_2 is likely the most important and interacts with peroxidases, producing a potent oxidant that is toxic to many bacteria (Hillier, 1998; Alvarez-Olmos *et al*., 2004). The pathogenesis of bacterial vaginosis still unclear, but appears to be related to factors that disrupt the normal acidity of the vagina and the equilibrium between the different constitute of the normal vaginal flora. The decrease of *Lactobacilli* results the increase the pH of vaginal due to low production of lactic acid and simultaneously low production of toxic hydrogen peroxide results in decreasing of vaginal nonspecific defense system (Alvarez-Olmos *et al*., 2004; Aroutcheva *et al*., 2001). In contrast, BV causing microorganism normally occurs in small number becomes increase and gain access to multiply and produce their own virulence

factors for pathogenesis (Bradshaw *et al*., 2006). Microbial product of anaerobic bacteria like proteolytic carboxylase enzymes, which break down vaginal peptides into a variety of amines that are volatile, malodorous, and associated with increased vaginal transudation and squamous epithelial cell exfoliation, resulting in the typical clinical features observed in patients with BV. *Mobiluncus* in addition produces trimethylamine serve as the source of the fishy odor. Succinic acid produced by *Prevotella* and *Mobiluncus* may inhibit movement of immune cells to the site of infection. Synergetic relation between *Prevotella bivia* amino acid conversion to ammonia and utilization of ammonia for the production of amino acid by *Gardnerella* results the increase in vaginal pH due to the increase in ammonia and decrease in lactic acid production (Spiegel, 2002a). Mucin digestion by Sialidase production is also important virulence factors for adhesion as result of homogenous discharge. Sialidase also cleaves IgA produced in response to the *G. vaginalis* hemolysin leaving this cytolytic agent to lyse white blood cells indicated by the increase in amount of lactofferin in the vagina fluid of women with BV (Rein *et al*., 1996).

1.2.5. Clinical Features of Bacterial Vaginosis

According to UK and US guidelines (BASHH, 2006; CDC, 2008), the most common symptom of BV is an excessive white to grey, malodorous vaginal discharge (often described as 'fishy'). The odour may be particularly noticeable after sexual intercourse (CDC, 2008; Sobel, 2000). However, determining whether discharge and odour are "normal" can be difficult, as assessment is subjective, and both vary through the menstrual cycle, between individuals, with age, and with other factors such as pregnancy. At low pH the amines are non-volatile and therefore non-odorous. Increased pH causes the amines to be volatile and thus results malodorous. The odour is therefore a result of both the increase in anaerobes (and hence an increase in production of amines), and the change in pH which makes the amines volatile (Carr *et al*., 1998; Sobel, 2000).

In addition, many women with BV are asymptomatic even though the balance of their vaginal microflora is disturbed (BASHH, 2006; Sobel, 2000). It is commonly estimated that approximately 50% of women with BV are asymptomatic (BASHH, 2006; Joesoef and Schmid, 2005), although higher levels have been reported (Nelson, 2007b), and some

authors describe “most” women as being asymptomatic (CDC, 2008). One US study found that only 25% of women with BV reported odour and 42% reported discharge in the previous 6 months, compared with 18% and 43% respectively for women without BV. In other study, 58% of women with BV had noticed symptoms of odour, discharge and/ or wetness, compared with 57% of women without BV (Klebanoff *et al*., 2004). Therefore although odour and discharge are typical symptoms of BV, their presence does not necessarily indicate BV, and their absence does not necessarily rule BV out.

There is disparity in accepting itching, irritation or soreness as symptom of BV. This disparity may arise from misdiagnosis, mixed infections, different individual perceptions or descriptions of the discomfort caused by BV. According to UK guideline (BSAHH, 2006) BV is not associated with such symptoms while according to US fact sheet (CDC, 2008) bacterial vaginosis sometimes associated with these symptoms.

1.2.6. Sequelae of Bacterial Vaginosis

Bacterial vaginosis can be a factor for many pregnancy complications including preterm rupture of membranes, preterm birth, LBW and postpartum sepsis especially in women with a previous history of preterm delivery (Mullick *et al*., 2005; CDC, 2008). Among these preterm birth is the most common type of complication. A pregnant woman diagnosed Bacterial vaginosis during the second trimester of pregnancy have an increased risk of to give premature of birth (less than 37 weeks of gestational age) and low birth weight (less than 2500 mg) (Hillier *et al*., 1995). These prematurity birth increases the risks of mortality and morbidity. Bacterial vaginosis infections during the early pregnancy also increase second term pregnancy loss (Nelson *et al*., 2007a).

BV is also a risk factor for non-obstetrical infections including postpartum endometritis, cuff cellulitis, post-abortal and spontaneous pelvic inflammatory disease. PID due to uterus (womb) and fallopian tubes infection by bacteria causing BV can cause infertility or damage the fallopian tubes enough to increase the future risk of ectopic pregnancy and infertility (CDC, 2008).

1.2.7. Diagnosis of Bacterial Vaginosis

I. Vaginal discharge characteristics

Specimen collection process for the diagnosis of BV can be done during pelvic examination using sterile unlubricated speculum. At the time of speculum examination, an evaluation of the nature of the discharge is made by the clinician to differentiate physiological discharge from abnormal, pathological discharge. The classic BV discharge adheres to the vaginal wall and often accompanied by odour. The discharge is thin, homogenous and grey/yellow in color (Eschenbach *et al*., 1988). On the other hand swab can be taken as a blind vaginal swab collected by the clinician or the patient itself. Self-sampling techniques are also acceptable to women, may improve efficiency in diagnosing BV, and have been shown to be both reliable and valid in diagnosing BV among pregnant women (Boskey *et al*., 2004; Nelson *et al*., 2003).. The site for specimen collection is lateral vaginal wall following the insertion of a speculum is recommended. But for Prepubertal women and that declining speculum examinations posterior vaginal wall sample, and for pregnant women vaginal wall smear can be taken. The swab is collected from lateral wall and posterior fornix using sterile cotton tip applicator swab (Keane *et al*., 2006).

II. Clinical diagnosis (Amsel's criteria)

Clinical diagnosis is done at bed side in hospital. The most widely used clinical diagnosis criteria is Amsel's criteria which require three out of the following four criteria: first, a vaginal pH of greater than pH 4.5; second, the presence of clue cells in the vaginal fluid; third, a milky, homogeneous vaginal discharge; and finally, the release of an amine (fishy) odour after addition of 10% potassium hydroxide to the vaginal fluid (Amsel *et al.,* 1983*).* The health care provider must examine the vagina for the sign of BV (CDC, 2008). The pH can be determined directly with the use of pH paper placed on the vaginal wall or with the use of a vaginal swab which is touched on pH paper. The vaginal swab sample also extracted into drop of physiological saline either on a glass slide or in a test tube; a drop of the extract is then placed on a glass slide. Additionally a drop of 10% potassium hydroxide is placed on another glass slide. The swab is then stirred in the 10% potassium hydroxide and immediately the presence of a fishy odour can be detected. Both drops are then covered

with a cover slip and examined at 400× magnification using microscope. Clue cells are identified as vaginal epithelial cells with such a heavy coating of bacteria that the peripheral borders are obscured from saline preparation of vaginal swab (Money, 2005). Small vaginal sample (discharge) with adequate amount of saline wet mount preparation is suitable in order to disperse the epithelial and facilitate finding of clue cells (Eschenbach *et al*., 1988). The presence of Candida yeast can be identified from KOH preparation after whiff test. If three of the four criteria are occurred, then a clinical diagnosis of BV is said to be positive. However, a recent study found that any two of four clinical criteria combination could be used without loss of sensitivity or specificity (Gutman *et al.*, 2005).

III. Microbiological diagnosis (Nugent scoring of vaginal Gram Stain)

Gram Stain laboratory testing methods of vaginal swab/discharge for BV are mostly used as the gold standard in the research setting. Gram-stained smear alone, without culture, can be used to evaluate vaginal swab specimens for bacterial vaginosis. Vaginal swab specimen smeared over the slide and air dried, then transported to microbiology laboratory. Then the slide is heat fixed and stained by standard gram staining procedure. The stained slide is read, and the number of morphotypes counted and evaluated using standardised scoring system. The diagnostic criteria developed by Spiegel *et al*. (1983) and later modified by Nugent *et al*. (1991) has been a well- reproduced standardized Gram stain scoring method. Nugent scoring of gram stain categorize the morphotypes if it is large gram positive rod as *Lactobacilli*, small gram variable rod as *Gardnerella vaginalis*, small gram negative rods as *Bacteroids* species and curved gram variable rods as *Mobiluncus* (Table 1.1).

A score of zero to three is considered to be normal, four to six is considered intermediate and seven to ten is defined as BV (Nugent *et al*., 1991). Intermediate scoring occur when the number of *Lactobacilli* morphotype decrease while other morphotypes are normal or outnumber of *G. vaginalis* morphotype without the decrease of *Lactobacilli*. Majority of the patients with an intermediate score will proceed to BV positive and a slight small to normal flora. Due to this high rate of transition there is debate to include in to abnormal flora, but there is a consensus to be rechecked and treated according to the clinical risk to be positive for BV (Vogel *et al*., 2006).

Comparing the Clinical criteria with the Gram staining methods, most of the time the clinical criteria results are under diagnosed due to the subjective nature of the methods but it is easy to perform and not time consuming. In addition the result of the Clinical diagnosis varies widely (inconsistent) at different setting while the Gram staining methods appears to be more sensitive, reliable and accurate (Gillet *et al.,* 2011).

Table 1.1: Nugent Scoring Vaginal Gram's Stain for Bacterial Vaginosis (Nugent *et al*., 1991)

Scale	Lactobacillus	Score	Gardnerella	Score	Mobiluncus	Score
4+ ≥30 organisms /field	4+	0	4+	4	4+	2
3+=5-30 organisms /field	3+	1	3+	3	3+	2
2+=1-4 organisms /field	2+	2	2+	2	2+	1
0or1+≤1 organisms/field	0	4	0	0	0	0

According to Spiegel, if *Lactobacillus* spp. Morphotypes fewer than five per oil immersion field and if there is five or more *G. vaginalis* morphotype together with five or more other morphotypes (gram-positive cocci, small gram negative rods, curved gram-variable rods, or fusiforms) per oil immersion field, the Gram stain interpreted as indicating bacterial vaginosis. If five or more *Lactobacillus* specius and fewer than five other morphotypes are present per oil immersion field, the Gram stain considered as normal (Spiegel *et al*.,1983).

IV. Culture

The use of Culture to diagnose bacterial vaginosis is not advocated. However, in clinical practice a vaginal culture is frequently obtained from the patient with symptoms when a conclusive diagnosis cannot be obtained from the wet mount preparation during the clinical diagnosis. A culture result of absence of normal flora and abundant *Gardnerella* can be

helpful in pointing to a diagnosis of bacterial vaginosis in such patient (Al-Muk and Hasony, 2001). Culture of *G. vaginalis* is not recommended as a diagnostic tool because it is not specific (CDC, 2010). *G. vaginalis* presence in culture alone cannot be diagnostic because it can be detected in up to 50-60% of healthy asymptomatic women (Sobel, 2012). Culture technique diagnosis of bacterial vaginosis is less sensitive than other methods (Udayalaxmi *et al*., 2011).

V. Other diagnosis methods

There have been alternative diagnostic methods suggested, but none are currently better than the standardized Gram stain methodology (Money, 2005). PCR also has been used in research settings for the detection of a variety of organisms associated with BV, but evaluation of its clinical utility is uncertain. Cervical Pap tests have no clinical utility for the diagnosis of BV because of their low sensitivity (CDC, 2010). Rapid point-of-care diagnostic kits for BV have become available commercially. The first kit has two cards, FemExam pH plus Amines test card and the FemExam *G. vaginalis* proline aminopeptidase activity test card. The second diagnostic tool, the BVBlue system is a chromogenic test for the detection of sialidase activity in vaginal fluid specimens. In both case high sensitivity and specificity were found in comparison with the gold standard of gram-stain diagnosis of BV, and these also performed better than Amsel's clinical criteria (Myziuk *et al*., 2003; West *et al*., 2003).

1.2.8. Treatment and Prevention of Bacterial Vaginosis

There is no consensus for whether to screening for or treat bacterial vaginosis among the general pregnant population to prevent adverse outcome. All pregnant women who have ever had a premature delivery or low birth weight baby should be considered for a BV examination, regardless of symptoms, and should be treated if they have BV. All pregnant women who have symptoms of BV should be checked and treated (Yudin and Money, 2008; BASHH, 2006; CDC, 2008). Despite the association between BV and preterm birth, screening and treatment of asymptomatic BV during pregnancy is controversial. But women undergoing a hysterectomy or abortion should be screened and treated for BV prior to the

procedure, regardless of symptoms, to reduce their risk of developing an infection (CDC, 2006).

