Determinants of Bacterial Vaginosis (BV) in HIV Negative Women Living in Cape Town, South Africa: A Cross-Sectional Study.

By

Felicity Pinkie Gopolang

(GPLFEL001)

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Supervisor: Professor Landon Myer

School of Public Health & Family Medicine

Infectious Disease Epidemiology Research Unit

University of Cape Town, South Africa.

DECLARATION

I, Felicity Pinkie Gopolang (GPLFEL001), hereby declare that the work in this mini-dissertation is based on my original work (except where acknowledgements indicate otherwise) and has not, in whole or in part, been submitted towards another degree, at this University or elsewhere.

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ABSTRACT

Bacterial vaginosis (BV) is a common cause of vaginal symptoms among women of childbearing age, although some women may be asymptomatic. The etiology of BV has been uncertain for many years, but several studies have related an increased frequency of BV to multiple diverse factors such as smoking, sexual behavior, vaginal douching, black race, sexually transmitted infections, and certain socio-economic factors. BV infection may lead to adverse reproductive health outcomes and may also increase the susceptibility of women to human immunodeficiency virus (HIV) and sexually transmitted infections (STIs). It is therefore of importance to know the risk factors for BV among a population so that mechanisms of prevention can be developed.

The objective of this study was to determine the socio-demographic and behavioral risk factors of BV at baseline particularly, sexual behavior and contraceptive use, among HIV negative women living in a peri-urban area in Cape Town, South Africa.

A cross-sectional sub-study to analyze data collected using questionnaires at enrollment from a Prospective, Observational Feasibility Study to Determine HIV Incidence in Preparation for a Future Preventative HIV Vaccine Efficacy Trial from June 2006 to December 2007, in Cape Town, South Africa was conducted. Smears prepared from self-collected vaginal swabs at baseline, from 295 women aged 16 to 40 years, were also examined for BV diagnosis using Nugent's gram stain method. Logistic regression

analysis was performed using Stata10 statistical software to identify possible factors that determined BV diagnosis.

Over half (57%) of the women were diagnosed with BV. Women who reported washing inside the vagina with soap and water were found to be at risk of being diagnosed with BV (OR=1.64, 95%CI=0.99-2.71), whereas, those who reported using tobacco were found to be at a significant risk after adjusting for possible confounders (AOR=1.81, 95%CI=0.99-3.32). None of the sexual behavior factors, socio-economic factors, or contraceptive use were identified as risk factors. However, women who reported having had a past pregnancy(s) were found to be protected from being diagnosed with BV (AOR=0.56, 95%CI=0.35-0.92).

Intra-vaginal washing with soap and water and tobacco use were associated with the risk of BV among women in the study. Since these are behavioral factors, interventions around behavior change could be developed which may reduce the high BV prevalence among women in this community, which may in turn, lead to a reduction in adverse reproductive health outcomes and susceptibility to HIV and other STIs.

ABBREVIATIONS

ACC	Amsel's composite criteria
AIDS	Acquired immunodeficiency syndrome
AOR	Adjusted odds ratio
BTL	Bilateral tubal ligation
BV	Bacterial vaginosis
95%CI	95% Confidence interval
FDA	Food and drug administration
GUM	Genitourinary medicine
HIV	Human immunodeficiency syndrome
hpf	High power field
ICH	International Conference on Harmonization
IUD	Intra uterine contraceptive device
NBM	Nugent's gram stain evaluation of bacterial morphotypes
OCP	Oral contraceptive pill
OR	Odds ratio
P	P-value
PI	Principal investigator
PID	Pelvic inflammatory disease
RCT	Randomized controlled trial
SES	Socio-economic status
STD	Sexually transmitted disease
STI	Sexually transmitted infection
TCC	Thomason's clue cell criteria
UCT	University of Cape Town
USA	United States of America
VCT	Voluntary counseling and testing
WSW	Women who have sex with women

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Definition of bacterial vaginosis

Bacterial vaginosis (BV) is a common condition (Yen et al. 2003, Weir 2004, Hillier 2006) among women of childbearing age (Hillier & Holmes 1999, Smayevsky et al. 2001). While nearly half of the women with bacterial vaginosis are asymptomatic (Georgijević et al. 2000, Hillier 2006), those with symptoms commonly present with a malodorous vaginal discharge (Hillier & Holmes 1999, Georgijević et al. 2000, Wier 2004, Hillier 2006), and on occasion, vaginal burning or itching (Weir 2004).

