

**ADDIS ABABA UNIVERSITY  
SCHOOL OF GRADUATE STUDIES  
BIOLOGY DEPARTMENT**



**PREVALENCE OF BACTERIAL VAGINOSIS,  
CHARACTERIZATION OF LACTIC ACID BACTERIA  
ISOLATES AND PATTERNS OF ANTIMICROBIAL TEST  
FROM WOMEN VAGINAL FLUID IN ADDIS ABABA**

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**BY**

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**ABBREVIATIONS**

AIDS	Acquired Immunodeficiency Syndrome
Amp	Ampicillin
BHI	Brain Heart Infusion
BV	Bacterial Vaginosis
Clind	Clindamycin
Gen	Gentamycin
HIV	Human Immunodeficiency Virus
IL	Interleukin
Kan	Kanamycin
KOH	Potassium Hydroxide
LAB	Lactic Acid Bacteria
Meth	Methicillin
MRS	de Man, Rogosa and Sharpe Agar
Pen	Penicillin
STD's	Sexual Transmitted Diseases
Strep	Streptomycin
Van	Vancomycin

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## ABSTRACT

The human vagina is a complex integrated environment containing an abundance of microorganism. Bacterial vaginosis (BV) is a disorder characterized by a reduction in or absence of *Lactobacillus* colonization, and overgrowth of several facultative and obligate anaerobic bacteria. BV has been reported as one of the most common vaginal infections in women at reproductive age from different part of the world. This study was conducted to evaluate the prevalence of BV and some associated risk factors in Addis Ababa. Moreover the study examined the composition of lactic acid bacteria (LAB) genera isolated form vaginal specimen culture and the antimicrobial sensitivity pattern of some of the isolated LAB. Among 100 women randomly enrolled in this study a BV prevalence of 32% was found based on Gram-stain scoring of vaginal flora according to Nugent criteria. The prevalence of BV was higher among women at age group 31-40 (44%), Christian women (34%), who are married (33%), non-pregnant (34%) and women who had a previous history of abortion (37%). Lactic acid bacteria (LAB) were isolated from 70% of women. Out of 350 colonies which were isolated and characterized, 40% were lactococci, 29% were lactobacilli, 23% were pediococci and 8% were leucnoscoc. From a total of 60 lactic acid bacteria isolates that were subjected to antimicrobial activity test, both the most frequent resistance and the least sensitivity result was noted for Clindamycin (42% and 50% respectively), where as the most frequent sensitivity and the least resistance was recorded for Vancomicine (80% and 20% respectively). This study has provided evidence for a considerable prevalence of BV in women. Therefore, the health sector and other responsible bodies should give the concern to screening and treating women for bacterial vaginosis. On top of that creating the awareness among the society will add its value on the public health profile of women and probably would contribute to reduce the risk of acquisition of other more serious STDs. Moreover, the knowledge of lactic acid bacteria composition and pattern of antimicrobial activities of human vaginal fluid would help to design better therapies and show future directions to maintain the healthy nature of human vaginal ecosystem.

## 1. INTRODUCTION

The adult human vagina is a complex ecosystem containing an abundance of microorganisms. The vagina and its unique microflora form a finely balanced ecosystem. The vaginal environment controlling the microbes present in the system where as the microflora, in turn, controlling the vaginal environment (Zhou *et al.*, 2003).

It is important to understand the structure of vaginal communities for, at least, two reasons. First, some of these microbes may have physiological functions that directly affect the health of women. They play the role in colonization resistance, protection against invasion by overt pathogens and dominance by potentially pathogenic species among the normal flora. Secondly, disruption of the vaginal community structure may predispose individuals to various infectious diseases (Morris *et al.*, 2001).

According to Zhou *et al.*, (2003), in the past 100 years since the first microbiological study of the human vagina, lactobacilli have been thought to be the predominant members of normal post pubertal vaginal microflora. However, a diverse array of other bacteria such as *Staphylococcus*, *Ureaplasma*, *Corynebacterium*, *Streptococcus*, *Peptostreptococcus*, *Gardnerella*, *Bacteroides*, *Mycoplasma*,

*Enterococcus*, *Escherichia*, *Veilloella*, *Bifidobacterium* and *Candida* can be present but in much lower numbers.

In women of childbearing age, the vaginal system is dominated by *Lactobacillus* species, a genus of Gram-positive, non motile rod-like bacteria (Zhou *et al.*, 2003). A general opinion is that species of the *Lactobacillus acidophilus* complex constitute most of the healthy vaginal *Lactobacillus* flora. However, other species have been encountered, such as *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus jensenii*, *Lactobacillus casei*, *Lactobacillus delbrueckii*, *Lactobacillus vaginalis*, and *Lactobacillus salivarius* (Vasquez *et al.*, 2000).

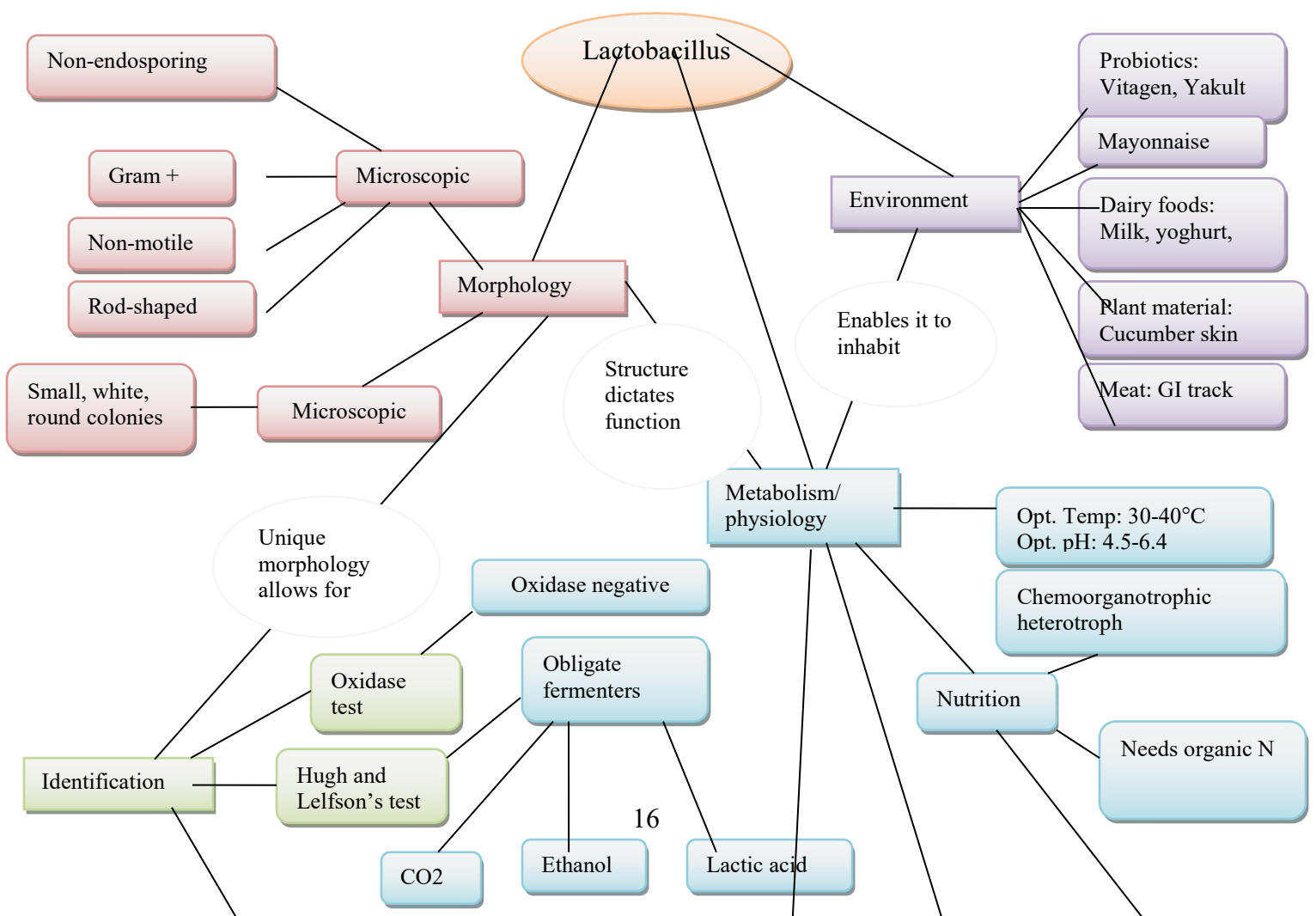
In the healthy human vagina, lactobacilli plays an important protective role against endogenous bacteria (such as the anaerobic Gram-negative rods *Bacteroides* and *Prevotella*, genital mycoplasmata, and *Gardnerella vaginalis*) and exogenous pathogens (such as *Neisseria gonorrhoeae* and *Chlamydia trachomatis*). Lactobacilli produce hydrogen peroxide and lactic acid, both of which lower the vaginal pH to a level inhospitable to many other bacteria. A low pH has been shown to have a direct microbicidal and virucidal effect (Vasquez, *et al.*, 2000). Lactobacilli can also adhere onto vaginal epithelial cells thus blocking the attachment of any pathogenic bacteria onto these cells. Lactobacilli are known to produce biosurfactants, bacteriocins and coaggregation molecules, all of which contribute to the maintenance of a healthy vaginal micro-environment (Hillier *et al.*, 1993).

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) also has a direct antimicrobial effect by inhibiting the growth of *Bacteroides*, *Gardnerella*, *Mobiluncus*, *Mycoplasma* and other vaginal organisms. H<sub>2</sub>O<sub>2</sub> has a direct effect on these organisms using its toxic effect, or by reacting with halide ions in the presence of vaginal peroxidase as part of the H<sub>2</sub>O<sub>2</sub>-halide peroxidase antibacterial system (Hawes *et al.*, 1996, Wilks *et al.*, 2004).