Developing countries manage different STD or GTI based on the syndromic management guidelines developed by world health organization (WHO). The syndromic management of STDs in Ethiopia also simply follows the chart to treat those women who have vaginal discharge without any laboratory diagnosis (Ethiopia, 2006). This flow chart has poor performance which results in either overtreatment of those without infection and inappropriate treatment due to it does not rely on identification of specific pathogen or under treatment due to presence of asymptomatic cases (Wolday *et al*., 2004). The syndromic flowchart for the management of vaginal discharge does not work well for controlling bacterial vaginosis in women because mostly BV infection is asymptomatic and it does not consider risk status of the pregnant women (Romoren *et al*., 2007).

I. Antibiotics

The established benefit of therapy for BV in pregnant women is to relieve vaginal symptoms and signs of infection. Additional potential benefits of therapy are reducing the risk for infectious complications associated with BV during pregnancy and for other infections (e.g., other STDs or HIV). The treatment protocol for bacterial vaginosis varies from the use of synthetic drugs to the use of probiotics. The most widely used drugs are metronidazole and clindamycin that can be administered either orally or topically (BASHH, 2006; CDC, 2006) (Table 1.2.). Oral and vaginal (topical) route metronidazole treatment of bacterial vaginosis in early pregnancy produces comparable efficacy in most BV-associated bacteria, even fastidious species (Mitchell *et al*., 2009b). Although topical clindamycin and metronidazole have similar clinical efficacy, using topical metronidazole advisable rather than clindamycin because treatment of BV with clindamycin is associated with significant development of antimicrobial resistance of anaerobic isolates (Austin *et al*, 2005). Topical (vaginal) therapy is not recommended for prevention of adverse pregnancy outcome (Yudin and Money, 2008). Regardless of the antimicrobial agent used to treat pregnant women, oral therapy is preferred because of the possibility of subclinical upper-genital–tract infection (CDC, 2010).

Table 1.2: National guidelines for the treatment of bacterial vaginosis (BASHH 2006; CDC 2010)

Regime	**UK (BASHH, 2006)**	**US (CDC, 2010)**
Metronidazole		
Oral, 400-500mg twice daily, 5-7 days	R	R
Oral, 2g, single dose	R	NR
Intravaginally gel (0.75%), 5g, once daily, 5 days	A	R
Clindamycin		
Oral, 300mg twice daily, 7 days	A	A
Intravaginally cream (2%), 5g once daily, 7 days	A	R
Intravaginal sustained release cream (2%), 5g, single dose	NA	NA
Intravaginal ovules, 100mg, once daily, 3 days	NA	A
Tinidazole		
Oral, 2g, single dose	A	NA

R = Recommended regimen, NR = Not recommended, A = Alternative regimen supported in the guidelines,
NA = Not applicable (not covered in guidelines)

II. Probiotics

Recurrence of BV is frustrating in reproductive women. One of the main reasons the occurrence of recurrence after treatment is that inability of the treatment to promote colonization of the vagina with hydrogen producing L*actobacilli*. Due to this and the increase in antimicrobial resistant pathogenic microorganism, additional measurement is necessary to completely restore a healthy vaginal environment (Mitchell *et al*., 2009a). Using *Lactobacilli* as a probiotics play an important role in the inhibition of growth, adhesion, and spread of pathogenic microbes due to its ability to form biofilms over the mucosal layer of the vagina and thereby compete for the nutrients and receptors with the pathogenic microbes. In addition to this, they secrete lactic acid, H_2O_2, bacteriocins, and biosurfactants which have good antimicrobial property. Apart from the antimicrobial

property of lactic acid, they help in maintaining the pH of vagina less than 4.5 thereby not allowing conducive environment for the growth of the pathogenic microbes (Aroutcheva *et al*., 2001). Besides this consecutive supplementation of *Lactobacilli* probiotics gives a significant improvement in treatment efficacy for bacterial vaginosis (Larsson *et al*., 2008).

III. Preventive measures

There is no clear and cut preventive measure to prevent development of BV. Because the factor that initiate is not well understood. However, be abstinent and use all of the medicine prescribed for treatment of BV, even if the signs and symptoms go away (CDC, 2008), avoiding of the expected factors like vaginal douching (Bahram *et al.,* 2009*;* Brtoman *et al.,* 2008a*;* Brtoman *et al.,* 2008b; Klebanoff *et al*., 2010;), multiple sexual partner and smoking (Smart *et al*., 2004) and factors that disturbs the normal vaginal flora (Brotman *et al*., 2008a and 2008b) can prevent from bacterial vaginosis.

1.3. Significance of the study

Bacterial vaginosis is an important gynecologic problem of child bearing age group of women worldwide. The presence of bacterial vaginosis has consistently been shown to be a risk factor for adverse obstetric outcomes, such as preterm labor and delivery, preterm premature rupture of membranes, spontaneous abortion, Chorioamnionitis, and postpartum infections such as Endometritis and Caesarean section wound infections (Nelson and Macones, 2002; Mullick *et al*., 2005; CDC, 2008). BV diagnosed at first trimester associated with a twofold increased risk of second trimester pregnancy loss of among women at the highest level of BV (BV scores of 7–10), compared to women with normal vaginal flora (BV scores of 0–3) (Nelson *et al*., 2007a). It also associated with miscarriage during the first trimester (Ralph *et al*., 1999). In addition BV has been associated with post partum Endometritis, vaginal cuff Cellulitis following abdominal hysterectomy and post abortion PID (Charonis and Larsson, 2006; CDC, 2008). Preabortal screening and subsequent treatment those who test clinically positive does lower the incidence of post abortion PID (Charonis and Larsson, 2006). Due to these realities screening of pregnant women will be beneficial to both the pregnant women as well as the health care provider.

BV is estimated to be the most prevalent vaginal infection particularly in countries with high HIV prevalence. BV is also a common disorder in women throughout the world, regardless of HIV status. A result from one Meta analysis indicates that BV increases the risk of HIV acquisition by approximately 60 percent and Studies of HIV prevalence tended to find higher HIV prevalence in women with BV (Atashili *et al*., 2008). The presence of bacterial vaginosis in HIV-infected women increases HIV genital shedding with in discharge and results in increased concentration of HIV in genital secretions. The increase in concentration in the genital secretion facilitates both vertical and sexual HIV transmission (Msuya *et al*., 2009; Sha *et al*., 2005). In addition, the organism causing bacterial vaginosis can result upper genital tract infections such as Chorioamnionits, Endometritis, and Placental compromise. Due to this infection there is strong association between BV and in utero HIV transmission (Farquhar *et al*., 2010). If BV is confirmed to increase the risk of *HIV* infection, screening and treatment of BV could be a meaningful intervention to prevent *HIV* acquisition. If better BV treatment strategies can be devised and these can be shown to lower the risk of HIV acquisition, the public health benefits would be enormous. BV also increases susceptibility to other STDs, such as HSV, *Chlamydia* and *Gonorrhea* (CDC, 2008). Although it is still an issue of controversy, there is a positive association BV and cervical HPV (Gillet *et al*., 2011).

Since maternal and neonatal complications resulting from poor pregnancy outcomes like preterm birth, PROM, and low birth weight can be costly to health care systems, the cost of screening and treating positive BV is certainly justified and advantageous (Lata *et al*., 2010).

Although many studies of BV have been done in different country, currently we know of no published studies that have been conducted in Ethiopia to describe the changes in vaginal flora or BV prevalence during pregnancy. In order to avoid the above aforementioned problem screening and treating of pregnant women is the crucial part. In addition to knowing the prevalence of BV, the diagnosis method of BV in our population perspective also should be assessed. Therefore this study was undertaken to assess the prevalence of bacterial vaginosis and to compare the diagnostic accuracy of individual and combined clinical diagnosis criteria using Nugent scoring as a standard among pregnant women

visiting Tikur Anbessa University Hospital, Addis Ababa, Ethiopia. Finding from this study will help as a standing point for further study concerning BV and provide baseline information for the diagnosis and management of bacterial vaginosis.

1.4. Objectives of the Study

General objective

- To determine the prevalence of Bacterial Vaginosis among pregnant women attending ANC at Tikur Anbessa University Hospital, Addis Ababa, Ethiopia.

Specific objectives

- To assess the prevalence of Bacterial Vaginosis.
- To assess the prevalence of Bacterial Vaginosis among symptomatic and asymptomatic pregnant women.
- To compare the diagnostic performance of clinical criteria with microbiological diagnosis methods.
- To assess risk factors associated with Bacterial Vaginosis.

CHAPTER II: MATERIALS AND METHODS

2.1. Study Design and Area

A hospital based cross sectional, observational study was conducted from November 2011 to April 2012 at Tikur Anbessa University Hospital, Addis Ababa, Ethiopia. Tikur Anbessa University hospital represents the highest tertiary level of referred patients in the country and located in Lideta sub-city. It receives referred and some directly visiting patients from all parts of the country and provides emergency service. The hospitals have 22 different departments including obstetrics and gynecology wards where this study has been conducted.

2.2. Study population

During the period from November 2011 to April 2012, a total of 252 pregnant women attending for ANC at Tikur Anbessa University Hospital at any gestational week were screened for bacterial vaginosis. Among these, 57 were symptomatic pregnant women (A pregnant women having abnormal vaginal discharge with or without unpleasant odour) while the remaining 195 pregnant women were present without known syndrome (Asymptomatic: women that don't complain abnormal vaginal discharge with or without unpleasant smell). Pregnant women with vaginal bleeding, on antibiotic treatment in the preceding two weeks and did not give consent were excluded from the study. After physical and gynecological examination by the attending Physician all eligible pregnant women were referred (requested) with their card to participate in the study. The Nurse interviewers explained the purpose and practice of the study, and obtained informed consent. Standard Questionnaire was used to get relevant information and the existing Clinical data was recorded for each participant (see Appendix I). The questionnaire was filled in a confidential location within the office by a female Nurse interviewer.

The sample size (n) was calculated by taking prevalence of bacterial vaginosis 32% in previous Ethiopian study (Aynalem *et al*., 2010). The margin of error (d) taken was 0.05 and the confidence interval (Zα/2) was 95%.

$$n = \frac{(1.96)^2 \text{ X } (0.32)\ (0.68)}{(0.05)^2} = 334.5$$

We had planned to collect 335 samples. We could only collect 252 samples from November 2011 up to April 2012.

2.3. Sample Collection, Handling and Transport

After obtaining the required information, two vaginal swabs/discharges were collected from posterior vaginal fornix with sterile cotton tipped applicator swabs by trained Nurse. One swab specimen was used for pH measurement and for whiff test after addition of 10% KOH while the second swab was smeared on the slide for gram staining and after that the remaining swab was placed into test tubes containing 0.5 ml saline for wet mount microscopic evaluation of clue cells. After labeling, all materials were transported immediately to Microbiology Teaching Laboratory for Microscopic wet mount examination and Gram staining.

2.4. Diagnosis of Bacterial Vaginosis

2.4.1. Clinical diagnosis based on Amsel's criteria

a. Vaginal pH

After collection of vaginal swab/discharge, the pH of vaginal swab/discharge was measured using a colorimetric indicator pH paper (RANKEM, RFCL Limited, New Delhi), placed in contact with secretions on swab for one minute. After one minute the color developed on the paper was compared with a standardized colorimetric reference chart to estimate the actual pH. Vaginal pH greater than 4.5 was taken as positive for bacterial vaginosis.

b. Whiff test

An odour was noted as elicited with the addition of two drop of 10% KOH to a first vaginal swab/discharge on the slide after measurement of pH. It was considered positive for Whiff test when characteristic "foul, fishy odor" is detected. This slide also was examined using high-power (400x) magnification power for detection of Yeast cells.

c. Wet smear

The second swab placed into test tube containing saline was used to make a wet mount preparation. A drop of saline prepared vaginal swab/discharge was placed on glass slide and then covered with a cover slip and examined under high-power (400x) microscopy to identify the clue cells. Clue cells are vaginal epithelial cells surrounded by many short rod shaped (cocobacilli) bacteria (Money, 2005). The slide was considered as positive for Clue cells when at least 20 % of vaginal epithelial cells are coated by cocobacilli bacteria. These wet mount preparations were also examined for detection of *Trichomonas vaginalis.*

d. Interpretation of Amsel's criteria

Clinical diagnosis of bacterial vaginosis was considered positive if two of the following three of Amsel's criteria were fulfilled:

- Vaginal pH exceeded 4.5.
- The presence of foul smelling after 10% KOH addition (whiff test positive)
- The presence Clue cells on saline wet smear preparation.

2.4.2. Microbiological Diagnosis based on Nugent Scoring system

a. Gram stain of vaginal swab/discharge

The smear prepared form vaginal swab by rolling over on the labeled slide by patient serial number was air dried and was transported to Microbiology Teaching Laboratory for gram staining. The slides were stained by gram staining solution on arrival at the Microbiology Laboratory (procedure of gram staining see Appendix IV).