Biochemical analysis of vaginal fluid from women with bacterial vaginosis usually shows characteristic changes thought to be caused by bacterial metabolism. (Hillier & Holmes 1999: 563). Hence, the malodor found in bacterial vaginosis has been attributed to the altered pattern of organic acids (e.g., increased succinate, decreased lactate) and abnormal amounts of putrescine, cadaverine, and trimethylamine found in vaginal fluids. (Hillier & Holmes 1999). It is believed that these amines raise the vaginal pH and are involved in creating the characteristic vaginal discharge, hence, producing the signs and symptoms of bacterial vaginosis. (Josey & Schwebke 2008: 152)

1.2 Prevalence

The prevalence of BV varies among pregnant and non-pregnant populations, depending on several factors which include clinical setting, socio-demographic factors, diagnostic

criteria, and gestational age. (Trabert & Misra 2007). The overall prevalence of BV recorded in asymptomatic pregnant women, from 8 institutions (located in Colombia, Ireland, Myanmar, The Philippines, Thailand, The United States, and Zimbabwe) participating in the Global Network for Perinatal and Reproductive Health (www.gnprh.org) ranges from 6 to 24%, with Ireland having the lowest prevalence, and Zimbabwe having the highest. (Tolosa et al. 2006). On the other hand, in non-pregnant women, the prevalence of BV in various countries (the United States, United Kingdom, Sweden, Australia, India, and the Gambia) ranges from 12 to 47%. (Holzman et al. 2001, Allsworth et al. 2007, Ness et al. 2002, Morris et al. 2001, Brabin et al. 2005, Shoubnikova et al. 1997, Moi 1990, Bradshaw et al. 2005, Demba et al. 2005, Madhivanan et al. 2008).

1.3 Historical names

Several names were used to describe bacterial vaginosis in the past, and these included: leukorrhea or non-specific vaginitis, *Gardnerella vaginalis* vaginitis, *Haemophilus vaginalis* vaginitis, *Corynebacterium* vaginitis, and anaerobic vaginosis (Hillier & Holmes 1999, Georgijević et al. 2000, Smayevsky et al. 2001, Hillier 2006).

1.4 Characteristic flora

Bacterial vaginosis is characterized by an imbalance of the normal vaginal flora, the shift resulting from a decrease in the normal dominant acid-producing (H_2O_2 –producing) Lactobacilli, and an overgrowth of mixed anaerobic bacteria. (Yen 2003, Weir 2004)

The concentration of bacteria increases from 100 to1000 fold in women with BV. (Georgijević et al. 2000) This mixed flora consists of *Gardnerella vaginalis,* and other anaerobes such as *Bacteroides* species, *Mycoplasma hominis*, *Mobiluncus* species, (Koumans & Kendrick 2001, cited in Weir 2004, Yen 2003) *Prevotella* species, and *Ureaplasma urealyticum*. (Koumans & Kendrick 2001, cited in Weir 2004).

1.5 Aetiology of BV

The etiology of BV has remained uncertain for many years. However, two leading hypotheses of causation of bacterial vaginosis have been cited. (Josey & Schwebke 2008). One is that *Gardnerella vaginalis* (originally designated *Haemophilus vaginilis*) is the specific and sexually transmitted aetiological agent (Gardner & Dukes 1955 and Josey & Hill 1988, cited in Josey & Schwebke 2008), and the other is the polymicrobial aetiological hypothesis, which postulates that G. *vaginalis* acts in concert with other bacteria, principally anaerobes, to produce the disease. (Hill 1993, cited in Josey & Schwebke 2008).

Since its introduction, the polymicrobial hypothesis has governed most reported studies on BV and its public health impact, but despite this, no host factors that initiate BV have been identified as yet. (Josey and Schwebke 2008).