As reports show, H<sub>2</sub>O<sub>2</sub> producing lactobacilli strains are found in about 96% of women with normal vaginal microflora but only in about 6% of women with bacterial vaginosis suggesting it has a protective role against bacterial vaginosis and other sexually transmitted diseases(STD's) (Eschenbach *et al.*, 1989, Taha, *et al.* 1998).

In general, the vagina is a complex integrated environment. The study of this environment is essential, with the goal of understanding that there are inherent safeguards to maintain a state of healthy balance. On the other hand, the vaginal environment is susceptible to influences that can alter its state of balance. Through understanding this system, we can gain control of vaginal ecosystem, keeping it healthy by supporting the natural systems (Taha *et al.* 1998).

### Concept map of *Lactobacillus*



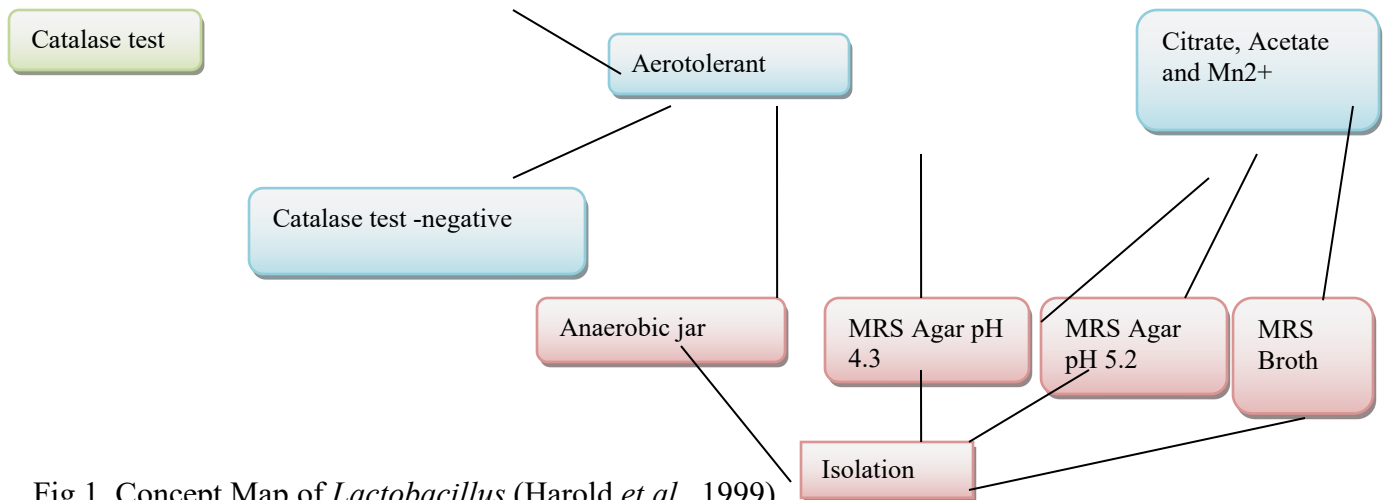


Fig.1. Concept Map of *Lactobacillus* (Harold *et al.*, 1999)

### 1.1. Factors that Affect Vaginal Ecosystem

The vaginal ecosystem is nature's gate-keeper for reproductive health of women. The vagina has a potentially healthy structure that can defend against invasion by infection. A normal vagina is hot and at core body temperature. It is moistened by a normal discharge from sets of glands in the cervix and the cells lining the walls. This fluid does not itch, burn or smell bad. It varies in color from clear to white, depending on the woman's menstruation cycle. When it dries it may appear yellowish. Pre-pubertal girls and post-menopausal woman have drier and less varying vaginal fluid. In women who are having normal fertility cycles the amount of the fluid varies from scant to moderate (Schwebke *et al.*, 1999).

When all the factors that influence the vagina are in a state of balance, the vagina feels good, has a faint, pleasant odor and a small amount of discharge. When factors are present that can influence the environment and cause a shift, a healthy vagina can compensate for the temporary imbalance and restore a state of health. If the vagina is unhealthy or out of balance, the health promoting mechanisms will be overwhelmed, the balance can not be remedied and result in vaginal infections (Barbara, 1998).

The vaginal ecosystem is dynamic with changes in structure and composition being influenced by age, menarche, time in the menstrual cycle, pregnancy, infections, methods of birth control, frequency of sex as well as number of sexual partners, various habits and practices such as douching, pH (acid/base balance), local immune factors, female hormones, sexual maturity and the types of organisms present in the vagina (Vitali *et al.*, 2007).

There is a striking correspondence between stage of the lifecycle and vaginal pH levels. Vaginal secretions are acidic at birth and vaginal microorganisms present in the newborn female are similar to those in the mother. Shortly after birth, when maternally derived estrogen levels decline, vaginal pH rises to about seven. A variety of microbial species colonize the vagina but at lower concentrations than in adults and lactobacilli are absent (Schwebke, *et al.*, 1999).

As stated by Hillier and Holmes (2006), at puberty, with the onset of menstruation, the female genital tract undergoes changes due to the influence of the female sex hormone, estrogen. The previously thin and fragile vaginal mucosa grows fleshy and elastic, and becomes rich in glycogen. Then, healthy organisms, the lactobacilli, begin to thrive the vaginal ecosystem. *Lactobacillus* uses glycogen as an energy source, breaking it down into glucose and lactic acid. Under the influence of lactic acid, the vagina maintains acidic pH of approximately 4.0. This acidic condition does two important things, first it kills pathogenic organisms, and second it causes squamous epithelial cells (mucous membrane) to cover over the exposed, fragile columnar cells of the cervical canal. *Lactobacillus* also produces hydrogen peroxide, which kills pathogens.

Adolescence represents a period of hormonal instability, which could affect vaginal pH. Adolescents have lower estrogen levels than adults and irregular menstrual cycles persist for varying periods after menarche. Adolescents with high pH levels may be at increased risk of bacterial vaginosis. An abnormal adolescent pH could indirectly increase susceptibility to genital tract infections (Brabin *et al.*, 2005).

The vaginal environment also shifts slightly during the course of the normal female cycle. Just prior to menses, the vagina is driest and most tender, with a slight shift

towards a less acidic level due to decreased populations of the good bacteria and other hormonal influences. This is frequently a time when women are most prone to vaginitis. Also just after the period, when there is still some blood present is another time when the environment is more at risk of being shifted out of balance (Morison *et al.*, 2005).

Hormone levels during menstrual cycling also affect susceptibility to infection. A recent report (Dumestre, *et al.*, 2005), postulated the presence of a waxing and waning of protection against HIV during the menstrual cycle. In the second half of the cycle, the immune system is suppressed so that sperm will not be destroyed, and there is an increased risk of infection. Low estrogen levels directly affect the vaginal mucosa, making it thin and friable.

Certain forms of contraception can also affect the vaginal environment. One woman out of 3 will be sensitive to the chemical and will have inflammation as a result of its use. This includes condoms with spermicide as well as all spermicidal creams, jellies and suppositories. Hormonal birth control methods (pills, the depo shot, Norplant implants, progesterone-containing IUDs), all work by tricking the body system into thinking that it is already pregnant and therefore does not need to ovulate. So just like in actual pregnancy, there may be slightly higher amounts of natural sugars in the vaginal discharge and hormonal shifts in the pH that may promote vaginal imbalance and infection (Demba *et al.*, 2005).

After menopause, the vaginal environment gradually reverts to the pre-menarcheal state. This lifecycle suggests that hormonal factors have a critical role in regulating vaginal pH levels. For post-menopausal women, the vaginal walls tend to become thinner, there is less vaginal lubrication and the pH may also shift slightly, making them more prone to imbalance (Brabin *et al.*, 2005).

## 1.2. Vaginal Infections

Vaginal infections are responsible for an estimated 10% of all visits by women to health care institutions (Vitali *et al.*, 2007). There are three general categories of vaginitis; hormonal, irritant and infectious.

*Hormonal vaginitis:* includes the atrophic vaginitis usually found in postmenopausal or postpartum women, but occasionally in young girls before puberty.

*Irritant vaginitis:* can be due to allergies including condoms, spermicides, deodorants, soaps, perfumes, semen, or douches. Irritants may also be due to hot tubs, mechanical abrasion, sanitary napkins, tampons, toilet tissue, topical medications and foreign bodies.

*Infectious vaginitis:* more than 90% of vaginitis in reproductive age women is due to an infectious agent. Vaginal infectious can be one of the three types: trichomoniasis, candidiasis or bacterial vaginosis (Thorsen, *et al.*, 1998, Vitali *et al.*, 2007).

### 1. 2.1. Trichomoniasis

Trichomoniasis is a common STD caused by a single-celled protozoan parasite called *Trichomonas vaginalis*. Trichomoniasis is primarily an infection of the urogenital tract, the urethra is the most common site of infection in men, and the vagina is the most common site of infection in women (Soper, *et al.*, 1990, Xueqiang, *et al.*, 2008).

The symptoms in women include a heavy, yellow-green or gray vaginal discharge, discomfort during intercourse, vaginal odor, and painful urination. Irritation and itching of the female genital area, and on rare occasions, lower abdominal pain can be present. The symptoms in men, if present, include a thin, whitish discharge from the penis and painful or difficult urination (Vitali *et al.*, 2007).

### 1.2.2. Vulvovaginal Candidiasis

Vaginal yeast infection or vulvovaginal candidiasis is a common cause of vaginal irritation. Vulvovaginal infections are among the most common infections caused by

*Candida albicans* occur most often when pH changes because of hormonal fluctuations, such as prior to and after menstruation, during per-menopause, or while taking oral contraceptives. It is estimated that approximately 75% of all women will experience at least one symptomatic yeast infection during their lifetimes (Dumestre *et al.*, 2005).