The result of Gram stained smear was categorized into three categories:

- *Lactobacilli* if it was large gram positive rod,
- *Gardnerella / Bacteroids* species if it was small gram variable or gram negative rod (short rod) and
- *Mobiluncus* species if it appeared as curved gram variable rods.

b. Interpretation of Nugent scoring

The standard used for evaluating of smear (diagnosis of BV) was Nugent scoring system. This is a standardized 0–10 point scoring system for evaluation of Gram stained vaginal smears based on three morphotypes: large gram positive rods (*Lactobacilli*), small gram negative/variable rods (*G. vaginalis* and Anaerobic rods) and curved gram variables (*Mobiluncus* species). After counting the total number of the three morphotypes from five non consecutive oil immersion fields the average number was taken and evaluated for the point score (see Appendix I). The sum of the three morphotype score was taken to categorize the vaginal smear. In this study, Microbiological diagnosis of BV was a score of 7–10 by Nugent's method. A score of 4-6 were intermediate, and a score of 0-3 were considered normal. Quality control of the readings was checked by rereading 10% of the slides by experienced Medical Laboratory Technologist for Nugent's score.

2.5. Variables

Table 2.1: Dependent and Independent variables of the study subjects

Dependent variable	**Independent variable**
Bacterial vaginosis	Age
	Education
	Religion
	Marital status
	Number of sexual partners
	Occupation
	Contraceptive use
	Gestational age
	Gravidity
	Previous pregnancy outcome
	Hygiene behavior
	Clinical feature

2.6. Statistical Analysis

Once all information is recorded, it was entered into a database system. The database used for data entry was formed on EPI data then exported to SPSS version 16.0 for analysis. Univariate analysis was done to calculate frequencies and proportions. Bivariate analysis for association of selected exposure variables was done with the outcome variable. Multivariate analysis was used for association of bacterial vaginosis and other factors for adjusting for potential confounding factors. Logistic regression was performed using SPSS. In addition sensitivity, specificity, positive and negative predictive value of Amsel's criteria was calculated using OpenEpi soft ware.

2.7. Ethical Considerations

The M.Sc. research project proposal was ethically cleared by the Department Research and Ethical Review Committee (DREC) and approved by Department of Microbiology, Immunology and Parasitology, School of Medicine, Addis Ababa University. The project also approved by department of Obstetrics and Gynecology School of Medicine, Addis Ababa University. Official permission from the study site was obtained. Written informed consent was obtained from willing pregnant women (Appendix III). No name was used on questioner and the participants were identified in the data system as a number, which corresponds to the consent, questionnaire, laboratory report sheet (Appendix I). Laboratory diagnosis results were reported to physicians if it was positive. Symptomatic pregnant women diagnosed positive were treated by 500mg oral metronidazole twice daily for seven days for BV while yeast infected pregnant were treated by Miconazole 2% cream 5 g intravaginally for 7 days.

CHAPTER III: RESULTS

3.1. Sociodemographic characteristics of study subjects

A total of 263 pregnant women visiting obstetrics and Gynecology OPD for ANC in Tikur Anbessa hospital were screened for bacterial vaginosis between November 2011 and April 2012. Of these 252 are included in the analysis while 11 participants were not included due to incompleteness of information. The baseline sociodemographic information of study participants are presented in Table 3.1.

The mean age of the study participant was 27.6 ($\pm$ 4.7) and all are resident in urban. Of 252 study participant 180(71.4%) were Orthodox, 144(57.1%) are grade 12 completed and above, 105(41.7%) were government or NGO employed while 118(46.8%) were house wife. Overall 92(36.5%) of the study participant had no or had less than 500 ETB personal monthly income. In addition nearly all 241(95.6%) of the study participants are married and 201(79.8%) had only one life time sexual partner. Approximately half of the study participants used contraceptive before the present pregnancy.

Table 3.1: Sociodemographic characteristics of 252 pregnant women screened for bacterial vaginosis in Tikur Anbessa Hospital (November 2011-April 2012).

Variables	No.	Percent
Age (years)		
≤20	17	6.7
21-29	156	61.9
30+	79	31.3
Mean ± SD	27.6 ± 4.7 years	
Religion		
Orthodox	180	71.4
Muslim	51	20.2
Protestant	21	8.3
Education		
< Grade 12	108	42.9
≥ Grade 12	144	57.1
Occupation		
Employed	105	41.7
House wife	118	46.8
Others	29	11.5
Personal income (ETB)		
0-500	92	36.5
501-1500	73	29.0
>1500	87	34.5
Marital status		
Married	241	95.6
Unmarried	11	4.4
No. of LTSP		
One	201	79.8
Two and above	51	20.2
Use of Contraceptive		
Yes	120	47.6
No	132	52.4
Type of Contraceptive used		
Loop/ IUD	3	2.5
Injection	50	41.7
Pills	41	34.2
Norplant	26	21.6

ETB – Ethiopian birr, LTSP- Life time sexual partner, IUD- intrauterine device

3.2. Obstetric data and Hygienic Practice of the study subjects

At the time of data collection 36(14.3%) of the study participants were at their first trimester gestational age while 121(48%) were at their third trimester gestational age. Ninety one (36.1%) pregnant women were Primigravidia. Among 161(63.9%) pregnant women with Multigravidia, 24 (14.9%) of pregnant women had previous history of spontaneous abortion. None of the study participants had vaginal douching practice in the preceding month. However, almost all study participants had a daily vaginal bathing practice.

Table 3.2: Obstetric data and hygienic practices of 252 pregnant women screened for bacterial vaginosis in Tikur Anbessa Hospital (November 2011-April 2012).

Variables	No.	Percent
Gestational age		
1st trimester	36	14.3
2nd trimester	95	37.7
3rd trimester	121	48
Gravidity		
Primigravidia	91	36.1
Multigravidia	161	63.9
History of abortion		
Yes-spontaneous	24	14.9
Yes-induced	6	3.7
No	131	81.4
History of Preterm birth		
Yes	6	3.7
No	155	96.3
History of Still birth		
Yes	7	4.3
No	154	95.7
Vaginal bathing		
Daily	245	97.2
Less than daily	7	2.8
Frequency of showering		
Daily	28	11.1
Less than daily	224	88.9

3.3. Prevalence of Bacterial Vaginosis

I. Clinical Diagnosis (Amsel's Criteria)

The overall prevalence of bacterial vaginosis in the present study was 46/252 (18.3%) by Amsel's criteria. Majority of BV infected women (65.2%) had no clinical symptoms. Only sixteen BV infected women were report abnormal discharge with or without unpleasant smell. Being symptomatic increases the chance to be diagnosed positive for bacterial vaginosis.

Table 3.3: prevalence of bacterial vaginosis based on Amsel's criteria among pregnant women attending ANC in Tikur Anbessa hospital (November 2011 - April 2012)

	Amsel's 2 of 3 criteria			Odd ratio	p- value
	Positive No. (%)	Negative No. (%)	Total No. (%)		
Symptomatic	16 (34.8)	41 (19.9)	57 (22.6)	2.15	< 0.05
Asymptomatic	30 (65.2)	165 (80.1)	195 (77.4)	1.00	(0.039)
Total	**46 (18.3)**	**206 (81.7)**	**252 (100)**		

II. Microbiological Diagnosis (Nugent scoring system)

The Gram stain result of 183/252 (72.6%) of the study participant classified as normal, 20 (7.9%) of participant Gram stain result were graded as an intermitted while the remaining 49 (19.4%) were positive for bacterial vaginosis by Nugent scoring system. As shown in Table 3.4, the prevalence of bacterial vaginosis among symptomatic pregnant was 18/57 (31.6%) but among asymptomatic 31/195 (15.9%). Majority (63.3%) of Bacterial vaginosis diagnosed pregnant women were asymptomatic. Gram stain result of vaginal smear is shown in figure 3.1 and 3.2.

Table 3.4: Prevalence of bacterial vaginosis based on Nugent scoring system among pregnant women attending ANC in Tikur Anbessa hospital (November 2011 - April 2012)

	Microbiological diagnosis			Odd ratio	p- value
	Positive No. (%)	**Negative No. (%)**	**Total No. (%)**		
Symptomatic	18 (36.7)	39 (19.2)	57 (22.6)	2.44	< 0.05
Asymptomatic	31 (63.3)	164 (80.8)	195 (77.4)	1.00	(0.032)
Total	**49 (19.4)**	**203 (80.6)**	252 (100**)**		

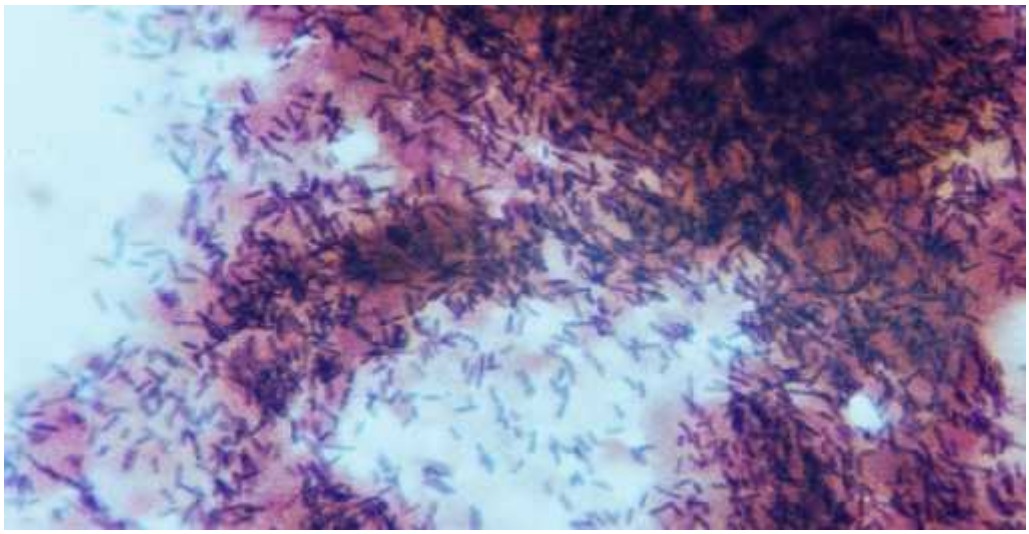

Figure 3.1: Photograph of gram stained smear of vaginal swab showing *Lactobacilli* Morphotypes

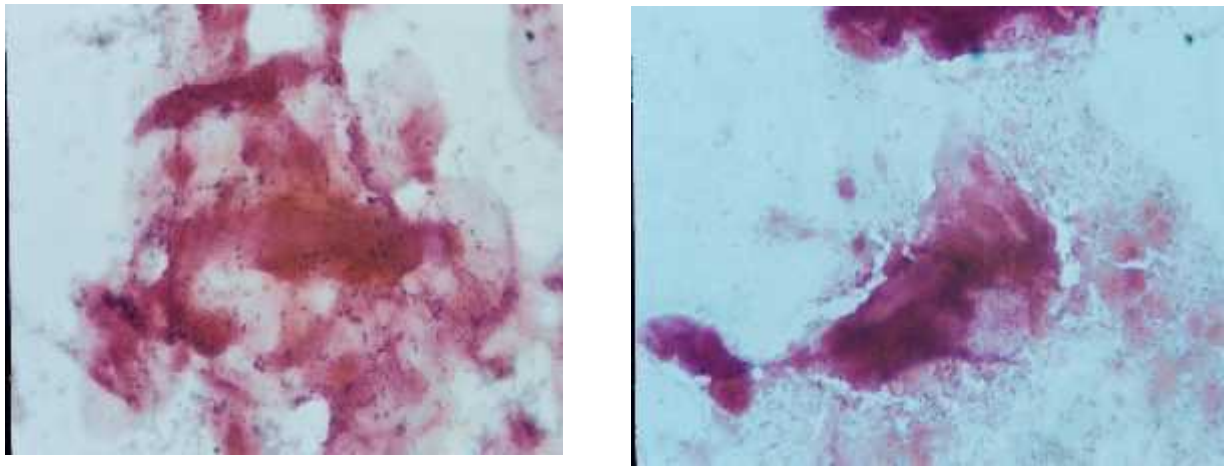

Figure 1.2: Photograph of gram stained smear of vaginal swab showing clue cells

3.4. Reported vaginal symptoms and bacterial vaginosis

As shown in Table 3.5 more vaginal symptoms were reported from BV negative pregnant women than BV positive pregnant women except for abnormal discharge with unpleasant smell. Out of unusual vaginal discharge complained women only 27 (47.4%) were suspected to be positive for bacterial vaginosis due to the presence of non-Candida like discharge. From BV positive only 18/49 (36.7%) pregnant women were reporting the presences of abnormal vaginal discharge with or without unpleasant smell. Among the type of discharge non Candida like discharge is significantly associated with BV.