However, there is substantial body of evidence demonstrating that factors associated with BV include age, ethnicity, socioeconomic status (Morris et al. 2001), contraceptive use

(Calzolari et al. 2000), and those related to sexual activity, which dominate. (Fethers et al. 2008).

1.6 Diagnosis of BV

Diagnosis of BV is mainly according to Amsel's composite criteria (Amsel et al. 1983) in routine clinical settings, and the Nugent's Gram stain evaluation of bacterial morphotypes (NBM) (Nugent et al. 1991), which is more suited for diagnosis in research. Amsel's criteria uses any three of four clinical signs of BV, being the presence of homogeneous vaginal discharge, the presence of "clue cells" in a wet mount preparation of vaginal fluid, pH of vaginal fluid of greater than 4.5, and the presence of an amine (fishy) odour after the addition of 10% potassium hydroxide (KOH). (Amsel et al. 1983).

Nugent's criteria, on the other hand, scores Gram stained bacterial morphotypes on a 0- to 10-point scale for the evaluation of vaginal flora, with scores of 0-3 considered as normal or negative for BV, 4-6 being intermediate flora, and 7-10 considered as positive for BV. (Nugent et al. 1991).

1.7 Adverse effects of BV

BV has been linked to serious public health consequences including pelvic inflammatory disease (PID) and spontaneous abortion (Allsworth et al. 2007), preterm delivery (Hay et al. 1994) with low birth weight (Hillier et al. 1995), postpartum endometritis (Allsworth et al. 2007), and acquisition of human immunodeficiency virus (HIV) by women (Myer et al. 2005, Atashili et al. 2008). Hence, the importance of understanding the causes of

BV is clearly evident. Until the pathogenesis is clarified, it will not be possible to improve the diagnosis, treatment, and prevention of this disease. (Josey & Schwebke 2008: 152).

Study aim

The aim is therefore, to conduct a sub-study to identify risk factors for bacterial vaginosis at baseline, in HIV negative women enrolled in a Prospective, Observational Feasibility Study to Determine HIV Incidence in Preparation for a Future Preventative HIV Vaccine Efficacy Trial in Cape Town, South Africa.

Study objectives

The objectives of this study are:-

1. To determine the baseline prevalence for BV.
2. To determine the socio-demographic risk factors for BV.
3. To determine the behavioral risk factors for BV, focusing particularly on sexual behavior and contraceptive use.

CHAPTER TWO

2.0 LITERATURE REVIEW

Literature on BV will be reviewed in this chapter, particularly that relating to the prevalence, diagnosis, and risk factors of BV.

2.1 Prevalence of BV

In a study conducted by Tolosa et al. (2006) in 7 countries participating in the Global Network for Perinatal and Reproductive Health from July 1999 to September 2001, the prevalence of BV among 1466 asymptomatic pregnant women, ranged from 6 to 24%. Later, Trabert & Misra (2007) supported his argument that BV prevalence varied according to several factors, and cited a wider range of BV prevalence (5 to 49%) in pregnant women, from studies (Hillier et al 1995, Klebanoff et al. 2005, Cristiano et al. 1996, Culhane et al. 2001, Kurki et al. 1992) carried out in different countries between 1992 and 2007.

Previous authors (Morris et al. 2001) had also contended that BV prevalence varied according to the population studied and geographic location, and cited prevalences recorded by Gibbs in 1990, ranging from 4% in university students to 33% in genitourinary medicine (GUM) clinics. Prior to that, Georgijević et al.'s (2000) report on the average incidence of BV had been recorded as follows: 10-35% in patients visiting gynaecological wards, 10-30% in patients visiting obstetric wards and 20-60% in patients visiting services of sexually transmitted diseases. A very recent study (Madhivanan et al.

2008) on Indian women attending reproductive health clinics obtained a similar prevalence of 19%.

On the other hand, Evans et al. (2007) cited high prevalences (reported by Edwards & Thin 1990, Skinner et al. 1996, Berger et al. 1995, McCaffrey et al. 1999, Bailey et al. 2004), of between 29 and 52% in UK and US studies on lesbians attending a Sexually transmitted infection (STI) clinic, which proved to be much higher than in heterosexual women.