According to Limia *et al.*, (2007), Vulvovaginal candidiasis is the second most common cause of vaginitis in the United States and the most common cause in Europe. Yeast is always present in the vagina in small numbers, and symptoms only appear with overgrowth. Symptoms of a vulvovaginal infection include itchiness, occasionally accompanied by irritation or burning. Additional symptoms may include painful intercourse or urination and redness of the vulva and inner thighs (Vitali *et al.*, 2007).

Several factors are associated with increased symptomatic infection in women, including pregnancy, uncontrolled diabetes mellitus, and the use of oral contraceptives or antibiotics. Other factors that may increase the incidence of yeast infection include using douches, perfumed feminine hygiene sprays, and topical antimicrobial agents, and wearing tight, poorly ventilated clothing and underwear (Hillier and Holmes, 2006).

### **1.3. Bacterial Vaginosis**

Bacterial vaginosis (BV) is a poly-microbial syndrome recognized since 1950s and characterized by a shift in vaginal flora from a predominant population of lactobacilli to their gradual or total replacement with anaerobes such as *Gardnerella vaginalis*, *Prevotella*, *Bacteroides* and *Mobiluncus* spp. and with other bacteria including *Mycoplasma* and *Ureaplasma* species (Demba *et al.*, 2005). These condition leads to an increase in vaginal pH and decrease in H<sub>2</sub>O<sub>2</sub> production. Incidence rates of BV range from 5 to 50% among both non-pregnant and pregnant women (Vitali *et al.*, 2007).

BV is associated with an imbalance in the bacteria that are normally found in a woman's vagina. The vagina normally contains mostly good bacteria, and fewer harmful bacteria (Hill, 1993). BV develops when there is an increase in harmful bacteria. Any woman can get BV. However, some activities or behaviors can upset the normal balance of bacteria in the vagina and put women at increased risk. These include, having a new

sex partner or multiple sex partners, using an intrauterine device for contraception and douching (Eschenbach, 1989). Women do not get BV from toilet seats, bedding, swimming pools, or from touching objects around them. Women that have never had sexual intercourse are rarely affected (Sobel, 2000).

### **1.3.1. Pathogenesis of Bacterial Vaginosis**

BV represents a complex change in vaginal flora characterized by a reduction in the prevalence and concentration of H<sub>2</sub>O<sub>2</sub> producing lactobacilli and an increase in the prevalence and concentration of anaerobes. The massive overgrowth of vaginal anaerobes is associated with increased production of proteolytic carboxylase enzymes, which act to break down vaginal peptides to a variety of amines which, in high pH, become volatile and malodorous, especially trimethylamine (Figure 1). The amines are associated with increased vaginal transudation and squamous epithelial cell exfoliation, creating the typical discharge. In conditions of elevated pH, *Gardnerella vaginalis* more efficiently adheres to the exfoliating epithelial cells, creating clue cells. Amines further provide a suitable substrate for *Mycoplasma hominis* growth (Sobel, 2000).

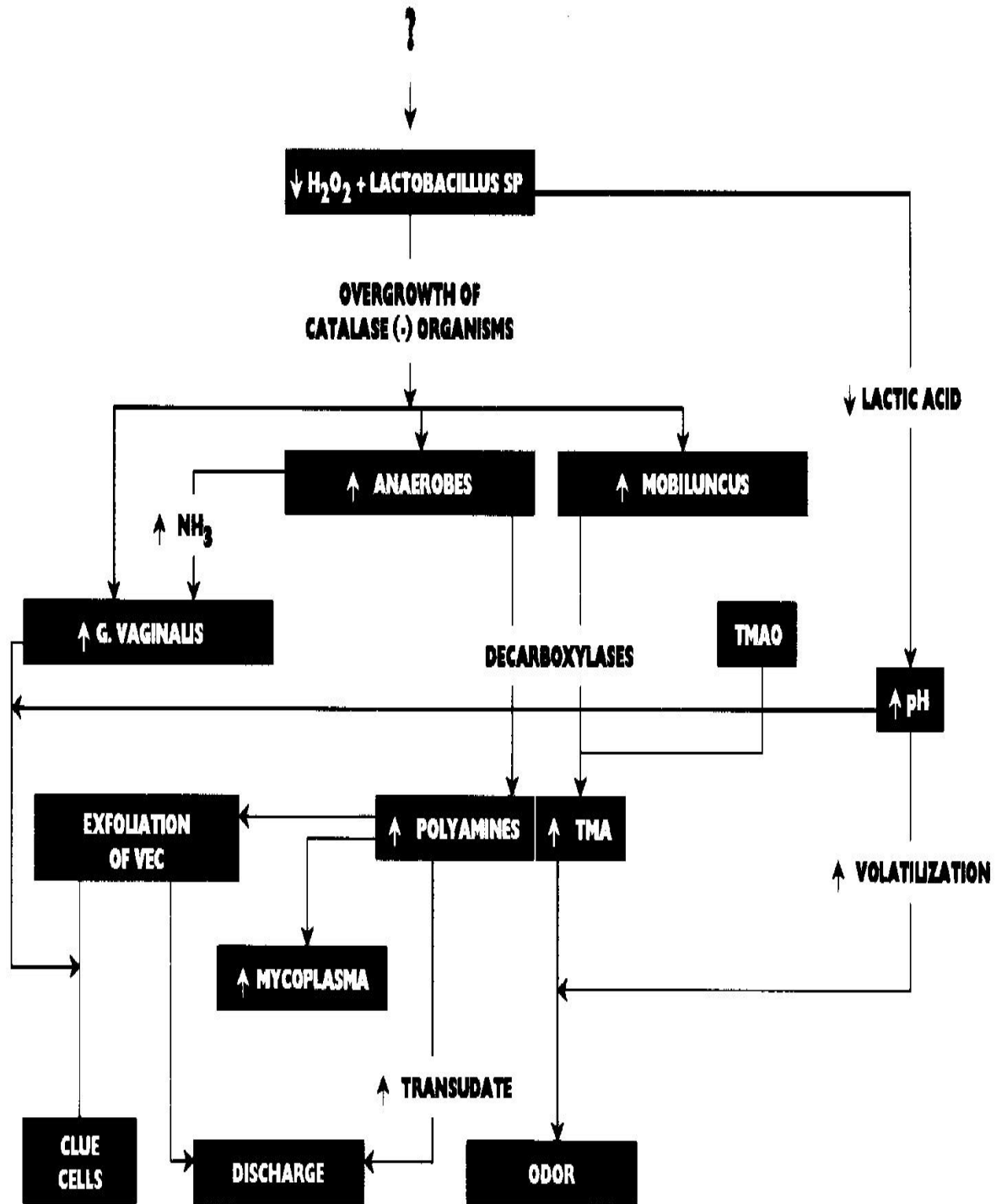


Fig. 2: Pathophysiology of Bacterial Vaginosis (Sobel, 2000).

### 1.3.2. Symptoms of Bacterial Vaginosis

Women with BV may have an abnormal thin, homogeneous white or grey thin vaginal discharge with an unpleasant odor. Some women report a strong fish-like odor, especially after intercourse. Women with BV may also have burning during urination or itching around the outside of the vagina. Increased discharge without an inflammatory response, abdominal pain, inter-menstrual bleeding, menorrhagia or prolonged menses can also be observed (Barbara, 1998). The normal pH of vaginal secretions is less than 4.5. In women with bacterial vaginosis, the pH is usually greater than 4.5. Some women with BV report no signs or symptoms at all and up to 50% of women are asymptomatic (Morris *et al.*, 2001).



Fig.3. Abnormal thin, homogeneous, white vaginal discharge due to BV

(Senok *et al.*, 2006)

### 1.3.3. Prevalence of Bacterial Vaginosis

Bacterial vaginosis is the commonest feminine woe that afflicts women of all age group and is twice the incidence of vaginal yeast infection (candidiasis). BV is the most common cause of vaginitis in women of childbearing age between 17-30 years. It predominantly affects young, sexually active females but can also occur in the absence of sexual intercourse (Sobel, 2000).

In African women, prevalence rates of more than 50% have been reported (Taha *et al.*, 1998). BV appears to be particularly common in Sub-Saharan Africa where several studies have reported high prevalence rates, ranging from 20–49% among women presenting to STD clinics with vaginal discharge, from 21–52% among pregnant women attending antenatal clinics, and from 37–51% in community-based studies. These are very much higher than the rates reported from industrialized countries, 13% in clinic attenders in the UK, 11% in gynecology clinic attendees in London, and 15% to 30% in studies of non-pregnant women in USA (Demba *et al.*, 2005).

Demba *et al.* (2005) reported the prevalence of BV and the pattern of vaginal micro-flora among women with vaginal discharge syndrome in an African setting, using Nugent's score as the gold standard. A BV prevalence of 47.6% was found in Gambia population, 20–23% in Burkina Faso and Malawi, 37% in Tanzania and 49% in Kenya. High prevalence of BV (21–29%) has also been observed among pregnant women in Kenya and South Africa.

The reasons for these disparities are not entirely clear, but may arise in part with different case definitions for BV, and because the pattern of vaginal microflora associated with this condition may differ in different populations. Moreover, it has been argued that lifestyle practices such as vaginal douching, levels of education and other socio-economic factors confound these associations. On the other hand, additional possible explanations for the high prevalence or incidence of BV in African populations have to be sought and the role of hormonal factors should be explored (Smart *et al.*, 2004, Demba, *et al.*, 2005).

### **1.3.4. Risk Factors Associated with Bacterial Vaginosis**

#### **1.3.4.1. Sexual Activity**

Several risk factor studies for bacterial vaginosis have focused on whether it is a sexually transmitted infection. Epidemiologic studies have found that early sexual activity, a high number of lifetime sexual partners, high frequency of partner change, women with a new sexual partner, homosexuality and women with a prior sexually transmitted disease are at increased risk of BV (Nelson, *et al.*, 2002, Brabin, *et al.*, 2005).