Table 3.5: Reported vaginal symptoms associated with bacterial vaginosis among pregnant women attending ANC in Tikur Anbessa Hospital (November 2011 – April 2012).

Vaginal symptom	BV positive (%)	BV negative (%)	Total (%)	p- value
Vaginal pain				
Yes	2 (33.3)	4(66.7)	6(2.4)	0.39
No	47(19.1)	199(80.9)	246(97.6)	
Itching or burning				
Yes	11(25.6)	32(74.4)	43(17.1)	0.27
No	38(18.2)	171(81.8)	209(82.9)	
Abnormal discharge				
Yes	18(31.6)	39(68.4)	57(22.6)	0.01
No	31(15.9)	164(84.1)	195(77.4)	
Type of discharge				
Candida like	5(16.7)	25(83.3)	30(52.6)	0.014
Non Candida like	13(48.1)	14(51.9)	27(47.4)	
Unpleasant smell				
Yes	7(87.5)	1(12.5)	8(14)	0.005
No	11(22.4)	38(73.8)	49(86)	

3.5. Comparison of Amsel's Criteria and Nugent Scoring system for the diagnosis Bacterial Vaginosis

The prevalence of bacterial vaginosis was 18.3% by Amsel's 2 of 3 criteria and 19.4% by Nugent scoring (Table 3.6). Amsel's criteria had sensitivity of 85.7% and a specificity of 98% when compared using Gram stain evaluated by Nugent scoring method as standard.

Table 3.6: comparison of Amsel's criteria and Nugent scoring for the diagnosis of bacterial vaginosis among pregnant women attending ANC in Tikur Anbessa Hospital (November 2011 – April 2012)

		Nugent scoring		P-value	SN	SP	PPV	NPV
		Positive	**Negative**					
Amsel criteria	**Positive**	42	4	<0.05	85.7	98	91.3	96.6
	Negative	7	199					
	Total	49	203					

3.6. Diagnostic Accuracy of Individual Amsel's criteria

When we compare individual Amsel's criteria with Nugent scoring, in the present study we found that clue cells was the criteria with the highest sensitive and specificity (Table 3.7). The sensitivity of the remaining individual criteria ranged from 69% to 82%. All criteria had high negative predictive value (93-97.5%).

Table 3.7: Diagnostic accuracy of individual clinical criteria among pregnant women attending in Tikur Anbessa Hospital (November 2011-April 2012).

Amsel's Criteria	N (%)	SN (95% CI)	SP(%)(95% CI)	PPV	NPV
Vaginal pH	65 (25.8)	81.6 (68.6, 90)	87.7 (82.5, 91.5)	61.5	95.2
Amine test	39 (15.5)	69.4 (55.5, 80.5)	97.5 (94.4, 98.9)	87.2	93
Clue cells	48 (19.0)	89.8 (78.2, 95.6)	98 (95, 99.2)	91.7	97.5

N - Number
SN - Sensitivity
SP - Specificity
PPV - Positive predictive value
NPV - Negative predictive value

3.7. Diagnostic accuracy of combination of two of the Amsel's criteria

Specificity of the combination of any two Amsel's criteria as shown in Table 3.7 ranged from 99% -100%. The Combination of Amine test with clue cells had hundred percent specificity and positive predictive value but had less specificity. Even though combinations of clue cells with other criteria increase its specificity and predictive value of positive, it markedly decreases its sensitivity. The combination of pH with clue cells had the highest sensitivity and negative predictive value.

Table 3.8: Diagnostic accuracy of combination of two Amsel's criteria among pregnant women attending Tikur Anbessa Hospital (November 2011-April 2012).

Amsel's Criteria	N (%)	SN (95% CI)	SP (95% CI)	PPV	NPV
Vaginal pH +Amine test	32(12.7)	61.2(47.3, 73.6)	99(96.5, 99.7)	93.8	91.4
Vaginal PH + clue cells	40(15.9)	77.6(64, 87)	99(96.5, 99.7)	95	94.8
Amine test + Clue cells	32(12.7)	65.3(51.3, 77)	100(98, 100)	100	92.3

N - Number
S - Sensitivity
SP - Specificity
PPV - Positive predictive value
NPV - Negative predictive value

3.8. Risk Factors Associated with Bacterial Vaginosis

In the Bivariate analysis (Table 3.9) personal monthly income ≤ 500 ETB OR (CI) 5.26 (2.16, 12.8), multiple sexual partners history OR (CI) 14.04(6.7, 29.43), first trimester gestational age OR (CI) 3.46 (1.48, 8.1) and previous history of spontaneous abortion OR (CI) 4.8 (2.03, 11.31), daily showering OR (CI) 7.72 (3.35, 17.78), were associated with bacterial vaginosis. Yeast infection was seems like a risk for bacterial vaginosis among pregnant women without a significant association. However, after we had adjusted for confounders in multivariate analyses (Table 3.10), more than one lifetime sexual partners (OR: 8.6; 95% CI: 2.5, 29; p=0.01) and previous history of spontaneous abortion (OR: 5.9; 95% CI: 1.5, 23; p=0.012) remained independently associated with increased likelihood of BV positive. Some factors that lacked independent associations with BV during pregnancy remained important confounders and were therefore retained in the final model.

Table 3.9: Bivariate analysis of factors association with bacterial vaginosis among pregnant women attending ANC in Tikur Anbessa Hospital (November 2011 - April 2012).

Variables	BV positive	BV negative	COR (95% CI)
Age			
≤20	3(17.6)	14(82.4)	1.09(0.27, 4.33)
21-29	33(21.2)	123(78.8)	1.36(0.67, 2.77)
30+	13(16.5)	66(83.5)	1.00
Religion			
Orthodox	40(22.2)	140(77.8)	1.00
Muslim	7(13.7)	44(86.3)	0.56(0.23, 133)
Protestant	2(9.5)	19(90.5)	0.37(0,08, 1.65)
Education			
< Grade 12	25(23.1)	83(76.9)	1.51(0.81, 2.82)
≥ Grade 12	24(16.7)	120(83.3)	1.00
Occupation			
Employed	19(18.1)	86(81.9)	1.00
House wife	24(20.3)	94(79.7)	1.16(0.59, 2.26)
Others	6(20.7)	23(79.3)	1.18(0.42, 3.3)
Income (ETB)			
≤500	29(31.5)	63(68.5)	5.26(2.16, 12.8)
501-1500	13(17.8)	60(82.2)	2.48(0.93, 6.58)
>1500	7(8.0)	80(92.0)	1.00
No. of LTSP			
One	20(10)	181(90)	1.00
Two and above	29(56.9)	22(43.1)	11.9(5.8, 24.5)
Gestational age			
1st trimester	13(36.1)	23(63.9)	3.46(1.48, 8.1)
2nd trimester	19(20)	76(80)	1.53(0.75, 3.14)
3rd trimester	17(14)	104(86)	1.00
No. of pregnancy			
Primigravidia	17(18.7)	74(81.3)	1.00
Multigravidia	32(19.9)	129(80.1)	1.08(0.56, 2.08)
Abortion			
Yes-spontaneous	12(50)	12(50)	6.28(2.45, 16.11)
Yes-induce	2(33.3)	4(66.7)	3.14(0.54, 18.41)
No	18(14)	113(86.3)	1.00
Vaginal bathing			
Daily	46(18.8)	199(81.2)	0.31(0.07, 1.43)
Less than daily	3(42.9)	4(57.1)	1.00

Showering frequency			
Daily	16(57.1)	12(42.9)	7.72(3.35, 17.78)
Less than daily	33(14.7)	191(85.3)	1.00
Previous BV/GTI			
Yes	3(15)	17(85)	1.4(0.39, 4.99)
No	46(19.8)	186(80.2)	1.00
Yeast infection			
Yes	9(32.1)	19(67.9)	2.18(0.92, 5.17)
No	40(17.9)	184(82.1)	1.00

Table 3.10: Multivariate analysis of factors associated with bacterial vaginosis among pregnant attending ANC in Tikur Anbessa hospital (November 2011- April 2012).

Variables	**COR (95% CI)**	**AOR (95% CI)**	**P –value**
Personal income ≤500 ETB	5.26 (2.16, 12.8)	3(0.6, 14.6)	0.17
Multiple life time sexual partner	14.04 (6.7, 29.43)	8.6 (2.5, 29)	0.01 (sig)
First trimester gestational age	3.46 (1.48, 8.1)	1.9 (0.4, 8.6)	0.40
Previous abortion	4.8 (2.03, 11.31)	5.9 (1.5, 23)	0.012(sig)
Daily showering	7.72 (3.35, 17.78)	2.7 (0.6, 12)	0.19

COR – Crude Odd Ratio; AOR – Adjusted Odd Ratio; ETB- Ethiopian Birr; Sig- Statically Significant

3.9. Other organisms

During wet mount preparation *T. vaginalis* from saline wet mount and Yeast cells from KOH wet mount were assessed. Yeast cells were diagnosed from 28 (11.1%) pregnant women while none of the participants had *T. vaginalis*.

CHAPTER IV: DISCUSSION

Bacterial Vaginosis (BV) is the name of a condition in women where the normal balance of bacteria in the vagina is disrupted and replaced by an overgrowth of certain bacteria. It is sometimes accompanied by discharge, odor, and pain, itching, or burning (CDC, 2008). It is an extremely prevalent vaginal condition and the number one cause of vaginitis among both pregnant and nonpregnant women (CDC, 2010). Bacterial vaginosis has been associated with a variety of adverse health and poor pregnancy outcomes. It can cause increased risk in preterm birth, low birth weight and PID (BASHH, 2006; CDC, 2008; Nelson and Macones, 2002). Bacterial vaginosis also has been found that it is associated with increased incidence and prevalence of different STI (Allsworth and Peipert, 2007; Cherpes *et al*., 2008; Schwebke, 2005). Although an extensive research is available worldwide, there is no published study conducted in Ethiopia about BV prevalence among pregnant women.

The present study was carried out to determine the prevalence of bacterial vaginosis and to evaluate diagnostic performance of Amsel's criteria for Clinical diagnosis of bacterial vaginosis among 252 pregnant women attending ANC in Tikur Anbessa University Hospital from November 2011 to April 2012. The study subject includes 18-50 years old pregnant women at any gestational age and lives in urban.

In this study of bacterial vaginosis among pregnant women, 18.3% prevalence of bacterial vaginosis determined by Amsel's two of three criteria was comparable with the study done among pregnant women with preterm labor in Pakistan in which the prevalence of BV was 21% (Islam *et al*., 2009). Consistent with our study, 19.6% prevalence of bacterial vaginosis was documented among HIV negative pregnant women in Tanzania (Msuya *et al*., 2009). In contrast to our finding higher prevalence of bacterial vaginosis was diagnosed on the basis of three of four Amsel's criteria as 38.5% among symptomatic pregnant women (Neelam and Sohail, 2010). This high prevalence of bacterial vaginosis may be due to that all study participants are symptomatic women with complaints vaginal discharge. In addition difference of the number of Amsel criteria used for diagnosis of bacterial vaginosis can be a factor for this prevalence variation from our study.

In the present study, the overall prevalence of bacterial vaginosis by Gram stains Nugent scoring criteria was 19.4%. This result is comparable with other studies done among pregnant women in different countries e.g. in India 20.5% (Lata *et al*., 2010) and in Denmark 17% (Vogel *et al*., 2006).

Our data suggest that prevalence of BV is low comparing with the previous studies done in Addis Ababa which was 32% (Aynalem *et al.*, 2010), although it was not exclusive report of pregnant women. Bacterial vaginosis prevalence of 19.4% in pregnant women in our study is also lower than the 25.3% reported prevalence among women with genital discharge seeking primary care in Addis Ababa (Wolday *et al*., 2004). Similar with our method, these two studies use Gram stain of vaginal smear evaluated by Nugent scoring. However, low prevalence of bacterial vaginosis in the present study may be due difference in study subjects since the majority of previous study subjects were non pregnant women who are seeking primary care due to some problem like genital discharge and gynecologic problems.

In addition to this, the current study showed lower prevalence of bacterial vaginosis than reports from different sub-Saharan countries like Kenya (37%) (Marx *et al*., 2010), Botswana (38%) (Romoren *et al*., 2007), Zimbabwe (32.5%) (Kurewa *et al*., 2010) using microbiological diagnosis methods. One reason for this lower prevalence of BV in our study may be due to that absence of vaginal douching in our study population, difference in vaginal hygiene practices and sexual behaviors; because these factors are the main determinants of BV most likely to vary between populations and geographical locations (Trabert and Misra, 2007). The decreased prevalence in our finding also can be due to most of the study in the above mentioned countries were done among specific gestational age not include all gestational age. Besides these, the lower prevalence of BV in this study can be also explained as most of the women were in third trimester gestational age since as gestational age increase the prevalence of BV decrease.