Other studies on non-pregnant women, had recorded BV prevalences ranging from 15 to 40% in the United States (Holzman et al. 2001, Allsworth et al. 2007, Ness et al. 2002), 12 to 33% in the United Kingdom (Morris et al. 2001, Brabin et al. 2005), 13 to 27% in Sweden (Shoubnikova et al. 1997, Moi et al. 1990), and 46% in Australia (Bradshaw et al. 2005).

2.2 Polymicrobial flora

The constituents of BV have been described as polymicrobial flora, because of the characteristic mixed flora it presents as, consisting of *Gardnerella vaginalis* and other anaerobes, as listed in the introduction. In a clinical study in which part of the objective was to compare the prevalence of *Gardnerella vaginalis*, anaerobic bacteria, and *Mycoplasma hominis* in women with bacterial vaginosis and those without it, Smayevsky et al. (2001) demonstrated that there were no isolates of *Prevotella* species, *Bacteroides* species, *Mobiluncus* species, nor *Mycoplasma hominis* in the vaginal fluid of women

without bacterial vaginosis, and in contrast, these microorganisms, together with *Gardnerella vaginalis* showed a strong association with bacterial vaginosis in women with the condition. A more recent study (Tamrakar et al. 2007) also showed that BV-related bacteria, including BVAB2, *Megasphaera*, *Leptotrichia*, and *Eggerthella*-like bacterium were common in the vagina of pregnant Japanese women with BV. Tamrakar et al. (2007) also cited other authors (Ferris et al, 2004, Zhou et al. 2004, Frecricks et al. 2005) who reported detecting these microorganisms by molecular analyses in BV flora. In addition, Josey & Schwebke (2008) also cited Fredricks et al.'s (2005) addition of *Atopobium vaginae*, to the multitudinous list of associated bacteria.

On the other hand, despite Hillier et al. (1992) demonstrating that women colonized by H_2O_2-positive lactobacilli were less likely to have BV compared to those with H_2O_2-negative lactobacilli, in contrast, a study by Rosenstein et al. (1997) revealed the presence of H_2O_2 –producing strains of lactobacilli in 11 out of 12 pregnant women with BV, indicating that it is possible for a change to abnormal flora to occur before the complete disappearance of lactobacilli. Thus, it became more recognizable that BV is microbiologically heterogeneous, with ~1 in 4 women with bacterial vaginosis having H_2O_2 producing lactobacilli present. (Agnew & Hillier 1995, cited in Beigi et al. 2005)

Despite extensive study of the altered vaginal bacterial environment associated with BV, the microbiological cause remains controversial. (Josey and Schwebke 2008: 152)

In a recent review, Josey and Schwebke (2008) reassessed one of the two leading hypotheses of causation of bacterial vaginosis, the polymicrobial hypothesis, which has

held sway for more than two decades. In this review, it was argued that the cause and effect association of anaerobes with bacterial vaginosis was unjustified. To justify this, Josey and Schwebke (2008) cited Tabaqchali et al. (1983) who argued that increased anaerobic concentrations are associated with other vaginal conditions, particularly trichomoniasis, and are not specific to bacterial vaginosis.

2.3 Diagnosis

Two most widely used methods of diagnosing bacterial vaginosis are the Amsel's composite criteria (Amsel et al. 1983) and the Nugent's Gram stain evaluation of bacterial morphotypes (NBM) (Nugent et al. 1991).

2.3.1 Amsel's composite criteria (ACC)

Amsel's criteria defines bacterial vaginosis using three of the following four signs: the presence of homogeneous vaginal discharge, the presence of "clue cells" in a wet mount preparation of vaginal fluid, pH of vaginal fluid of greater than 4.5, and the presence of an amine (fishy) odour after the addition of 10% potassium hydroxide (KOH). (Amsel et al. 1983). Nugent et al. in 1991, saw it most fitting for bacterial vaginosis to be defined primarily by these clinical signs, reasoning that it is a clinical syndrome associated with a group of genital microorganisms rather than a single etiologic agent. Nevertheless, they further went on to highlight that, despite the assessment of clinical signs being the "gold standard", the signs are subtle and their detection is dependent on the acuity of the clinician performing the test. It was therefore, foreseen that this method would provide