Bacterial vaginosis often coincides with a new sexual partnership. Changes in the vaginal environment induced by sexual intercourse with a new partner may increase susceptibility to abnormal colonization. The condition may simply follow disruption of the woman's established vaginal flora by intercourse with a new partner. Although bacterial vaginosis is more common in sexually active women, it has been also found in virgins (Morris *et al.*, 2001). Although sexually transmitted diseases and BV commonly coexist, BV is not considered a sexually transmitted disease. For example, a study among school age girls found similar rates of BV among virgin girls and non-virgin girls (12 % and 15 % respectively) (Hillier and Holmes 2006).

#### **1.3.4.2. Douching**

Douching is rinsing or cleaning out the vagina by squirting water or other water based solutions (such as vinegar, baking soda, or commercial douching solutions) into the vagina. The water or solutions are held in a container and are squirted through tubing and a nozzle (Jancin, 2000).

Many women have been grown up in believing douching is good and it would give them "fresh and natural" feeling. However, different researches show that douching is not the best thing for women (Jancin, 2000). Douching, a remarkably common practice in women may enhance the risk for vaginal infections like BV. The mechanism by which douching predisposes to BV is by disrupting the normal vaginal flora. When the flora is disrupted, the H<sub>2</sub>O<sub>2</sub> producing lactobacilli decrease in concentration and are replaced by an overgrowth of anaerobic and facultative aerobic bacteria (Ness *et al.*, 2004).

To date, several cross sectional epidemiologic studies have shown an association between douching and bacterial vaginosis. Studies found that douching was independently associated with a two-fold risk of acquiring bacterial vaginosis and a 3.5 fold elevated risk of getting *Trichomonas vaginalis*. The more often women douched, the greater risk for BV (Ness et al., 2007).

#### **1.3.4.3. Contraceptive Practice**

Some studies found significant associations between bacterial vaginosis and the use of intrauterine devices compared with oral contraceptive or barrier contraceptive users (Moi, 1990). Bacterial vaginosis is common among women at reproductive age who are also the most likely to use contraceptive device, meaning any association could be the result of the age structure of the study (Sobel, 2000).

#### **1.3.4.4. Socioeconomic Status**

Women with lower socioeconomic status and women self-reporting higher levels of psychosocial stress also have increased rates of BV. In recent studies among obstetrics populations, the reported prevalence of BV ranged from a low of 10% to a high of 35% among women reporting low monthly incomes and low educational levels (Nelson *et al.*, 2002, Demba *et al.*, 2005).

#### **1.3.4.5. Racial Origin**

Black ethnic groups are found to have the highest prevalence of bacterial vaginosis. Vaginal douching is more commonly practiced by African-American women in the United States and African-Caribbean women in the UK than among women from white ethnic groups. On the other hand, douching has been independently associated with acquiring bacterial vaginosis. It suggests that differing rates of bacterial vaginosis between racial groups may be due to cultural differences rather than genetic and socio-economic variations (Holzman *et al.*, 2001). Numerous studies have confirmed at least a twofold increased risk of BV among African-American women presumably due to environmental and behavioral factors (Nelson *et al.*, 2002).

#### **1.3.4.6. Smoking**

Smoking has been consistently associated with bacterial vaginosis and sexually transmitted diseases in UK and Swedish studies. The results from an antenatal population in London gave a percentage population attribute risk of 8.5% for current smokers against former and non-smokers. Smoking may suppress the immune system facilitating infection, but may simply reflect poor health seeking behavior. Smoking could be a marker for sexual behavior as significantly more of the UK populations with more than five lifetime sexual partners are smokers than non-smokers (Holzman *et al.*, 2001).

### **1.3.5. Complications Associated with Bacterial Vaginosis**

Bacterial vaginosis is associated with an increased risk of several pathologic conditions. It is responsible for considerable morbidity among reproductive age women. It has been consistently associated with the following complications;

#### **1.3.5.1. Preterm Delivery**

Pregnant women with BV are considered to be at increased risk for having preterm birth, infants with low birth weight, premature rupture of the membranes, post-caesarean section and postpartum endometritis (Martius *et al.*, 1988, Oleen-Burkey and Hillier, 1995, Thorsen, *et al.*, 1998).

Mechanisms that may initiate preterm birth in these circumstances are not fully understood. Bacteria may induce prostaglandin synthesis in amniotic cells via several ways. Many genital tract organisms associated with BV synthesize Phospholipase A2, an enzyme that liberates arachidonic acid (Kurki *et al.*, 1992). Bacteria may induce prostaglandin synthesis via direct invasion of the extraplacental membranes that will lead to disruption of the amniotic cells and release of lysosomal phospholipase. Another possible mechanism that may initiate labor is the migration of maternal inflammatory cells, which metabolize arachidonic acid (Kurki *et al.*, 1992, Morris *et al.*, 2001).

One study examining several pregnancy outcomes related to BV diagnosed during the first trimester of pregnancy reported a 2.6-fold increased risk of preterm labor, a 6.9-fold increased risk of preterm delivery and a 7.3-fold increased risk of preterm, premature rupture of the membranes (Sobel, 2000).

Moreover, a growing body of literature has begun to suggest an increased risk of spontaneous abortion among pregnant women with BV. Studies have reported a three to fivefold increased risk of spontaneous abortion among pregnant women with BV in the first trimester (Wilks *et al.*, 2004).

#### **1.3.5.2. Tubal Infertility**

Studies have investigated the association between bacterial vaginosis and tubal infertility after noting that the prevalence of bacterial vaginosis tends to be higher in women attending infertility clinics than among the general population. Observational studies found that one-third of women with tubal factor infertility had bacterial vaginosis (Morris *et al.*, 2001).

#### **1.3.5.3. Pelvic Inflammatory Disease**

*Bacterial vaginosis is common among women with upper genital tract inflammation and pelvic inflammatory disease (PID).* The similarity in micro-organisms characteristic of both bacterial vaginosis and pelvic inflammatory disease makes an association between the two and progression from bacterial vaginosis to pelvic inflammatory disease is biologically plausible. Studies have found bacterial vaginosis to be more common among women with pelvic inflammatory disease (Morris *et al.*, 2001).

#### **1.3.5.4. Increase Risk of HIV/AIDS Susceptibility**

Several propositions have been elucidated to understand the association between BV and HIV. The lactobacilli in healthy women produce lactic acid which maintains a low vaginal pH and inhibits the growth of potentially pathogenic organisms. Absence of these aerobic lactobacilli and an increase in anaerobic microorganisms like, *Gardnerella vaginalis*, results in an increase in vaginal pH and a decrease in H<sub>2</sub>O<sub>2</sub> production, which creates a conducive environment for HIV replication (Sweankambo *et al.*, 1997, Harold, *et al.*, 1999).

The lactobacilli produce H<sub>2</sub>O<sub>2</sub>, which is toxic to a number of microorganisms, including HIV (Cohn *et al.*, 1995). *In vitro*, it has been shown that H<sub>2</sub>O<sub>2</sub> producing

lactobacilli are critical to HIV-1. This may be due to the reaction of H<sub>2</sub>O<sub>2</sub> with myeloperoxidase and halides present in vaginal fluid (Dumestre *et al.*, 2005).

In addition, low vaginal pH has been postulated to inhibit CD4 lymphocyte activation and reduce HIV target cells in the vagina. Conversely, an elevated pH due to lack of lactobacilli may make the vagina more conducive to HIV survival and adherence (Sweankambo *et al.*, 1997). BV has also been shown to increase intravaginal levels of IL-10, which increases susceptibility of macrophages to HIV (Taha *et al.*, 1998).

### 1.3.6. Diagnosis of Bacterial Vaginosis

Generally two approaches are available to diagnosis BV;

#### 1.3.6.1. Amsel Criteria

Simple diagnostic criteria established by Amsel *et al.* (1999), have proved useful in clinical practice. Amsel criteria include (i) homogenous adherent grayish-white discharge, (ii) release of fishy amine odor or whiff test on addition of 10% potassium hydroxide, (iii) an elevated vaginal pH of 4.5 and (iv) the presence of clue cells on microscopy (Figure 4). Clue cells are vaginal epithelial cells that have a stippled appearance due to adherent coccobacilli. The edges of the cells are obscured and appear fuzzy compared with the normally sharp edges of vaginal epithelial cells. To be significant for bacterial vaginosis, more than 20 percent of the epithelial cells on the wet mount should be clue cells (Barbara, 1998).