In contrast to the present study, lower prevalence of bacterial vaginosis was reported in Burkina Faso (6.4%) (Kirakoya-Samadoulougou *et al*., 2008), India (8.6%) (Dadhwal *et al*., 2010), Sweden (9.3%) (Larsson *et al*., 2007), Boston (11%) (Delaney and Onderdonk, 2001)

and Washington (12%) (Krohn *et al*., 1989). This may be due to environmental, behavioral, socioeconomic status and stressor differences in the geographical variation.

Bacterial vaginosis is mostly present without sign and symptoms. The most common clinical sign and symptoms of BV are the presence of abnormal vaginal discharge which is thin white or gray homogenous in color with or without unpleasant smell. The smell of the discharge mostly noticed after sexual intercourse (BASHH, 2006; CDC, 2008; Eschenbach *et al*., 1988). Other sign and symptoms sometimes occur in bacteria vaginosis infected women includes burning during urination or itching around the outsides of vagina, or both (CDC, 2008). In the current study we found that 63.3% of participants who were diagnosed positive for BV by gram stain had no symptom for BV. This result has a consistency with other studies done in different countries (Amsel *et al*., 1983; Romoren *et al*., 2007; Aynalem *et al*., 2010). Vaginal discharge complains by women has less value as diagnostic algorithm because approximately one third (31.6%) of BV positive participant only were report abnormal discharge when asked. These are consistent with other study which report that only very few women reported vaginal symptom when asked (Marx *et al*., 2010). In addition, higher proportions of vaginal symptoms including vaginal pain, abnormal discharge, itching or burning were reported form BV negative women than BV positive women of this study. In contrast to our result, slightly higher proportion of BV positive compared to BV-negative women presented with abnormal discharge or odour and equal vaginal symptom of burning/itching, pain was reported between the two groups (Nelson *et al*., 2007b). These high vaginal symptom reports from bacterial vaginosis negative pregnant women in our study may be due to that presence of other genital tract infection that cannot be detected by the method used in the current study.

In our study we did not examine the vaginal environment using speculum to evaluate the presence and type of discharge used for diagnosis of bacterial vaginosis among pregnant women who do not complain vaginal discharge. However, women having abnormal discharge were examined by attending physician and the types of discharge were characterized in to Candida like and non Candida like. Out of all vaginal discharge complaint pregnant women 27 cases were suspected for bacterial vaginosis and the other 30

were categorized as Candida like discharge. Comparing with a women having Candida like discharge, pregnant women with non Candida like abnormal discharge had a greater chance (OR: 4.6; 95% CI: 1.4, 15.7; p=0.014) to be positive for bacterial vaginosis. In addition we also found that the presence of abnormal vaginal discharge (p=0.01) and unpleasant smell (p=0.005) are also reported vaginal symptoms associated with bacterial vaginosis. In general abnormal vaginal discharge is not accurate sensitive indicator of BV status in this study which confirms the suggestions by other studies (Nelson *et al.*, 2007b; Romoren *et al*., 2007) but symptomatic pregnant have more than a twofold increased chance to be positive for bacterial vaginosis than asymptomatic. Eventhough further studies are necessary to prove, use of syndromic diagnostic algorithms can reduce detection and prediction of bacterial vaginosis because the syndromic diagnosis algorithms advocated by the WHO for vaginal infections including BV and other STIs often begin with a patient's complaint of vaginal discharge (WHO, 2005). Such algorithms were demonstrated to be of limited value among our study, as small BV positive women complained of vaginal discharge when asked, but it may have higher validity in other populations such as women clinics where patients present specifically due to reproductive health symptoms such as vaginal discharge.

The importance of diagnosis and treatment of BV in various clinical settings is increasingly recognized. Treatment with antibiotics might be helpful in some cases of idiopathic preterm labor but at present knowledge and diagnostic methods are not sufficient in recommending antibiotic therapy in routine clinical practice. Rapid screening with available resource is essential for a favorable health care outcome. Classical initial method of BV diagnosis was done by isolation of *G. vaginalis* from clinical specimen (Sobel, 2012; Al-Muk and Hasony, 2001). Later on with the advent of the anaerobic culture technique other organisms are also detected from those women with disturbed flora. The diagnostic approach of bacterial vaginosis varies from time to time and at different clinical setting and purpose. Despite this, Amsel's clinical diagnosis and gram stain evaluation by Nugent methods are mostly used worldwide particularly in developing countries. However, in resource-poor settings the World Health Organization (WHO) syndromic management protocol for vaginal discharge is most commonly used for management of STD or BV infected women. Since most BV infected women are asymptomatic, this management protocols have poor sensitivity and

specificity for bacterial vaginosis infected women (Wolday *et al*., 2004). Failure to screen BV has been associated with poor pregnancy outcome and different infection. The Nugent scoring test requires health care experts, laboratory support, and access to high-power microscopy to obtain timely results for the diagnosis of BV. Since these necessities are not always available in developing countries, it is important to have simple and reliable clinical criteria that clinicians can use in practice. Therefore knowledge of best diagnostic approach in a given area using the available resource helps to inform the preference method.

Taking into account this, we compared the Amsel's diagnosis method and its accuracy with the standard Gram stain Nugent scoring method. In addition to Amsel's criteria we evaluated individual and combination of Amsel's criteria using Gram stain Nugent criteria as a gold standard, for the diagnosis of bacterial vaginosis. The diagnostic accuracy of clinical diagnosis, individual and combined Amsel's criteria is depicted in Tables 3.6, 3.7 and 3.8 respectively.

Clinical diagnosis by Amsel's criteria has been reported that a good sensitivity and specificity (Amsel *et al*., 1983). However, the subjective nature in the evaluation of clinical criteria and since many cases of bacterial vaginosis is asymptomatic result in significant under diagnosis of BV in some centers and in some study groups (Goyal *et al*., 2005). In the present study, we found that almost equal prevalence of BV by Amsel's criteria and Gram stain (18.3% vs. 19.4%). Consistent with our study, almost equal prevalence of bacterial vaginosis (6.7% vs. 8.6%) by the two methods was reported from the study that was conducted among 502 New Delhi pregnant women (Dadhwal *et al.*, 2010). The researcher was also considering three of four Amsel's criteria for clinical diagnosis of bacterial vaginosis. In addition to these our BV prevalence finding also similar with the findings from general population in South India (18% vs. 19%) (Udayalaxmi *et al*., 2011). In contrast to our finding, the study conducted among 200 symptomatic women in the rural setting had documented higher prevalence of bacterial vaginosis by three of four Amsel's criteria than Nugent gram stain (49% vs. 35%) (Posner *et al*., 2005). These differences may be due to difference in number of Amsel's criteria used. The other possible reason the presence of

high prevalence of abnormal vaginal discharge in the study population increases the probability of a woman to be positive by Amsel criteria.

When we correlate any two of three Amsel's criteria (excluding type of discharge) with Gram stain Nugent criteria for diagnosis of bacterial vaginosis, we found that the sensitivity, specificity, PPV and NPV Amsel's was 85.7%, 91.3%, 98 and 96.6% respectively which is almost equal with the clue cells performance. The result from Texas (Mastrobattista *et al*., 2000) among 69 asymptomatic pregnant women which compared clinical criteria to Gram stain in the diagnosis of bacterial vaginosis by considering two of the following three criteria: vaginal pH more than 4.5, Amine test positive and clue cells positive on wet smear; clinical diagnosis had a lower sensitivity of 56%, a comparable specificity of 96% and a lower positive and negative predictive value of 83% and 85%, respectively than the current study clinical diagnosis. This difference in sensitivity and predictive value may be due to difference of study population clinical case and higher prevalence of bacterial vaginosis (27%). The sensitivity and specificity of Amsel's criteria comparing with Gram stain result was 35% and 99% respectively (Grantacos *et al*., 1999). Amsel's method was found to be 78% sensitive and 95.6% specific as compared to Nugent's method (Udayalaxmi *et al*., 2011). Consistent with these two study we found that almost equal specificity of Amsel's criteria.

From individual criteria for predicting the gram stain result, clue cells detection from wet mount microscopic examination is the single most reliable predictor of bacterial vaginosis. This is consistent with the study done by other researcher's for example Eschenbach *et al*., 1988; Goyal *et al*., 2005; Islam *et al*., 2009; Mittal *et al*., 2012. It had a higher sensitivity, specificity and positive and negative predictive value. But our observation is not consistent with other researcher like Dadhwal *et al* in which clue cells criteria is the least in sensitivity and negative predictive value (Dadhwal *et al.,* 20l0*)*. This difference may be due to the subjective nature inherent in the evaluation of the test.

Normal vaginal pH ranges from 3.8 to 4.5. The presence of blood, sperm (recent sexual intercourse), amniotic fluid or cervical mucus's are some factors which increase vaginal pH

without the presence of any vaginal infection (Carr *et al*., 1998). We found that vaginal pH had moderate sensitivity and negative predictive but it was the lowest in specificity and positive predictive value from the other two clinical diagnostic criteria. This result is similar with the finding by Mastrobattista and his colic's which was done among 69 asymptomatic pregnant women (Mastrobattista *et al.,* 2000). In comparison with this study we found that higher sensitivity, specificity, PPV and NPV of vaginal pH. This difference may be due to that our study participants include both symptomatic and asymptomatic pregnant women. Many studies suggest that raised vaginal pH is recognized as the least specific criteria (Mastrobattista *et al.,* 2000; Mittal *et al*., 2012) and it is also confirmed in our investigation. In our study, increased vaginal pH (> 4.5) had lower sensitivity (81.6% vs. 85.7%) and specific (87.7% vs. 98%) than the Amsel's criteria. The lower specificity and positive predictive value of vaginal pH compared to others clinical criteria in this study indicates the presence of other genital tract infection or factor which increases pH without the presence of disturbed vaginal flora.

In our study whiff test diagnostic criteria was the lowest in sensitivity (69.4%) but its specificity (97.5%) was as good as that of Amsel's criteria. This finding is comparable with the result from Dadhwal *et al.* in which sensitivity was 60.4% and specificity was 96.7% (Dadhwal *et al*., 2010). However, our findings did not support the suggestions in which the whiff test was a highly sensitive and specific method (Gutman *et al.,* 2005; Neelam and Sohail, 2010). The decrease in sensitivity of whiff test may attribute to subjective nature of the test due sensation ability of the person doing the test. The other factor may be absence or presence of low number of amine producing abnormal microorganism.

Our results indicate that clue cells from individual criterion by its own sufficient to diagnose BV, but if we modify Amsel criteria by using a combination of any two criteria, there is decreased sensitivity and increased specificity. Combination of any two Amsel's criteria for clinical diagnosis can simplify the more cumbersome and time consuming Amsel's technique. The combination of two criteria had sensitivity of 61.2% to 77.6%, specificity of 99% to 100%. Amine test plus pH as diagnosis of clinical test is mostly recommended among the study done in different setting, population and countries (Posner *et al*., 2005;

Dadhwal *et al*., 2010; Mittal *et al*., 2012). But these two Amsel's criteria can be present without the disturbance of the vaginal flora or as a result of other genital tract infection example during T. vaginalis (Sobel, 2012). In contrast to these three studies we found that combination of clue cells and pH had the highest sensitivity and very good specificity than pH and amine test. In addition, in our study we found that raised vaginal pH lacks specificity and whiff test lacks sensitivity in comparison with clue cells. So in conditions where there is no enough time and gram stain procedure, combination of vaginal pH and clue cells detection can be used with only seven false negative diagnosis but comparative sensitivity and specificity. Eventhough vaginal pH can be affected by other factors; clue cells are the best specific for bacterial vaginosis (Sobel, 2012).

With attention to the above findings, it can be concluded that bacterial vaginosis has a varying degree of prevalence rate among people of different communities which might be due to certain factors such as hygiene behaviors and sociodemographic characteristics. Therefore, it is important to try to establish a correlation between BV and factors affecting its prevalence. Because our sample population was exclusively urban living and nearly all women were married, had no previous history of preterm and still birth and had daily vaginal bathing, we cannot effectively examine these risk factors. Additionally it may be in appropriate to compare the frequency of bacterial vaginosis among religious demonstrations due lack comparability in the number of participants.