problems of standardizing observations and determining the comparability of results, if used in a large study with more than one clinician. (Nugent et al. 1991). Hence, it was with this in mind, that in 1991, Nugent et al. examined the intercenter variability in the interpretation of Gram-stained vaginal smears from pregnant women participating in a large multicenter study. They developed a new scoring system which allowed gradations in severity, for the morphotypes that were most reliably identified at all centers, and hence, the proposal of a standardized scoring system for the interpretation of Gram-stained vaginal smears. This has since formed the basis of Nugent's Gram-stain method for diagnosing bacterial vaginosis. In later years, Calzolari et al. (2000), in agreement, argued that interpretation of clinical signs (hence, Amsel's criteria) can be difficult because factors unrelated to infection could also elevate the vaginal pH or mask the amine odour. Examples of these factors include recent intercourse, menstruation, recent douching, presence of large amounts of cervical mucous, or the physician's ability to recognize amine odour. Hence, this further justified the utilization of Nugent's Gram stain method, as an alternative diagnostic criterion.

2.3.2 Nugent's Gram stain evaluation of bacterial morphotypes (NBM)

In this method, the scoring system provides a 0- to 10-point scale for the evaluation of vaginal flora. That is, the number of Lactobacilli (medium to large, gram positive rods), Bacteroides/*Gardnerella vaginalis* (small gram-variable rods, coccobacilli), and Mobiluncus (thin curved, gram-variable rods) morphotypes per oil-immersion microscope field. A score of 0-3 is normal vaginal flora, 4-6 is intermediate, and 7-10 is positive for bacterial vaginosis. (Nugent et al. 1991).

However, one disadvantage of this method is that it does not permit an immediate treatment at the moment of consultation. (Calzolari et al. 2000).

2.3.3 Thomason's clue cell criteria (TCC)

According to Hilmarsdóttir et al. (2006), Amsel's method, is cumbersome and not easily subjected to quality control and Nugent's method's apparent complexity may have limited its adoption by clinical laboratories. Hence, their study in 2006, was the first to assess the utility of a third method, named Thomason's clue cell criteria (TCC), after its initial publication by Thomason et al. in 1992. This study evaluated Thomason et al.'s description of simple interpretation of Gram-stained smears against Amsel's and Nugent's methods for the diagnosis of bacterial vaginosis, and also examined interobserver agreement for the Gram stain methods. The rapid Gram stain evaluation was found to be reliable, easy to perform, and well suited for the routine clinical laboratory.

2.4 Adverse health effects caused by BV

A number of adverse health outcomes have been linked to bacterial vaginosis, the majority being related to women's reproductive health morbidity, and others being sexually transmitted diseases (STDs).

Adverse pregnancy outcomes and infectious complications of certain gynaecologic procedures which have been reported to be linked to bacterial vaginosis (Koumans et al.

2001), include pelvic inflammatory disease (PID) (Koumans et al. 2001, Allsworth et al. 2007), postoperative infections (Allsworth et al. 2007), late miscarriage / spontaneous abortion (Hay et al. 1994, Allsworth et al. 2007), preterm delivery (Hay et al. 1994, Allsworth et al. 2007, Madhivanan et al. 2008) with low birth weight (Hillier et al. 1995, Madhivanan et al. 2008), and postpartum endometritis (Allsworth et al. 2007). However, a recent intervention study (Larsson et al. 2007) found no correlation between bacterial vaginosis and history of preterm deliveries, previous miscarriages, maternal under-weight, extra-uterine pregnancies, and infertility problems, among other things. They also found no association between BV (in both treated and non-treated patients) and obstetric outcomes of women who delivered at term.

The multiple STDs who's prevalence and incidence BV has been found to be associated with (Allsworth et al. 2007), include HIV (Koumans et al. 2001, Myer et al. 2005, Atashili et al. 2008), chlamydia, gonorrhoea, herpes, and trichomoniasis. Because high bacterial vaginosis prevalence may result in a high number of HIV infections being attributable to bacterial vaginosis, authors have recommended further prospective studies to evaluate the role of BV in HIV acquisition in women (Atashili et al. 2008), and also in the area of BV prevention, as a possible means of preventing HIV infections in women. (Koumans et al. 2001, Myer et al. 2005, Atashili et al. 2008)

2.5 Etiology

Little is known about the natural history of bacterial vaginosis (Holzman et al. 2001), and despite extensive research being carried out over the years, the etiology of bacterial

vaginosis still remains uncertain. Several factors have been implicated, which fall into two groups, being behavioral and socio-demographic risk factors.