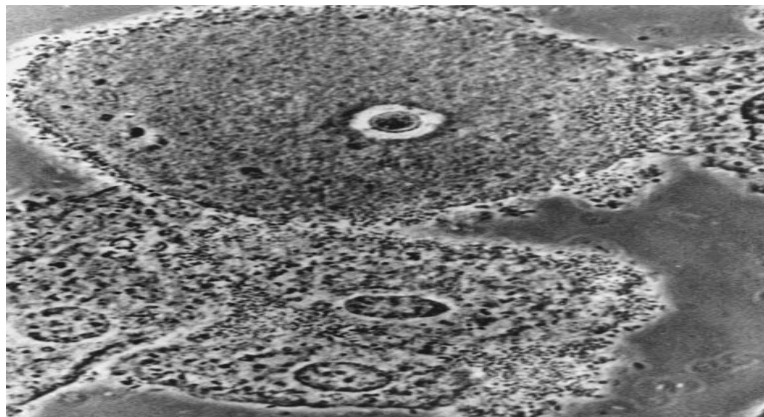


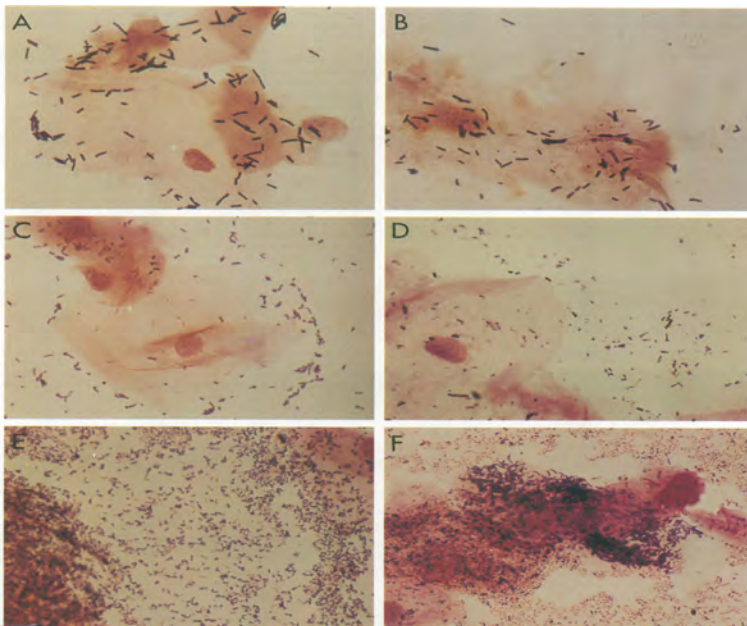
Fig. 4: High-power micrograph of clue cell (Sobel, 2000).

### 1.3.6.2. The Nugent's Method

According to Nugent scoring system Gram stained slides are examined under oil immersion (X 1000) (Nugent *et al.*, 1991). Smears are observed and quantified for the presence of the following morphotypes; Large Gram-positive bacilli (*Lactobacillus* morphotypes), small Gram-variable bacilli (*Gardnerella* morphotypes) and curved Gram-negative or gram-variable bacilli (*Mobiluncus* morphotypes) (Mary *et al.*, 2001, Tokyol *et al.*, 2004).

The Nugent score is derived from estimating the relative proportions of bacterial morphotypes to give a score between 0 and 10. A score of less than 4 is normal, 4-6 is intermediate, and greater than 6 is BV (Nugent *et al.*, 1991).

Gram stain of vaginal secretions is even more reliable than wet mount, with a sensitivity of 93% and specificity of 70%. The Gram stain provides an inexpensive, reliable, reproducible, fast and easy method to diagnose BV. It provides a direct look at the bacteriologic morphotypes and density, and is comparatively unaffected by factors such as menses or recent intercourse, which may alter vaginal pH (Sobel, 2000).



A and B: Normal vaginal flora  
(*Lactobacillus* morphotype)

C and D: Intermediate vaginal flora  
(Few *Lactobacilli* and many  
*Gardnerella* morphotype)

E and F: bacterial vaginosis  
(*Gardnerella* and *Mobiluncus*  
morphotype) (Nugent *et al.*, 1991).

FIG. 5 Gram-stained vaginal smears from women with various floras

### 1.3.7. Treatment of Bacterial Vaginosis

There are three areas of potential health gain from interventions for bacterial vaginosis: treating symptomatic cases, reducing infertility, pelvic inflammatory disease or HIV transmission and reducing infant mortality and morbidity associated with preterm birth (Morris *et al.*, 2001).

Poor efficacy of BV has been observed with triple-sulfa creams, erythromycin, tetracycline, and acetic acid gel. Moderate cure rates have been obtained with ampicillin (mean cure rate 66%) and amoxicillin. The most successful oral therapy remains metronidazole. The beneficial effect of metronidazole results predominantly from its anti-anaerobic activity and the susceptibility of *Gardnerella vaginalis* to the hydroxymetabolites of metronidazole (Sobel, 2000).

Treatment is especially important for pregnant women. 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 (Schwebke *et al.*, 1995). Treatment with metronidazole in women undergoing abortion who have bacterial vaginosis reduces the post-abortion risk. Oral metronidazole and oral clindamycin have been shown to reduce pregnancy-associated morbidity (Ahmed-Jushuf, *et al.*, 1995, Nelson and Macones, 2002).

On the other hand, though the dominant BV causing anaerobic organisms have been cultured from the male sexual partners of women with BV, treatment of male sexual partners is not a reliable way to reduce the recurrence of BV in these women. Despite indirect evidence of sexual transmission, no study has been documented with regard to reduced recurrence rates of BV in women whose partners have been treated (Ferris, *et al.*, 1995, Colli, *et al.*, 1997, Shalev, 2000).

### **1.3.7.1. Side Effect of Treatments**

Although antibacterial treatment is generally effective at eradicating the bacteria that cause BV, there is still a high rate of recurrence which affects the patient's quality of life. It is shown that between 5% to 20 % of women will experience repeated infections within 3 months of treatment (Morales, *et al.*, 1994). With repeated use of anti-bacterials, there is also the increased risk of resistance to the drugs. Recurrence more likely reflects vaginal relapse with failure to eradicate the offending organisms. At the same time the normal protective *Lactobacillus*-dominant vaginal flora fails to reestablish itself (Sobel, 2000).

In addition, metronidazole is often poorly tolerated due to side effects, including gastrointestinal upset, alcohol intolerance, metallic taste and infrequently neurological and haematological adverse reactions. Such findings provide a rationale for use of proven probiotic strains to maintain vaginal health and reduce the risk of recurrent symptomatic bacterial vaginosis (Shalev, 2000).

### **1.3.8. Probiotics Application for Bacterial Vaginosis Treatment**

Although beneficial microorganisms, as components of fermented foods, are an integral part of the human diet in many indigenous cultures, interest in the health benefits and therapeutic potential of probiotics in clinical medicine is relatively new. Probiotics which literally means "for life" because of their ability to favorably impact the local gastrointestinal tract ecology, are capable of promoting health and facilitate human resistance to opportunistic infection (Harish and Varghese, 2006).

The most commonly utilized probiotic preparation includes specific strains, either pure or in combination, lactobacilli, streptococci and bifidobacteria. These three genera are important components of the gastrointestinal flora, are considered to be harmless, and might be capable of preventing the overgrowth of pathogenic organisms (Shalev, 2000).

Probiotic agents exert a beneficial effect via a wide array of actions. These include resistance to colonization, production of anti-microbial substances, inhibition of pathogen adhesion, degradation of toxins, stimulation of local and peripheral immunity, Because of these varied actions, it is unlikely pathogens will develop resistance against probiotic agents (Cross, 2002).

Evidence from the available studies also suggests that probiotics can be beneficial for preventing recurrent vaginal infections in women including BV. So, natural practitioners have been advocate the consumption of live culture yoghurt or to insert a tampon soaked in yoghurt in to vagina to prevent BV. However a trial using yoghurt containing lactobacilli as a douche was ineffective in treating or preventing BV as the lactobacilli failed to colonize the vagina (Ocana *et al.*, 2006).

Recent trials using oral probiotic supplement comprising *Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* RC-14 found naturally in the vagina flora show that these two strains are able to restore the normal vaginal microflora due to their ability to adhere to the urogenital cells. This ability is thought to crowd out the anaerobes, thus impeding their growth. *Lactobacillus* GR-1 is also extremely good at inhibiting yeast that cause candidiasis, *E.coli*, the common culprit in urinary tract infection, as well as *Salmonella*, *Shigella* and bacteria that cause gut infections. *Lactobacillus* RC-14 is able to knock out the competition as it produces hydrogen peroxide which is a potent bactericidal agent. Both bacteria are also capable of killing HIV virus within minutes (Harish and Varghese, 2006).

### **1.3.9. Prevention of Bacterial Vaginosis**

Bacterial vaginosis is not completely understood by scientists, and the best way to prevent it is unknown. However, it is known that BV is associated with having a new sex partner or having multiple sex partners. Therefore basic prevention steps which can help reduce the risk of upsetting the natural balance of bacteria in the vagina and developing BV requires to be abstinent, to limit the number of sex partners, to use all of the medicine

prescribed for treatment of BV, even if the symptoms go away and avoiding douching (Vitali *et al.*, 2007).

## **2. OBJECTIVES OF THE STUDY**

### **2.1. General Objective**

The main aim of this study was to assess the prevalence of bacterial vaginosis in women visiting a gynecological clinic in Addis Ababa and to identify and characterize the most common Lactic acid bacteria isolated from vaginal fluid of women.

### **2.2. Specific Objectives**

- ✓ To examine the human vaginal microbial flora
- ✓ To identify the dominant microbial flora that colonize the human vagina
- ✓ To examine the association between vaginal colonization and normal flora, abnormal vaginal flora and occurrence of infection particularly bacterial vaginosis
- ✓ To assess the distribution of drug resistance among the dominant lactic acid bacteria isolates from vaginal microflora

### **3. MATERIALS AND METHODS**

#### **3.1. Study Population**

Sources of populations were women residents of Addis Ababa scheduled to have a vaginal examination at Yehulueshet Higher Clinic. The study sample comprised randomly selected 100 women age range between 18-60 years.

#### **3.2. Specimen Collection**

Sampling was carried out by the gynecology and obstetrics specialist through insertion of a sterile cotton swabs into the vaginal lateral wall, after placement of a non-lubricated speculum. The swab was rotated against the vaginal wall at the mid portion of the vault and was carefully removed. All women who had been positive for pathogenic infections were treated according to the prescription of the physician. The samples were immediately put in sterilized test tube, labeled with date, sample code number and transported and analyzed in the Microbiology laboratory of the Institute of Pathobiology, AAU within 2 hrs interval of sample collection.

#### **3.3. Specimen Processing**

The swab was first used for anaerobic culture preparation by streaking on the MRS agar plates. Then fluids taken from the swabs were used to prepare a smear on a glass slide for the purpose of Gram staining and grading was done according to Nugent criteria (Nugent *et al.*, 1991).