Consistent with other studies, in our study there was a significant correlation between number of life time sexual partner and prevalence of BV. Multiple life time sexual partners was more likely a risk factors for bacterial vaginosis. This is consistent with other researcher findings (Bailey *et al*., 2004; Brtoman *et al.*, 2008b; CDC, 2008; Smart *et al*., 2004), whereas other findings showed no significant correlation in this regard (Trabert and Misra, 2007). One explanation for this association may be the content and the pH of the semen may disturb the normal flora and increase the pH of the vagina. The other explanation to these may be transmission factors even though sexual transmission of BV is controversial. In addition the use of spermicidal condom or inconsistent condom use also can be a factor for this occurrence because consistent condom use prevents development of BV by women

(Hutchinson *et al*., 2007; Smart *et al*., 2004). Similar to other studies, there was no association observed between the prevalence of BV and age group, as almost equal prevalence was seen in our study age groups. However, other studies showed a significant correlation between BV and different age groups (Allsworth and Peipert, 2007). Some researchers have reported that more prevalence of BV among younger age (Lata *et al*., 2010), while others said BV is common as age increases (Jones *et al*., 2007; Aynalem *et al*., 2010; Allsworth and Peipert 2007).The causes for the age distribution patterns of BV are difficult to justify, as possibly a variety of behavioral, physiological (hormonal), and immunological variables interact. The results of current study also showed that there was no a significant correlation between BV and educational status, which is similar to other similar studies (Trabert and Misra, 2007); it was not evident that completing grade 12 has been found to be significantly associated with BV among women in Addis Ababa. Our data showed that among different monthly income in the current study, women who got greater than 1500 birr were proportional in number with who got less than or equal to 500. However, as compared to high income, BV was diagnosed significantly more frequent in women with low income. This difference of BV prevalence by income may be due to socio-economic and psychological factors. However, in our study personal monthly income had no independent association with bacterial vaginosis infection which is not similar with the finding of Allsworth and Peipert (Allsworth and Peipert, 2007). This contradiction may be due to that we consider personal monthly income only not family income or economic status.

Women diagnosed positive for BV at first trimester have a greater probability of second trimester pregnancy loss than BV negative pregnant women (Nelson *et al*., 2007a). On the other hands BV infection during pregnancy increases the probability of spontaneous abortion (Sobel, 2000). In the current study, there was also a significant correlation between previous history of spontaneous abortion and prevalence of BV. We found that previous spontaneous abortion is independently associated as a risk factor of bacterial vaginosis infection. This is consistent with other study done among pregnant women in Burkina Faso (Kirakoya-Samadoulougou *et al*., 2008). In addition, on univariate analysis, we found that first trimester stage of pregnancy is seems like to have increased risk of bacterial vaginosis

and as the age of pregnancy increase the prevalence of BV decrease. Although additional research on the mechanism for variation is required, this variation may be due to physiological change as gestational age increase. The other possible reason may be sexual behavior change like decrease in frequency of sexual intercourse as pregnancy age increase. We also found that being Primigravidia or Multigravidia had no risk for bacterial vaginosis, whereas other findings showed independent correlation of these issues with BV (Smart *et al*., 2004).

Different hygiene behavior affects the normal balance of vaginal environment. This factor can help for the development of bacterial vaginosis either by removing the normal inhabiting microorganism or disturb the normal pH of vagina. The most common cause of BV causing hygiene behavior is vaginal douching. Other hygiene behaviors including bathing and showering frequency do not have association with BV (Klebanoff *et al*., 2010). In the current study we found similar association between BV and hygiene behavior other than douching.

Different researchers showed that other organisms were present with BV like Yeast cell and *T. vaginalis* e.g. 59% candida *yeast* and 19% *T. vaginalis* by Romoren *et al*. (2007), 4.5% Candida infection (Lata *et al*., 2010). This finding also observed in our study the presence yeast cells among 11.1% of the study participants and none of them had *T. vaginalis.*

CONCLUSION AND RECOMMENDATIONS

The overall prevalence of bacterial vaginosis among pregnant women is 19.4%. The prevalence of BV among symptomatic pregnant women was 31.6% while among asymptomatic pregnant women it was 15.9% and BV infection is mostly asymptomatic. Bacterial vaginosis can be diagnosed using simplified method clinically. Combination of two Amsel's criteria including clue cells detection from wet mount preparation of vaginal swab and measurement of vaginal pH can be used for clinical diagnosis. Bacterial vaginosis is significantly associated with multiple lifetime sexual partner and previous history of spontaneous abortion.

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

- Routine screening of all pregnant women by Amsel's criteria is recommended because most bacterial vaginosis diagnosed women are asymptomatic and very few women are eager to report the presence of discharge to health care provider.
- Since BV infection is mostly asymptomatic, previous pregnancy out come for risk assessment should be included in the current syndromic management algorithm which is mostly applicable to women who attend STI/STD or gynecology clinic due to some problem in order to apply for ANC attendees.
- Clinical diagnosis of BV in pregnant women using combination of Clue cell and vaginal pH Amsel's criteria is recommended to simplify and for rapid screening of bacterial vaginosis among pregnant.
- Future research should be done to identify the association between bacterial vaginosis and pregnancy outcome.

REFERENCES

Allsworth JE, Peipert JF. (2007) Prevalence of Bacterial Vaginosis: 2001-2004 National Health and Nutrition Examination Survey data. *Obstet Gynacol*; **109**: 114-20.

Al-Muk JM, Hasony HJ. (2001) Isolation of *Gardnerlla vaginalis* from pregnant women with bacterial vaginosis in Basrah, Iraq. *Bahrain Med Bull*; **23*:***124-26.

Alvarez-Olmos MI, Barousse MM, Rajan L, Van Der Pol BJ, Fortenberry D, Orr D, Fidel PL. (2004) Vaginal Lactobacilli in Adolescents: presence and relationship to local and Systemic Immunity, and to Bacterial Vaginosis. *Sex Transm Dis*; **31**:393-400.

Amsel R, Totten PA, Spiegel CA Chen KC, Eschenbach D, Holmes KK. (1983) Nonspecific vaginitis: Diagnostic criteria and microbial and epidemiologic associations. *Am J Med*; **74**:14-22.

Antonio MD, Hawes SE, Hillier SL. (1999) The identification of vaginal Lactobacillus species and the demographic and microbiologic characteristics of women colonized by these species. *J Infect Dis*; **180**:1950-1956.

Aroutcheva A, Gariti D, Simon M, Shott S, Faro J, Simoes JA, Gurguis A, Faro S. (2001) Defense factors of vaginal lactobacilli. *Am J Obstet Gynecol*; **185**:375–9.

Atashili J, Poole C, Ndumbe PM, Adimora AA, Smith JS. (2008) Bacterial vaginosis and HIV acquisition: A meta-analysis of published studies. *AIDS*; **22**: 1493–1501.

Austin MN, Beiji RH, Meyn LA, Hillier SL. (2005) Microbiologic Response to Treatment of Bacterial Vaginosis with Topical Clindamycin or Metronidazole. *J Clin Microbiol;* **43**:4492–4497.

Ayenalem S, Yusuf L and Ashenafi M. (2010) Lactic Acid Bacterial Vaginosis among Outpatients in Addis Ababa. *Ethiop J Health Dev.*, **24**: 198-204.

Bahram A, Hamid B, Zohre T. (2009) Prevalence of Bacterial Vaginosis and Impact of Genital Hygiene Practices in Non-Pregnant Women in Zanjan, Iran. *Oman Med J;* **24**: 288-293.

Bailey JV, Farquhar C, Owen C. (2004) Bacterial vaginosis in lesbians and bisexual women. *Sex Transm Dis*; **31**:691-4.

BASHH (2006) National Guideline for the Management of Bacterial Vaginosis. Clinical Effectiveness Group, British Association for Sexual Health and HIV. www.bashh.org (Accessed: 29/09/2011).

BASHH draft BV guideline (2012) National Guideline for the Management of Bacterial Vaginosis. Clinical Effectiveness Group, British Association for Sexual Health and HIV. www.bashh.org (Accessed: 27/02/2012).

Bolstad AI, Jensen HB, Bakken V. (1996) taxonomy, biology, and periodontal aspects of Fusobacterium nucleatum. *Clin Microbiol Rev*; **9**: 55-71.

Boskey ER, Atherly-Trim SA, O'Campo PJ, Strobino DM, Misra DP. (2004) Acceptability of a self-sampling technique to collect vaginal smears for gram stain diagnosis of bacterial vaginosis. *Womens Health Issues*; **14**:14-18.

Bradshaw CS, Morton AN, Hocking J, Garland SM, Morris MB, Moss LM, Horvath LB, Kuzevska I, Fairley CK. (2006) High recurrence rates of bacterial vaginosis over the course of 12 months after oral metronidazole therapy and factors associated with recurrence. *J Infect Dis*; **193**:1478-86.

Brotman RM, Ghanem KG, Klebanoff MA, Taha TE, Scharfstein DO, Zenilman JM. (2008a) The effect of vaginal douching cessation on bacterial vaginosis: a pilot study. *Am J Obstet Gynecol*; **198**:628.e1-7.

Brotman RM, Klebanoff MA, Nansel TR, Andrews WW, Schwebke JR, Zhang J, Yu KF, Zenilman JM, Scharfstein DO. (2008b) A longitudinal study of vaginal douching

and bacterial vaginosis a marginal structural modeling analysis. *Am J Epidemiol*; **168**:188-96.

Carr PL, Felsenstein D, Frieidman RH. (1998) Evaluation and management of vaginitis. *J Gen Inter Med*; **13**: 335-346.

CDC Bacterial vaginosis: CDC Factsheet. Updated February 2008. (http://www.cdc.gov/std/BV) (Accessed: 29/09/2011).

CDC Sexual transmitted disease treatment guideline. (2010) *MMWR*; 59(No. RR-12):56-58.

CDC Sexually transmitted diseases treatment guidelines. (2006) http://www.cdc.gov/std/ treatment/2006/vaginal-discharge.htm#vagdis2 Accessed 14 February 2008.

Chapin KH, Lauderdale TL. Reagents, Stains, and Media: (2003) Bacteriology. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH. 8th ed. Manual of Clinical Microbiology. Washington, D.C: ASM press, Vol 1: 354-83.

Charonis G, Larsson PG. (2006) use of pH/whiff test or quick advanced pH and amine test for the diagnosis of bacterial vaginosis and prevention of postabortion pelivic inflammatory disease. *Acta Obstet Gynacol*; **85:**837-43.

Cherpes TL, Hillier SL, Meyn LA, Busch JL, Krohn MA. (2008) A delicate balance: risk factors for acquisition of bacterial vaginosis include sexual activity, absence of hydrogen peroxide-producing lactobacilli, black race, and positive herpes simplex virus type 2 serology. *Sex Transm Dis*; **35**:78-83.

Dadhwal V, Hariprasad R, Mitta S, Kapi A. (2010) Prevalence of bacterial vaginosis in pregnant women and predictive value of clinical diagnosis. *Arch Gynecol Obstet*; **281**:101–104.

Delaney ML and Onderdonk AB for the Microbiology and Prematurity Study Group. (2001) Nugent Score Related to Vaginal Culture in Pregnant Women. *Obstet Gynacol*; **98**:79-84.

Einarson A, Koren G. (2002) Bacterial vaginosis during pregnancy: should we screen for and treat? *Canadian family physician*; **40**:877-8.

Eschenbach DA, Hillier S, Critchlow C Stevens C, DeRoven T, Holmes KK. (1988) Diagnosis and clinical manifestation of bacterial vaginosis. *Am J Obstet Gynecol*; **158**: 819.

Eschenbach DA, Thwin SS, Patton DL, Hooton TM, Stapleton AE, Agnew K, Winter C, Meier A, Stamm WE. (2000) Influence of the normal menstrual cycle on vaginal tissue, discharge, and microflora. *Clin Infect Dis*; **30**:901-7.

Ethiopia Ministry of health. (2006) National guideline for the management of sexual transmitted infections using the syndromic approaches. HIV/AIDS prevention and control office: Ministry of health.

Farquhar C, Mbori-Ngacha D, Overbaugh J, Wamalwa D, Harris J, Bosire R, John-Stewart G. (2010) Illness during Pregnancy and Bacterial Vaginosis are Associated with *In Utero* HIV-1 Transmission. *AIDS*; **24**: 153–155.

Ferris MJ, Masztal A, Aldridge KE, Fortenberry JD, Jr PLF, Martin DH. (2004) Association of *Atopobium vaginae*, a recently described metronidazole resistant anaerobe, with bacterial vaginosis. *BMC Infect Dis*; **4**:5.

Gellar M, Nelson A. (2004) Diagnosis and Treatment of Recurrent and Persistent Vaginitis. *Women's Health: Gynecol;* **4**: 137-146.

Gillet E, Meys JFA, Verstraelen H, Bosire C, Sutter PD, Temmerman M, Broeck DV. (2011) Bacterial vaginosis is associated with uterine cervical human papillomavirus infection: a meta-analysis. *BMC Infect Dis*; **11**:10.

Goyal R, Sharma P, Kour I, AggarwaN, Talwar V. (2005) Diagnosis of Bacterial Vaginosis in Women in Labour. *JK SCINCE*; **7**:1-4.

Grantacos E, Figueras F, Barranco M, Ros R, Andreu A, L.Alonso P,Cararach V. (1999) Prevalence of Bacterial Vaginosis and correlation of Clinical to Gram stain Diagnosis criteria in low risk Pregnant women. *eur J Epidemiol*; **15**:913-916.

Gutman RE, Peipert JF, Weitzen S, Blume J.(2005) Evaluation of clinical methods for diagnosing bacterial vaginosis. *Obstet Gynecol*; **105**:551-556.