2.5.1 Socio-demographic risk factors

Socio-demographic factors which have been found to be associated with Bacterial vaginosis include age above 24 years (Go et al. 2000, Morris et al. 2001), low socio-economic status (SES)/education level (Holzman et al. 2001, Morris et al. 2001, Bukusi et al. 2006, Koumans et al. 2007, Allsworth & Peipert 2007), black ethnic group (Georgijević et al. 2000, Jancin 2000, Morris et al. 2001, Holzman et al. 2001, Yen et al. 2003, Koumans et al. 2007, Allsworth et al. 2007), and being unmarried (Bukusi et al. 2006, Koumans et al. 2007)

2.5.1.1 Age

However, a study by (Holzman et al. 2001) as well as a news report by Jancin (2000) reported no impact of age on the prevalence of bacterial vaginosis. Furthermore, a more recent study on Kenyan women (Bukusi et al. 2006) showed contrasting results, where bacterial vaginosis was, on the contrary, found in women who were on average, two years younger than those without the condition. Hence, in these Kenyan women, the odds of having BV decreased with increasing age. Subsequently, similar findings were obtained in an intervention study, where binary multiple logistic regression analysis revealed a significant correlation between bacterial vaginosis and pregnant women in the

younger age groups, i.e. under 26, (Odds ratio (OR) 0.97, 95% Confidence interval (CI): 0.96-0.99, $p < 0.001$). (Larsson et al. 2007)

2.5.1.2 Male partners

Bukusi et al.'s study (2006) had actually explored a different route and in effect, was the first comprehensive study which simultaneously looked at both females and their male partners to identify the risk factors for BV in Kenya. The results obtained were unexpected, showing that the general demographic characteristics of men such as age, marital status, and education were not associated with BV in their female partners. The combined analysis, however, showed that increased odds of bacterial vaginosis was significantly associated with HIV positivity in either partner, as well as certain female characteristics. The study concluded that characteristics of males and females were independently associated with BV.

2.5.2 Behavioral risk factors

Behavioral factors associated with bacterial vaginosis are diverse, the majority being related to sexual behavior, and others including female hygiene practices, contraceptive use, smoking, past pregnancies, and sexually transmitted infections.

2.5.2.1 Sexual practice risk factors

Recently, a systematic review and meta-analysis of the association between sexual risk factors and bacterial vaginosis was performed, this being the first review to summarize

available observational data for BV. (Fethers et al. 2008). In this review, Fethers et al. (2008) revealed that BV is significantly associated with sexual contact and with new and multiple male and female partners. For instance, a recent study by Trabert & Misra (2007) on pregnant African American women attending prenatal care clinics and a bacterial vaginosis prevalence estimation, also carried out in the USA (Allsworth et al. 2007), related BV acquisition to increased sexual activity. In addition, Cherpes et al. (2008) in a longitudinal (cohort) study (which produces better evidence), showed that BV acquisition was independently associated with, among other things, vaginal intercourse.

Cross-sectional studies carried out over the years, in Kenya (Bukusi et al. 2006), Australia (Bradshaw et al. 2005) and several in the United States of America (Georgijević et al. 2000, Yen et al. 2003, Koumans et al. 2007, Allsworth et al. 2007, Trabert & Misra 2007) provided evidence that bacterial vaginosis was associated with women having sexual intercourse with more than one male partner. Similar findings were obtained from women who have sex with women, (Smart et al. 2004, Fethers et al. 2008, Koumans et al. 2007, Marrazzo et al. 2002) in which bacterial vaginosis was found in those women who had sexual intercourse with one or more female partner(s). In fact, Marrazzo et al.'s study (2002) was specifically on women who have sex with women (WSW), and their findings supported the hypothesis that sexual exchange of vaginal secretions is a possible mechanism for acquisition of bacterial vaginosis, since BV was associated with, among other things, failure to always clean an insertive sexual toy before use. Furthermore, in this study, neither douching nor sexual practices with male partners were associated with bacterial vaginosis, this clearly suggesting that other risk factors of BV exist. This is more so that neither having a new female partner or new male partner had any impact on

the presence of BV. Unlike Bukusi et al.'s study (2006), where characteristics of females and their male partners were independently associated with BV, as already mentioned, Marrazzo et al.'s study (2002), in contrast, showed 55 (95%) of 58 monogamous couples having concordant vaginal smears from each partner.