#### **3.4. Gram-Staining, Grading of Slides and the Scoring System**

Vaginal fluid was transferred onto a glass microscope slide from the cotton-tipped swab. The slide was labeled with the date of collection and sample code. It was, then air dried, heat fixed and Gram-stained according to the procedure of Kopeloff ( ). Finally the slide was observed under oil immersion high power magnification of microscope(1000x).

The microbiological evaluation of vaginal microflora pattern was made using Nugent scoring system (table 1 ), based on counting bacterial cell types on Gram stained slides of vaginal smears.

According to the Nugent criteria interpretation, when the lactobacilli morphotype (Large Gram-positive rod) was present alone or in combination with very few *Gardnerella* morphotype (Small Gram-negative rod), the smear is interpreted as normal (score 0-3). When a more mixed flora, including not only the *Gardnerella* morphotype but also other Gram-negative and Gram-positive bacteria, such as curved rods, Gram negative rods and Gram-positive cocci, are present and when the *Lactobacillus* morphotype is completely absent, the smear is interpreted as consistent with BV (score 7-10). When lactobacilli found in low number and *Gardnerella* and *Mobiluncus* present in considerable large number, it is interpreted as an intermediate flora (score of 4–6) (Nugent *et al*, 1991).

Following the above steps, estimation of numbers of bacteria between 1–30 intervals per field of microscope was taken as approximate representative of bacteria in the whole field. The numbers of different bacterial morphotypes were then transformed to intervals in accordance with Nugent scoring system. Finally, the points achieved from the number of different bacterial morphotypes were added together.

Table 1: Scoring Vaginal Gram’s Stain for Bacterial Vaginosis (Nugent *et al*, 1991)

Scale	<i>Lactobacillus</i>	Score	<i>Gardnerella</i>	Score	<i>Mobiluncus</i>	Score
4+ = >30 organism per field	4+	0	4+	4	4+	2
3+ = 5-30 organism per field	3+	1	3+	3	3+	2
2+ = 1-4 organism per field	2+	2	2+	2	2+	1
0 = no organism per field	0	4	0	0	0	0

### **3.5. Plating, Isolation and Identification of Lactic Acid Bacteria (LAB)**

Plating was done within 2 hours of arrival of the sample in the laboratory. Enumeration of lactic acid bacteria was done after swabbing on de Mann Rogosa Sharp agar (MRS) and plates were incubated anaerobically using anaerobic jar at 37 °C for 48-72 hours. Five colonies were randomly selected from each MRS agar plate. The colonies were isolated and purified using Brain Heart Infusion (BHI) broth before being subjected to characterization. The isolates were grouped as lactic acid bacteria after examining for their Gram reaction, colony morphology, catalase reaction and glucose fermentation test. Finally representative of pure colonies from each plate were transferred to MRS agar slant, incubated for 24 hours and maintained in the refrigerator at 4°C.

### **3.6. Gram Reaction (KOH test)**

Two drops of 3% Potassium Hydroxide (KOH) solution were placed on a clean microscope slide. A pure culture colony was picked using a sterile wire loop from the MRS agar plate and stirred in the KOH solution, and the inoculating loop was then raised slowly from the mass. The Gram-negative ones show the thread of slime followed the loop, where as the Gram positives do not show slime and the watery suspension.

### **3.7. Catalase Test**

A drop of 3% solution of hydrogen peroxide ( $H_2O_2$ ) was placed on a clean microscope slide, and culture colony from the MRS plate was picked using sterile wire loop and the evolution of gas causing bubbles was indicative of positive test.

### **3.8. Fermentation Test (Gas Production):**

The production of gas during glucose fermentation was observed by placing an inverted Durham tube in Brain Heart Infusion (BHI) broth with 50% of glucose inoculated with pure culture isolates. The results were analyzed after 24hrs of incubation period.

### **3.9. Antibiotic Susceptibility Test**

The antimicrobial activities of the isolates were quantified by disc diffusion method as described by Bauer *et al.*, (1966). Well-isolated colonies were selected from MRS agar plate and transferred into a tube containing sterile 5ml BHI broth. The inoculated broth was incubated at 37°C for 24 hours. Broth culture was swabbed on the entire dried surface of a Mueller-Hinton agar plate. Eight antibiotic discs consisting of Ampicillin (10mg), Clindamycin (2mg), Gentamycin (10mg), Kanamycin (30mg), Methicillin (5mg), Penicillin (10 unit), Streptomycin (10mg) and Vancomycin (30mg) were placed on the surface of the agar plate using disk dispenser and incubated for 24 hours at 37°C. After incubation, the diameter of the zones of complete inhibition was measured to the nearest whole number in millimeter, using transparent ruler.

### **3.10. Data Analysis**

The Data obtained was processed using SPSS software for possible association between variables. Colonization and dominance rate of lactic acid bacteria and other pathogenic microflora was calculated. Intensity of bacterial vaginosis was determined by counting pathogenic bacterial density per-Gram stained slide.

### **3.11. Ethical Consideration**

Ethical clearance was obtained from ethical clearance committee of Biology Department in AAU. The purpose of the study was clearly explained to all subjects and they were included in the study after they consented. Any patient positive for BV was treated by the physician.

## 4. RESULTS

During the six month study period, from January 2008 - June 2008, samples were taken randomly from 100 women with age range from 18-65 (mean age; 35.92 and median age 34 years). Out of 100 women enrolled in the study 44% were in the age range of 18-30, 27% were in age range of 31-40, and 21% were in range between 41 to 50 and the remaining 8% were greater than 50. Among the 100 women 62 (62%) were Christians whereas 38(38%) were Muslims. About 93% of the women were married and the remaining 7% were singles. Form the total study population, 11% were pregnant, 11% were contraceptive users and 19% had experienced abortion at least once in their previous life time. Of all women who were enrolled in the study, only 15% were symptomatic with bacterial vaginosis (the presence of malodor or abnormal discharge was confirmed by the physician during clinical diagnosis) but the rest (85%) were asymptomatic.

Table 2. Socio-demographic profile of women involved in the study

<b>Age</b>	<b>18-30</b>	<b>31-40</b>	<b>41-50</b>	<b>&gt;51</b>
	44%	27%	21%	8%
<b>Religion</b>	<b>Christian</b>	<b>Muslim</b>		
	62%	38%		
<b>Marital Status</b>	<b>Married</b>	<b>Single</b>		
	93%	7%		
<b>Pregnancy</b>	<b>Pregnant</b>	<b>Non-pregnant</b>		
	11%	89%		
<b>Abortion History</b>	<b>Previous Abortion</b>	<b>No abortion</b>		
	19%	81%		
<b>Contraceptive use trend</b>	<b>Contraceptive users</b>	<b>Non-users</b>		
	11%	89%		
<b>Clinical Case</b>	<b>Asymptomatic</b>	<b>Symptomatic</b>		
	85%	15%		

#### **4.1. Gram Stain Patterns of Vaginal Micro-Flora**

Gram stain patterns based on Nugent criteria represent the presence of BV, intermediate or normal flora. Generally, an inverse relationship between the presence of *Lactobacillus* morphotype and *Gardnerella* morphotype was observed. When the *Lactobacillus* morphotype was dominant, the *Gardnerella* morphotype was usually absent. When the *Gardnerella* morphotype was dominant the quantity of the *Lactobacillus* morphotype was generally diminished.

According to this study, large Gram-positive bacilli were dominantly present in 42% of women. The typical appearance of the organisms identified as having the *Lactobacillus* morphotype on Gram stain was evident for the absence of bacterial vaginosis and generally interpreted as normal flora (score 0-3).

In 26% of the women, two or three bacterial morphotypes were evident. Stains showing the presence of few *Lactobacillus* and many Gram-negative morphotypes (*Gardnerella* and/or *Mobiluncus* morphotype) were interpreted as intermediate flora (score 4-6).

Thirty two percent of the women showed complete absence or few presence of *Lactobacillus* morphotype and a smear consistent with BV (score 7-10). The flora in this group of women was mainly composed of small Gram-negative organisms of the *Gardnerella* morphotype and curved Gram-negative rods (*Mobiluncus* morphotype).

#### **4.2. Prevalence of BV by Socio-demographic and other Associated Factors**

The rate of BV infection increased with age as observed in this study. A higher prevalence (52%) of BV was observed among the 31-40 aged group study participants than the other age groups. The prevalence decreased among study participants above 41 years old (Figure 6). The majority of the study participants who were under the age category of 18-30 had a high prevalence of normal flora as compared to the other age groups. The prevalence of intermediate flora was higher (75%) among those women above 50.