Hill GB. (1993) The microbiology of bacterial vaginosis. *Am J Obstet Gynecol*; **169**: 450-454.

Hillier SL, Krohn MA, Rabe L, Klebanoff SJ, Eschenbach DA. (1993) The normal vaginal flora, hydrogen peroxide producing lactobacilli and bacterial vaginosis in pregnant women. *Clin Infect Dis.*, **16**(Suppl):S273-81.

Hillier SL. (1998) The vaginal microbial ecosystem and resistance to HIV. *AIDS Res Hum Retrovirus*; S17-21.

Hillier SL, Nugent RP, Eschenbach DA, Krohn MA, Gibbs RS, Martin DH, Cotch MF, Endalman R, Pastork II JG, Rao AV, McNellis D, Regan JA, Carey JC, Klebanoff MA. (1995) Association between bacterial vaginosis and preterm delivery of a lowbirth-weight infant. *N Engl J Med*; **333**: 1737-42.

Holzman C, Leventhal JM, Qiu H, Jones NM, Wang J and the BV Study Group. (2001) Factors linked to bacterial vaginosis in non-pregnant women. *Am J Public Health*; **91**:1664–1670.

Hutchinson KB, Kip KE, Ness RB. (2007) Condom use and its association with bacterial vaginosis and bacterial vaginosis-associated vaginal microflora. *Epidemiology*; **18**:702-8.

Islam A, Safdar A, Malik A. (2009) Bacterial Vaginosis. *J Pak Med Assoc*; **59**:601-604.

Joesoef MR, Schmid G. (2005) Bacterial Vaginosis. *Clin Evid*; **13**: 1968-1978.

Jones FR, Miller G, Gadea N, Meza R, Leon S, Perez J, Lescano AG, Pajuelo J, Caceres CF, Klausner JD, Coates TJ. (2007) Prevalence of bacterial vaginosis among young women in low-income populations of coastal Peru. *Int J STD & AIDS*; **18**: 188–192.

Keane F, Ison CA, Noble H, Estcourt C. (2006) Bacterial vaginosis. *Sex Transm Infect;* **82**(Suppl IV):iv16–iv18.

Kirakoya-Samadoulougou F, Nagot N, Defer MC, Yaro S, Meda N, Robert A. (2008) Bacterial vaginosis among pregnant women in Burkina Faso. *Sex Transm Dis*; **35**: 985–989.

Klebanoff MA, Nansel TR, Brotman RM, Zhang J, Yu KF, Schwebke JR, Andrews WW. (2010) Personal Hygienic Behaviors and Bacterial Vaginosis. *Sex Transm Dis*; **37**: 94-99.

Klebanoff MA, Schwebke JR, Zhang J, Nansel TR, Yu KF, Andrews WW. (2004) Vulvovaginal symptoms in women with bacterial vaginosis. *Obstet Gynecol*; **104**:267-72.

Krohn MA, Hillier SL, Eschenbach DA. (1989) Comparison of methods for diagnosing bacterial vaginosis among pregnant women. *J Clin Microbiol*; **27**: 1266-1271.

Kurewa NE, Mapingure MP, Munjoma MW, Chirenje MZ, Rusakaniko S, Stray-Pedersen B. (2010) The burden and risk factors of sexually transmitted infections and reproductive tract infections among pregnant women in Zimbabwe. *BMC Infect Dis;* **10**:127.

Larsson PG, Fahraeus L, Carlsson B, Jakobsson T, Forsum U. (2007) Predisposing factors for bacterial vaginosis, treatment efficacy and pregnancy outcome among term deliveries; results from a preterm delivery study. *BMC women's health*; **7:**20.

Larsson PG, Stray-Pedersen B, Ryttig KR, Larsen S. (2008) Human Lactobacilli as supplementation of clindamycin to patients with bacterial vaginosis reduce the recurrence rate; a 6-month, double-blind, randomized, placebo-controlled study. *BMC Women's Health*; **8**:3.

Lata I, Pradeep Y, Sujata and Jain A. (2010) Estimation of the Incidence of Bacterial Vaginosis and other Vaginal Infections and its Consequences on Maternal/Fetal Outcome in Pregnant Women Attending an Antenatal Clinic in a Tertiary Care Hospital in North India. *Indian J Community Med*; **35**:285-89.

Marx G, John-Stewart G, Bosire R, Wamalwa D, Otieno P, and Farquhar C. (2010) Diagnosis of sexually transmitted infections and bacterial vaginosis among HIV 1 infected pregnant women in Nairobi. *Int J STD AIDS*; **21**: 549–552.

Mastrobattista JM, Bishop KD, Newton ER. (2000) Wet Smear Compared With Gram Stain Diagnosis of Bacterial Vaginosis in Asymptomatic Pregnant Women. *Obstet Gynacol*; **96**: 504-506.

Mitchell C, Balkus J, Agnew K, Lawler R, Hitti J. (2009a) Changes in the Vaginal Microenvironment with Metronidazole Treatment for Bacterial Vaginosis in Early Pregnancy. *J Women's Health*; **18**: 1817-24.

Mitchell CM, Hitti JE, Agnew KJ, Fredricks DN. (2009b) Comparison of oral and vaginal metronidazole for treatment of bacterial vaginosis in pregnancy: impact on fastidious bacteria. *BMC Infect Dis;* **9**:89.

Mittal V, Jain A, Pradeep Y. (2012) Development of modified diagnostic criteria for bacterial vaginosis at peripheral health centers in developing countries. *J Infect Dev Ctries*; **6**:373-377.

Money D. (2005) The laboratory diagnosis of bacterial vaginosis. *Can J Infect Dis Med Microbiol*; **16**:77-79.

Msuya SE, Uriyo J, Hussain A, Mbizvo EM, Jeansson S, Sam NE, Stray-Pedersen B. (2009) Prevalence of sexually transmitted infections among pregnant women with known HIV status in northern Tanzania. *Reproductive Health;* **6**:4.

Mullick S, Watson-Jones D, Beksinska M, Mabey D. (2005) Sexually transmitted infections in pregnancy: prevalence, impact on pregnancy outcomes, and approach to treatment in developing countries. *Sex Transm Infect*; **81**:294-302.

Myziuk L, Romanowski B, Johnson SC. (2003) BVBlue test for diagnosis of bacterial vaginosis. *J Clin Microbiol*; **41**: 1925-1928.

Neelam S, Sohail I. (2010) Rapid Clinical Diagnostic Tests for Bacterial Vaginosis and its Predictive Value. *Int J Pathol*; **8**: 50-52.

Nelson DB, Bellamy S, Gray TS, Nachamkin I. (2003) Self-collected versus provider-collected swabs for the diagnosis of BV: an assessment of validity and reliability. *J Clin Epidemiol*; **56**: 862-866.

Nelson DB, Bellamy S, Nachamkin I, Ness RB, Macones GA, Allen-Taylor L. (2007a) First trimester bacterial vaginosis, individual microorganism levels and risk of second trimester pregnancy loss among urban women. *Fertil Steril*; **88**: 1396–1403.

Nelson DB, Bellamy S, Odibo A, Nachamkin I, Ness RB, Allen-Taylor L. (2007b) Vaginal symptoms and bacterial vaginosis (BV): how useful is self-report? Development of a screening tool for predicting BV status. *Epidemiol Infect*; **135**:1369–75.

Nelson DB, Macones G. (2002) Bacterial Vaginosis in Pregnancy: Current Findings and Future Directions. *Epidemiol Rev*; **24**: 102–8.

Ness RB, Hillier SL, Richter HE, Soper DE, Stamm C, McGregor J, Bass DC, Sweet RL, and Rice P. (2002) Douching in Relation to Bacterial Vaginosis, Lactobacilli, and Facultative Bacteria in the Vagina. *Obstet Gynecol*; **100**:765-72.

Nugent RP, Krohn MA, Hillier SL. (1991) Reliability of diagnosing bacterial vaginosis is improved by standardized method of gram stain interpretation. *J Clin Microbiol*; **29:**297-301.

Pepien J, Deslands S, Giroux G, Sobe'la F, Khonde N, Diakite S, Demeule S, Labbe AC, Carrier N, Forst E. (2011) The complex vaginal flora of West African women with bacterial vaginosis. *PLoS One*; **6**: e25082. Epub 2011 sep 20.

Posner SF, Kerimova J, Aliyeva F, Duerr A. (2005) Strategies for diagnosis of bacterial vaginosis in a resource-poor setting. *Int J STD AIDS*; **16**: 52–55.

Ralph SG, Rutherford AJ, Wilson RO. (1999) Influence of bacterial vaginosis on conception and miscarriage in the first trimester: cohort study. *BMJ*; **319**:220-223.

Rein MF, Shih M, Miller JR, Guerrant RL. (1996) Use of a lactoferrin assay in the differential diagnosis of female genital tract infections and implications for the pathophysiology of bacterial vaginosis. *Sex Transm Dis*; **23**:517–521.

Romoren M, Velauthapillai M, Rahman M, Sundby J, Klouman E, Hjortdahl P. (2007) Trichomoniasis and bacterial vaginosis in pregnancy: inadequately managed with the syndromic approach. *Bull World Health Organ*; **85**:297-304.

Schwebke J. (2003) Gynecolocic consequences of bacterial vaginosis. *Obstet Gynecol Clin North Am*; **30**: 685-694.

Schwebke J. (2005) Abnormal vaginal flora as a biological risk factor for acquisition of HIV infection and sexually transmitted diseases. *J Iinfect Dis*: **192**:1315-1317.

Schwebke JR, Lawing LF. (2001) Prevalence of Mobiluncus species among women with and without bacterial vaginosis as detected by polymerase chain reaction. *Sex Transm Dis*; **28**: 195-9.

Schwebke JR, Morgan SC and Hillier SL. (1996) Humoral Antibody to *Mobiluncus curtisii*: a Potential Serological Marker for Bacterial Vaginosis. *Clin Diagn Lab Immunol*; **3**: 567–569.

Sha BE, Zariffard MR, Wang QJ, Chen HY, Bremer J, Cohen MH, Spear GT. (2005) female genital-tract HIV load correlates inversely with *Lactobacillus* species but positively with bacterial vaginosis and *Mycoplasma hominis. J Infect Dis*; **191**:25–32.

Smart S, Singal A, Mindel A. (2004) Social and sexual risk factors for bacterial vaginosis. *Sex Transm Infect*; **80**:58–62.

Sobel JD. (2012) bacterial vaginosis. (http://www.uptodate.com/contents/bacterial vaginosis). (Accessed: 01/06/2012).

Sobel, JD. (2000) Bacterial Vaginosis. *Annu Rev Med*; **51**: 349-356.

Spiegel CA, Amsel R, Holmes KK. (1983) Diagnosis of bacterial vaginosis by direct Gram stain of vaginal fluid. *J Clin Microbiol*; **18**:170-7.

Spiegel CA. (2002a). Bacterial vaginosis. *Rev Med Microbiol*; **13**: 43-51.

Spiegel CA. (2002b) Bacterial vaginosis. In: Gardner HL, Dukes CD. *Haemophilus vaginalis* vaginitis: A newly defined specific infection previously classified 'nonspecific' vaginitis. *Am J Obstet Gynecol*. 1955; **69**:962–976.

Tamrakar R, Yamada T, Furuta I, Cho K, Morikawa M, Yamada H, Sakuragi N, Minakami H. (2007) Association between *Lactobacillus* species and bacterial vaginosis-

related bacteria, and bacterial vaginosis scores in pregnant Japanese women. *BMC Infect Dis*; **7**:128.

Trabert B, Misra DP. (2007) Risk factors for bacterial vaginosis during pregnancy among African American women. *Am J Obstet Gynecol* ; **197**: 477.e1-.8.

Udayalaxmi, Bhat G, Kotigadde S, Shenoy S. (2011) Comparison of the Methods of Diagnosis of Bacterial Vaginosis. *J Clin Diagn Res*; **5**: 498-501.

Verstraelen H, Verhelst R, Vaneechoutte M, Temmerman M. (2010) The epidemiology of bacterial vaginosis in relation to sexual behavior. *BMC Infect Dis;* **10**:8.

Vogel I, Thorsen P, Jeune B, Jacobsson B, Ebbesen N, Arpi M, Bremmelgaard A, R.Moller B. (2006) Acquisition and Elimination of Bacterial Vaginosis During Pregnancy: Danish Population-Based Study. *Infect Dis Obstet Gynecol*; 2006: 94646.

Wayne WD. (1998) Determination of sample sizes: Estimation. Biostastics for analysis in health sciences. pp 180-181.

West B, Morison L, schim van der Loeff M, Gooding E, Awasana AA, Demba E, Mayau P. (2003) Evaluation of a new rapid diagnostic kit (FemExam) for bacterial vaginosis in patients with vaginal discharge syndrome in the Gambia. *Sex Transm Dis*; **30**: 483-489.

Wolday D, G-Mariam Z, Mohammoed Z, Meles H, Messele T, Seme W, Geyid A, Maayan S. (2004) Risk factors associated with failure of syndromic treatment of transmitted disease among women seeking primary care in Addis Ababa. *Sex Transm Infect*; **80**:392-394.

WHO (Geneva) sexually transmitted and other reproductive tract infections. (2005) A guide to essential practice. http://www.who.int/reproductive-health/publications/rtis_gep/rtis_gep.pdf.

Yudin MH and Money DM. (2008) Screening and Management of Bacterial Vaginosis in Pregnancy. *J Obstet Gynecol Can*; **211**:702-708.

Zhou X, Bent SJ, Schneider MG, Davis CC, Islam MR, Forney LJ. (2004) Characterization of vaginal microbial communities in adult healthy women using cultivation-independent methods. *Microbiology;* **150**: 2565-73.

Zhou X, Brown CJ, Abdo Z, Davis CC, Hansmann MA, Joyce P, Foster JA, and Forney LJ. (2007) Differences in the composition of vaginal microbial communities found in healthy Caucasian and black women. *ISME J*; **1**: 121-33. Epub 2007 May 10.

Zozaya-Hinchliffe M, Lillis R, Martin DH, Ferris MJ. (2010) Quantitative PCR Assessments of Bacterial Species in Women with and without Bacterial Vaginosis. *J Clin Microbiol*; **48**: 1812–1819.

Appendix I: Questionnaire

Questionnaire for investigation of the prevalence of bacterial vaginosis among pregnant women attending ANC in Tikur Anbessa specialized hospital, Addis Ababa Ethiopia.

Serial no---------- Card no----------

Demographic characteristics

1. Age ----------
2. Religion ----------
3. Residence ----------
4. Occupation A. daily servant B. gov/private employed C. house wife D. agriculture E. commercial F. jobless G. others ----------
5. Education ----------
6. Income ----------
7. Marital status ----------
8. Number of lifetime sexual partners ----------
9. Previous contraceptive use A. yes B. no
   If yes, mention ----------
10. Gestational age (LMP) ----------
11. Gravidity A. Primigravidia B. Multigravidia
12. Abortion A. yes B. no
    If yes what is the cause A. Spontaneous B. Induced
    At what gestational age ----------
13. History of preterm birth A. yes B. no
14. History still birth A. yes B. no
15. Have you done vaginal douching in the preceding month A. Yes B. No,
    If yes, how many times per week ----------
16. Frequency of vaginal bathing ----------
17. Frequency of showering ----------
18. Pain of vagina A.yas B. no
19. Burning or Itching A.yas B. no

20. Swelling A. yes B. no

21. History of GTI A. yes B. no

22. If yes, frequency ----------

23. Unusual Discharge A. yes B. no

If yes, types ----------

Does it have Odour? A. yes B. no

Result Report Sheet

Serial number ……

Clinical diagnosis

Type of swab/discharge ----------

P^H ----------

Whiff test ----------

Yeast ----------

Clue cell ----------

T. vaginalis ----------

Gram staining results

Morphotype	Number of morphotypes per field					Average	Point	Score
	1st	2nd	3rd	4th	5th			
Lactobacilli								
G. vaginalis								
Moblincus								
							Sum of score	------

Comment: ----------

Examiner signature ---------- Date ----------

Appendix- II: Information sheet for study participants

Purpose: The aim of this study is to determine prevalence of BV among pregnant women whose gestational age is 14-28 weeks.

Duration: The duration of this study depend upon the availability of study subjects it can probably take about two months or more.

Procedure: The procedure of sample collection is easy and straight forward; sample will be collected from vagina using cotton swab by attending health professional and then it will be analyzed clinically and microbiologically in the microbiology laboratory of Tikur Anbessa Hospital for the presence of bacterial vaginosis.

Risk and discomfort: almost there will no any risk associated during sample collection without little discomfort.

Benefits: If you participate in this research, you may not get direct benefit but you will get a clinical assessment of your health condition and treatable disease condition.

Compensation: No compensation will be provided by participating in this study.

Confidentiality: All your personal information collected for the purpose of the present study will be kept confidential.

Termination of the study: Participation in the study is voluntary, and refusal to participate involves no penalty or loss of benefits to which you are otherwise entitled. The study participants have a right to Keep hold information; decline to cooperate in the study, to refuse provision of specimens.

If you have any question about the right of the study participant the address is:

Faculty of Medicine Addis Ababa University

Office of Associate Dean, Postgraduate Programs and Research

P.O. Box 9086. Addis Ababa, Ethiopia

Tel. 251-011-551-28-765

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

Zemenu Mengistie
Department of Microbiology, Immunology and Parasitology
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የጥናቱ ተሳታፊዎች የመረጃ ቅጽ

<u>የጥናቱ ዓለማ</u> የዚህ ጥናት ዋና ዓላማ በነፋስ ጡር እናቶች ላይ የባክቴሪያል ቫጂኖሲስ ስርጭት ምን ያህል እንደሆነ ለማወቅና ለሚመለከተው አካል የመፍትሔ እርምጃ እንዲወስድ ለማሣወቅ ነው::

<u>የሚፈጀው ጊዜ</u> ይህ ጥናት እስከ 2 ወር ሊፈጅ ይችላል

<u>አጠቃቀም</u> በዚህ ጥናት ከሚሣተፉ እናቶች ናሙና ከሀፍረተ ስጋ በመውሰድ ከላይ የተጠቀሰውን በሽታ መኖሩ ይረጋገጣል::

<u>ሊደርስ የሚችል አደጋ</u> በዚህ ጥናት ውሰጥ አደጋ የሚያደርስ ድርጊት የለም::

<u>የሚገኝበት ጥቅም</u> በዚህ ጥናት ነፋስ ጡር እናቶች የጤና ሁኔተችውን በነፃ ማወቅ ይችላሉ:: ከላይ የተጠቀሰው በሽታ የተገኘባቸው እናቶች መድሐኒት ገዝተው እንዲወስዱ ይረዳቸዋል፡፡

<u>ሚስጢራዊነት</u> የማንኛውም የጥናቱ ተሳታፊዎች መረጃ በሚስጥራዊነት ይያዛል፡፡ የእያንዳንዱን ግለሰብ መረጃ ከዋናው ተመራማሪ እና አማካሪ በስተቀር ማንም ሊያገኝ አይችልም

<u>ፈቃደኝነትን ስለማቋረጥ</u> የጥናቱ ተሳታፊዎች መረጃን ያለመስጠት፣ በጥናቱ ለመሰተፋ ፋቃደኝነት የማሳየተ እንደሁም ናሙና ያለመስጠት መብታቸው የተጠበቀ ነው፡፡

ለማንኛው ጥያቄ ኣድራሽ ማወቅ ካስፈለገዎ

ህክምና ፋኩሊቲ፣ አዲስ አበባ ዩኒቨርሲቲ

የድህረ ምረቃ ፕሮግራምና ምርምር የተባባሪ ዲን ቢሮ፡፡

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ህክምና ፋኩልቲ አዲስ አበባ ዩኒቨርሲቲ

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Appendix- III: Consent form

(To be translated in to Amharic)

Serial no ……. Card no……

I consent to participate in the research study entitled: “prevalence of bacterial vaginosis among pregnant women attending at Tikur Anbessa specialized hospital”. I understand that the purpose of this study is to assess the prevalence of bacterial vaginosis among pregnant. I also understand that the result of the study is useful for my pregnancy outcome and used as information for other pregnant women. In addition I have the right to refuse or participate in the study and when I am willing to participate, I will be asked some question that will ask about personal information and I will give vaginal swab. Finally, I acknowledge that I understand all that is printed here on this consent form and the benefit of participating is getting free laboratory diagnosis. By signing below, I give my permission to be enrolled in the study. To protect human subject rights, I understand that this research proposal was reviewed and approved by the research and review committee of the department.

Participant Signature _____ Date _____

Witness name _____ signature _____ date _____

ቅጽ 2 **የስምምነት መግለጫ**

(ትርጉም በአማረኛ)

ተራ ቁጥር _____ የካርድ ቁጥር _____

እኔ የዚህ ጥናት ተሳታፊ የዚህ ጥናት ዋና ዓላማ የ ባ ክ ቴ ሪ ያ ል ቫ ጂ ኖ ሲ ስ ስ ር ጭት በ ነ ፍ ሰ ጡር እ ና ቶ ች ላ ይ ምን ያ ህ ል እ ን ደ ሆ ነ ለ ማ ጥ ና ት መ ሆ ኑ ን የ ጥ ና ቱ ውጤት ደ ግ ሞ ለ እ ኔ እ ር ግ ዝ ና ጤ ና ማ ነ ት አ ስ ፈ ላ ጊ እ ን ደ ሆ ነ ና ለ ሌ ሎ ች ነ ፍ ሰ ጡር እ ና ቶ ች ም ለ መ ረ ጃ ጥ ቅ ም እ ን ደ ሚ ያ ገ ለ ግ ል ተ ረ ድ ቻ ለ ሁ::

በ ተ ጨ ማ ሪ ም በ ጥ ና ቱ ውሳ ጥ የ መ ሳ ተ ፍ ወ ይ ም ያ ለ መ ሳ ተ ፍ መ ብ ቴ የ ተ ጠ በ ቀ እ ን ደ ሆ ነ ፣ ገ ብ ቼ ስ ሳ ተ ፍ የ ቃ ለ መ ጠ ይ ቅ እ ን ደ ሚ ደ ረ ግ ል ኝ እ ና ከ ሀ ፍ ረ ተ ስ ጋ ዬ ጥ ራ ጊ ና መ ና እ ን ደ ም ሰ ጥ በ ባ ለ መ ያ ፊ ቃ ደ ኝ ነ ቴ ን ተ ጠ ይ ቄ ያ ለ ሁ:: በ መ ሆ ኑ ም የ ጥ ና ቱ ን ዓ ላ ማ ና ጥ ቅ ም በ ሚ ገ ባ ስ ለ ተ ገ ነ ዘ ብ ኩ በ መ ሳ ተ ፌ የ ሚ ገ ኘ ው ጥ ቅ ም ም ነ ፃ የ ላ ብ ራ ቶ ሪ ም ር መ ራ መ ሆ ኑ ን ጭ ም ር አ ው ቄ ከ ላ ይ የ ተ ጠ ቀ ሱ ት ን ለ ጥ ና ቱ የ ሚ ያ ስ ፈ ል ጉ ነ ገ ሮ ች ን ሁ ሉ ለ መ ስ ጠ ት በ መ ሉ ፈ ቃ ደ ኝ ነ ት መ ስ ማ ማ ቴ ን በ ፊ ር ማ ዬ አ ረ ጋ ግ ጣ ለ ሁ::ይ ህ ጥ ና ት በ ት/ት ክ ፍ ሉ ጥ ና ት ና ም ር ም ር ኮ ሚ ቴ መ ጽ ደ ቁ ን ተ ነ ግ ሮ ኛ ል ፡ ፡

የ ጥ ና ቱ ተ ሳ ታ ፊ ፊ ር ማ _____

ቀ ን _____

የ እ ማ ኝ ስ ም _____

\ፊ ር ማ _____ ቀ ን _____

Appendix IV: Gram staining procedure (Chapin and Lauderdale, 2003):

1. Labeling the slides clearly with the patient's serial number.
2. Making smears of vaginal swab by spread evenly covering an area about 15-20mm diameter on a slide.
3. Drying of smears after making smears, the slide should be left in a safe place to air-dry, protected from flies and dust.
4. Fix the dried smear by using heat.
5. Cover the fixed smear with crystal violet stain for 30-60 seconds.
6. Rapidly wash off the stain with clean water.
7. Tip off all the water, and cover the smear with Gram's iodine for 30-60 seconds.
8. Wash off the iodine with clean water.
9. Decolorize rapidly (few seconds) with acetone alcohol. Wash immediately with clean water.
10. Cover the smear with neutral red or Safranine stain for 2 minutes.
11. Wash off the stain with clean water.
12. Wipe the back of the slide clean, and place in a draining rack for the smear to air-dry.
13. Examine the smear microscopically, first with the 40 x objective to check the staining and to see the distribution of materials and then with the oil-immersion objective to look for bacteria morphotype and cells.

The result of Gram stained smear was categorized in to three categories:

- *Lactobacilli* if it was large gram positive rod,
- *Gardnerella / Bacteroids* species if it was small gram variable or gram negative rod (short rod) and
- *Mobiluncus* species if it appeared as curved gram variable rods

DECLARATION

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

M.Sc. Candidate Zemenu Mengistie (B.Sc)

Signature _____

Date and place of submission _____

Addis Ababa, Ethiopia

Supervisor Yimtubezinash Woldeamanuel (MD, M.Sc., PhD)

Signature _____

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