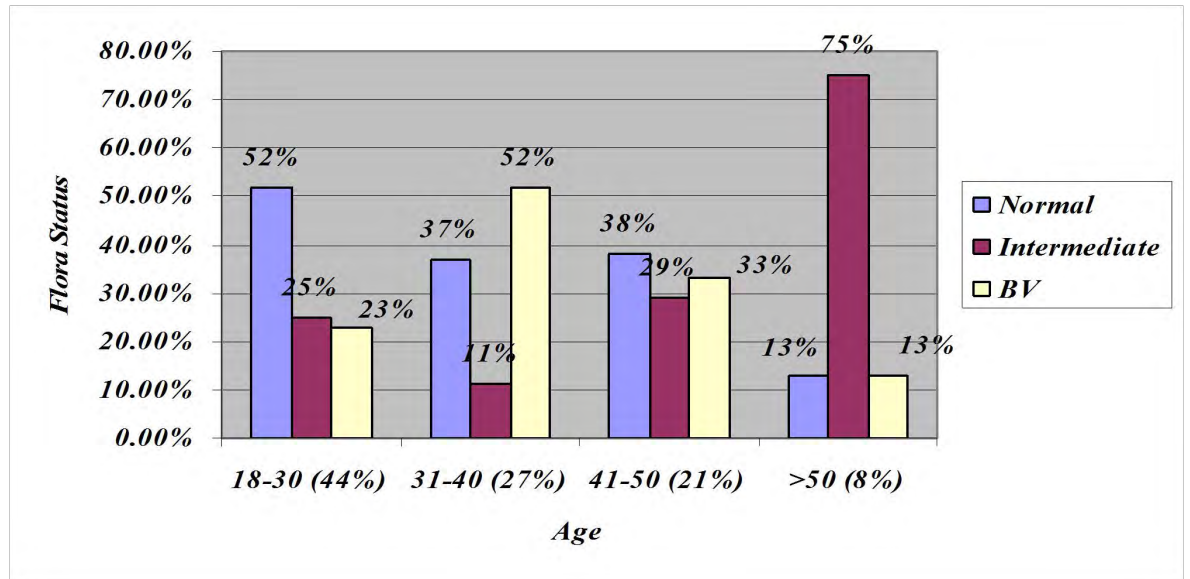


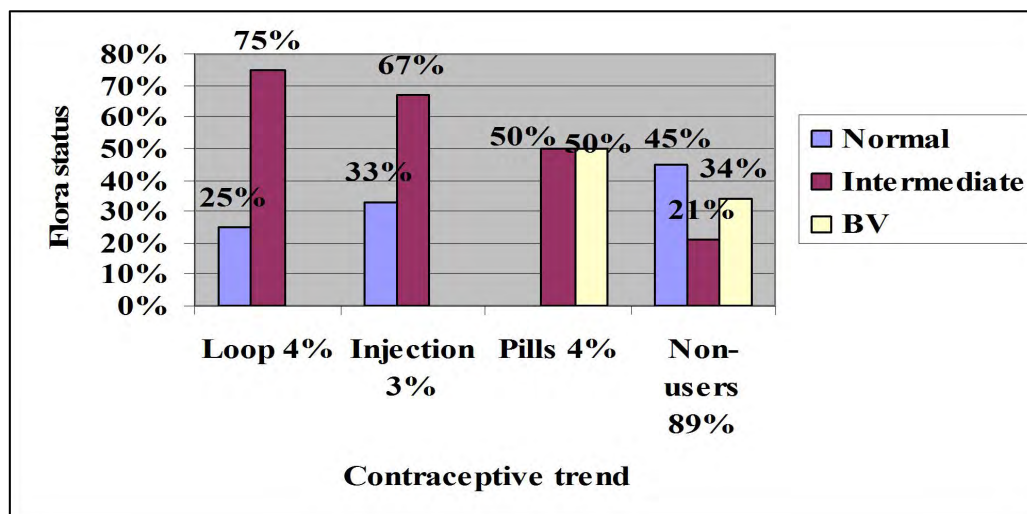
Fig. 6. Pattern of Vaginal flora with Age

Prevalence of BV by Socio-demographic and Clinical Status of women using Nugent's Criteria is presented in Table 3. Based on the study results, 21 (34%) of the 62 Christian women had BV as compared to 11 (29%) of 38 Muslim women. The results also show that 31 (33%) of 93 married women had BV as compared to 2(29%) of the 7 single women. Out of 85 women who had no symptom of bacterial vaginosis during clinical diagnosis, 24 (28%) had BV where as among 15 symptomatic women, a high prevalence 8 were positive for BV according to the Gram stain grading.

**Table 3: Prevalence of BV by Socio-demographic and Clinical Status of women using Nugent's Criteria**

		Pattern of Micro-flora		
		Normal	Intermediate	BV
<b>Total (100)</b>		<b>42%</b>	<b>26%</b>	<b>32%</b>
<b>Religion</b>	N	n/N (%)	n/N (%)	n/N (%)
Christian	62	29 (47%)	12 (19%)	21 (34%)
Muslim	38	13 (34%)	14 (37%)	11 (29%)
<b>Marital Status</b>				
Married	93	38 (41%)	24 (26%)	31 (33%)
Single	7	4 (57%)	1 (14%)	2 (29%)
<b>Clinical Case</b>				
Asymptomatic	85	40 (47%)	21 (25%)	24 (28%)
Symptomatic	15	2 (13%)	5 (33%)	8 (53%)

A high prevalence of intermediate flora was found among women who used loop (75%) or injection (67%) as contraceptive method. BV was observed among 2 of 4 oral contraceptive (pills) users. The majority of non-contraceptive users (45%) were found to have dominant normal flora.



**Fig. 7. Pattern of Vaginal flora with current contraceptive use**

Of the 100 women examined, 11 were pregnant. Of these, 6 had normal vaginal flora as compared to 36 of 89 non-pregnant women. BV was found in low prevalence (18%) among the pregnant women.

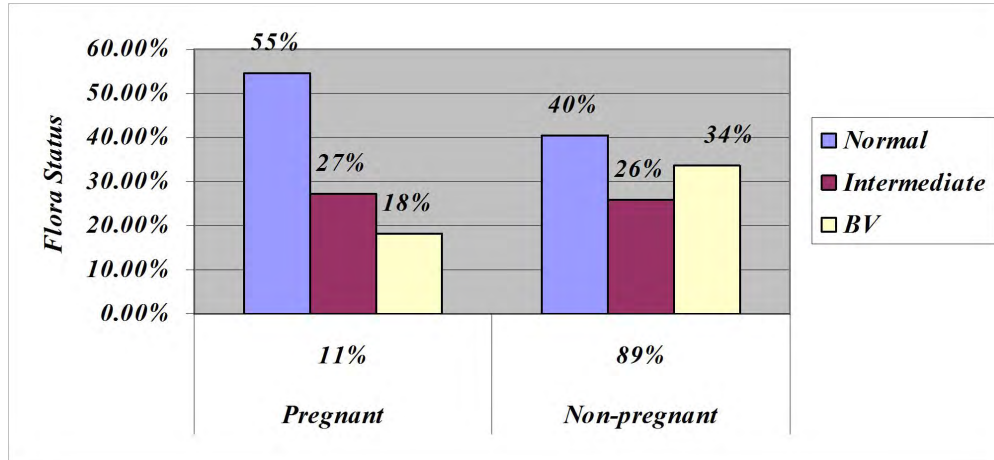


Fig. 8. Pattern of Vaginal flora with pregnant versus non-pregnant women

Of the 19 women who had a history of at least one abortion in the past, BV was found in high prevalence 37% compared to participants who had no abortion history (31%). The majority of women (44%), who had no abortion history, had normal flora status.

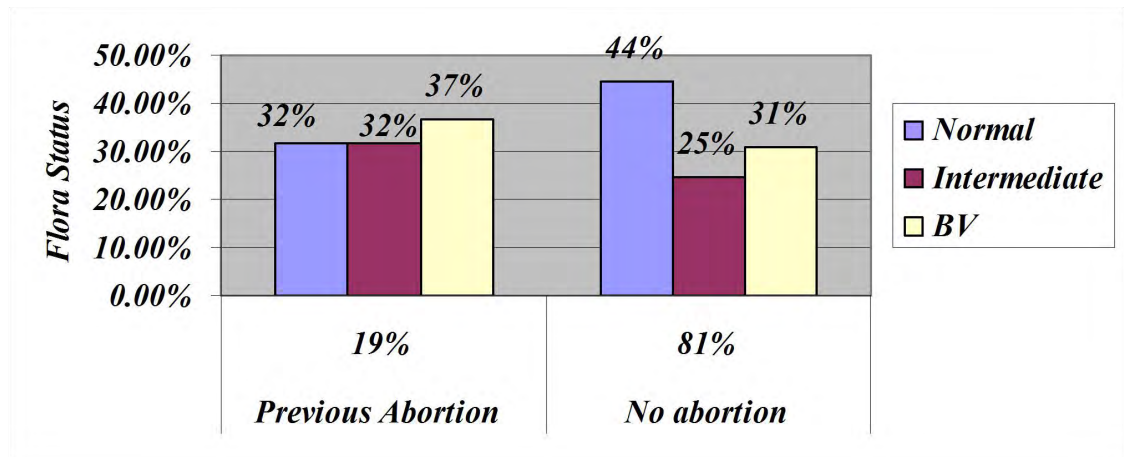


Fig. 9. Pattern of Vaginal flora with previous abortion history

### **4.3. Lactic Acid Bacteria Isolated from Vaginal Fluid Culture**

Results from microscopic examination of colony morphology, fermentation, catalase and KOH tests are summarized in table 4. LAB were isolated randomly from specimen culture of 70 women. Thirty women did not have genital cultures due to cases of either contamination or growth failure.

A total of 350 randomly picked colonies were purified and analyzed. Gram positive, catalase negative, cocci or rod shaped isolates which formed single, paired, tetrad or long chain colony arrangements were isolated and classified based on their colony morphology and fermentation test results to one of the following four genera: *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Pediococcus*.

The most predominant isolates were long chained, cocci-shaped homofermentative lactococci which comprised 140 (40%) of the 350 isolates. Lactobacilli constituted 29% of the total 350 colonies. Of this lactobacilli isolates, 84% were homofermentative and the rest were hetrofermentative. Colonies which formed either paired or tetrad chain arrangement and were homofermentative in their glucose utilization were categorized under pediococci and made up 23% of the total 350 LAB colonies. Finally, colonies which were cocci but shown hetrofermentative nature were identified as Leucnostoc and made up 8 % of the total LAB.

Table 4: Microscopic characteristics, KOH and Catalase test results of lactic acid bacteria isolated from women vaginal fluid

LAB Isolated	Colony morphology	Colony arrangement	Fermentation Test	KOH test	Catalase test	Isolates 350	%
Lactococci	Cocci	Long Chain	Homofermentative	Positive	Negative	140	40%
Lactobacilli	Rod	Single	84% Homo- fermentative 16% Hetro- fermentative	Positive	Negative	100	29%
Pediococci	Cocci	Pair or Tetrad	Homofermentative	Positive	Negative	82	23%
<i>Luconostoc</i>	Cocci	Pair or Short chain	Hetrofermentative	Positive	Negative	28	8%

#### 4.4. Antimicrobial Susceptibility/Resistance Patterns of LAB Isolates

In this study, the conventional methodology described by Bauer *et al.*, (1966) was applied using Muller Hinton medium to test the effect of the antibiotics on selected 60 LAB isolates. Based on size of the inhibition zones, the LAB were classified into susceptible, moderate susceptible (intermediate) and resistant isolates.

The antimicrobial activity of LAB isolates is given in Table 4. Among the 60 lactic acid bacteria subjected to antimicrobial sensitivity or resistance, the diameters of inhibition ranged from 0mm to 30mm. Over 50% of the isolates belonging to the various genera were sensitive to Ampicillin, Clindamycin, Gentamycin, Kanamycin, Methicillin, Since intermediate values are usually considered as sensitive for interpretation purposes, resistance to Clindamycin and Methicillin was observed in only about 40% of the isolates.

Table 5: The antimicrobial activity of LAB isolates from women vaginal fluid

	Amp	Clind	Gen	Kan	Meth	Pen	Strep	Van
Resistance	16 27%	25 42%	16 27%	18 30%	24 40%	20 33%	22 37%	12 20%
Intermediate	12 20%	5 8%	2 3%	0	0	34 57%	0	0
Sensitive	32 53%	30 50%	42 70%	42 70%	36 60%	6 10%	38 63%	48 80%

Where: Amp, Ampicillin; Clind, Clindamycin; Gen, Gentamycin; Kan, Kanamycin; Meth, Methicillin; Pen, Penicillin; Strep, Streptomycin; Van, Vancomycin

In general, it can be said that resistance of LAB to all antibiotic tested ranged between 20% and 42%.

## 5. DISCUSSION

In this study a BV prevalence of 32% found. This result concurs with the studies that have specifically assessed BV prevalence among African settings. Demba *et al.* (2005) reported the prevalence of BV and the pattern of vaginal micro-flora among women with vaginal discharge syndrome in an African setting, and recorded a BV prevalence of 47.6% in Gambia, 20–23% in Burkina Faso and Malawi, 37% in Tanzania and 49% in Kenya populations.

Findings in this study attempted to analyze the association of BV with some of the possible factors such as age, contraceptive use, pregnancy as well as previous abortion history. BV was found in higher proportion among the age group of 31-40. Some researchers have previously reported that the condition is more common in among younger women (Bukusi *et al.*, 2006). While others found that risk for BV increased with age (Morris *et al.*, 2001). However, most researchers agree on the instability of vaginal flora and the subsequent BV is common phenomena among reproductive age group women as compared to the pre-pubertal and post-menopausal women (Alesna *et al.*, 1996, Bhalla *et al.*, 2007).

This study show Muslim women had a BV prevalence of 34%. One study done in India reported a BV prevalence of 37% for Muslim women (Madhivanan *et al.*, 2008). Another study done to know the association of BV with religious practices suggested certain risk factors such as genital hygiene practices are known to be associated with BV and found to vary among women who differ in religion (Bhalla *et al.*, 2007).

The prevalence of pregnant women with BV was only 18%. According to different studies bacterial vaginosis is a common vaginal infection that affects 12–22% of pregnant women (Joan *et al.*, 2000). Pregnant women with BV are considered to be at increased risk for having preterm birth, infants with low birth weight and premature rupture of the membranes (Sobel, 2000, Senok *et al.*, 2006).

Women who had a previous abortion history show a high prevalence of BV (37%) as compared to non-aborted women (31%). A growing body of literatures have been reported an increased risk of spontaneous abortion among pregnant women with BV. This is due to the decrease in the protective role of normal flora and the overgrowth of pathogenic microorganisms lead to the disruption of the entire uterus wall which serve as an attachment for the fetus (Sobel, 2000, Wilks *et al.*, 2004).

Moreover, studies found significant associations between bacterial vaginosis and the use of contraceptive (Moi, 1990). In our study the majority of contraceptive users had dominant intermediate flora and BV recorded among oral contraceptive users only. Since, bacterial vaginosis is common among women at reproductive age who are also the most likely to use contraceptives, any association between BV and contraceptive use could be the result of the age structure of the women (Sobel, 2000).

In different studies women with BV often report no signs or symptoms at all and up to 50% of women were asymptomatic (Morris *et al.*, 2001). According to this study result, the presence of BV was confirmed among women who had shown clinical symptom. However, asymptomatic women with BV also shared a considerable percentage (28%). These asymptomatic women are less likely to seek treatment and thus are more likely to acquire other serious STDs including HIV/AIDS (Taha *et al.*, 1998, Senok *et al.*, 2006).

In the result presented in this study, a comparison of vaginal micro-flora isolates with Nugent's score showed significant positive associations with BV and a significant negative association with the presence of lactobacilli or vice versa. These findings are generally coinciding with findings in other studies (Nugent *et al.*, 1991, Demba *et al.*, 2005), since the method employed for the Nugent's score is based on the observation of BV-associated bacterial morphotypes.

This study found a considerable different proportion of LAB isolates which comprised the genera *Lactococcus*, *Lactobacillus*, *Pediococcus* and *Leuconostoc* respectively. Few literatures documented the species richness and relative abundance of *Lactobacillus* and other lactic acid bacteria in vagina (Jin *et al.*, 2007, Senok *et al.*, 2006). According to these, though many lactic acid bacteria colonize women, their genera and species distributions may be different in women of geographically separated communities.

Moreover, the knowledge of species richness and relative abundance of vaginal lactic acid bacteria may lead to the design of better probiotic products as bacterial replacement therapy.

The antimicrobial susceptibility of 60 LAB isolates was studied. The knowledge of the antimicrobial susceptibility or resistance of LAB isolated from human vagina is of interest to many researchers to predict the behavior of an exogenously applied probiotics in women subjected to bacterial vaginosis as well as to consider the application of the probiotic and antibiotics for the restoration of the normal genital flora (Senok *et al.*, 2006). In this regard, the performance of antimicrobial susceptibility testing of vaginal specimen may be considered as an effective guide for specific antimicrobial therapy for women with genital tract infections including BV (Reid *et al.*, 2003).

According to this study most of the LAB isolates were sensitive to vancomycin (80%). In different studies resistance or susceptibility to vancomycin has deserved a special consideration in terms of classification of lactic acid bacteria, mainly lactobacilli, associated with human infections (Senok *et al.*, 2006). Moreover, 41.67% of the isolates were resistant to Clindamycin. This result has interesting meaning because Clindamycin is one of the most commonly prescribed antibiotics for the treatment of bacterial vaginosis and supposed to be sensitive to most of the genital flora (Ocana *et al.*, 2006).

## 6. CONCLUSION

Based on the findings of this study, the following conclusions could be made:

- This study was the first of its kind on assessing the prevalence of BV and characterizing the major LAB isolates from women in Ethiopia.
- In this study a BV prevalence of 32% was found. Higher proportion of BV observed among women who were married, Christian, non-pregnant and had a previous history of abortion.
- LAB isolated from vaginal fluid of women in this study basically comprised four genera; *Lactococcus*(40%), followed by *Lactobacillus*(29%), *Pediococcus* (23%), and *Leuconostoc*(8%) respectively.
- The LAB isolated from vaginal specimen was resistance to various antibiotics with range of 20 % to 42% and sensitivity rang of 10% to 80%. However the majority of the isolates (41.6%) were resistant to Clindamycin where as 80% of the isolates were sensitive to Vancomycin.

## 7. RECOMMENDATIONS

Given the limited information regarding the factors and behaviors placing a woman at increased risk for BV, a considerable prevalence of BV (32%) was found in this study. However it is essential to conduct large-scale epidemiological studies to determine behavioral, sexual, or environmental factors that increase a woman's risk for BV in our country setting. It is only when microbiologic, epidemiologic, and sociologic determinants of BV are examined that fully high risk groups can be targeted more efficiently.

Future work must focus on exploring a causative association of BV to acquiring of STD's. At present different researches found a definite link between bacterial vaginosis and increased risk of HIV-1 acquisition. Hence higher prevalence of HIV/AIDS is found in Ethiopia, knowing the association of BV versus STD's would have implications for decreasing the vulnerability of women.

On top of that, Bacterial vaginosis is an important predictor of adverse reproductive outcomes. Bacterial vaginosis has yet to be shown as an independent risk factor for pelvic inflammatory disease, infertility, preterm delivery and miscarriage. If the association of such factors with BV is studied in the future, it will probably contribute a lot to minimize the public health profile of women who are at risk of reproductive morbidity and mortality.

Moreover, attempts should be made to supply standardized clinical diagnostic criteria of BV so that future case control studies will be more effective and comparable. The validation of clinical diagnosis compared with microbiological analysis would help in assessing the effectiveness of studies.

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Annex 2

VERBAL CONSENT FORM

God Morning/ Good Afternoon

I am Seblewongiel Aynalem, a second year graduate student in Department of Biology at Addis Ababa University. This research is designed aiming to identify and characterize the most common microbial microorganisms found in vagina of human. Moreover the study will examine the association between vaginal colonization with abnormal flora and occurrence of infection particularly bacterial vaginosis.

The intention of this research is only for academic purpose. Participation is completely voluntary. I will use codes and making secret your real name to keep the confidentiality. The information you will give is very much valuable for the success my research. As a result, I am kindly requesting your co-operation.

Are you willing to be participant of the research?

Yes \_\_\_\_\_

No \_\_\_\_\_

Thank You.

## DECLARATION

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

Name: Seblewongiel Aynalem

Signature: -----

This thesis has been submitted for examination with my approval as an advisor:



**Prof. Mogessie Ashenafi**

---

**Prof. Lukman Yusuf**