Another sexual risk factor for bacterial vaginosis which was revealed by Marrazzo et al. (2002), is oral-anal sex with female partners. However, conflicting results have also been obtained. That is, two other studies (Bukusi et al. 2006, Cherpes et al. 2008) also showed rectal sex to be a risk factor for bacterial vaginosis, whereas, others showed no impact of oral sex on BV acquisition in pregnant women (Trabert & Misra 2007), and no statistically significant association was seen between bacterial vaginosis and receptive oral-anal sex (Bradshaw et al. 2005).

2.5.2.2 Evidence against sexual transmission

The literature reviewed clearly indicates that sexual activity is associated with bacterial vaginosis acquisition. However, Hillier and Holmes (1999) argue that there is conflicting evidence on the role of sexual transmission of bacterial vaginosis, and this is well supported by other authors. For example, bacterial vaginosis has been found in women reporting no history of sexual intercourse, although the rate was half (15% versus 30%) that found in women who reported a history of sexual intercourse. (Allsworth et al. 2007). This had also been found in a much earlier study (Bump & Buesching 1988), where no significant difference was seen in the prevalence of BV or in the isolation of *Gardnerella vaginalis* in virginal and sexually active groups of adolescent females.

Bump & Buesching (1988), hence, argue that bacterial vaginosis should not be considered an exclusively sexually transmitted disease. In addition, in a United Kingdom study (Morris et al. 2001) to determine whether bacterial vaginosis was sexually transmitted in women attending a genitourinary medicine clinic (GUM), despite finding BV to be associated with some factors related to the acquisition of gonorrhoea, *Chlamydia trachomatis*, and history of a bacterial sexually transmitted infection (STI), Morris et al. (2001) contended that other factors other than sexual activity may also be important in the acquisition of bacterial vaginosis, and hence concluded that it is hard to confirm that its development is entirely determined by sexual transmission. Two years later in 2003, Yen et al., in a cross-sectional study on sexually experienced and non-sexually experienced young women entering the military in the United States of America, concluded that BV is not a sexually transmitted infection, but their findings followed the pattern of infection for a number of sexually transmitted diseases (STDs). This study, having the largest sample of non-sexually experienced young women (n = 263) evaluated for bacterial vaginosis, showed a positive Nugent's test in 48/263 (18.3%) of women answering no to ever having vaginal sex, compared to 468/1675 (27.9%) of women answering yes to ever having vaginal sex. These results are in agreement with those of Allsworth et al. (2007), hence, indicating the presence of BV in both women who have and have not experienced vaginal intercourse, and they are also similar to Morris et al.'s (2001) findings in that BV prevalence varied according to other non-sexual factors such as race, or ethnicity.

Lately, Verstraelen (2008) in his letter to the editor, similarly summarized the outcome of different studies carried out by other authors, in attempt to determine whether BV is sexually transmitted or not. He concluded that, despite the close relationship with sexual behavior, BV differs from true STDs, in that there is lack of evidence in support of simple male-female transmission of a known infectious disease agent. Verstraelen also cites Hill's (1965) causality criterion of experiment and intervention, concluding that, according to this criterion, there is further lack of evidence in support of an STD causal pathway for BV.

Accordingly, while BV epidemiology has challenged the traditional STD transmissibility model in many ways, an alternative infectious disease model emerges, in which BV may be referred to as a *sexually enhanced* rather than a *sexually transmitted* disease. (Verstraelen 2008: 576) In this alternative model, Verstraelen postulates that sexual contact alters BV susceptibility, by allowing anaerobic overgrowth of bacteria already present in the vagina or rectum, which in turn, possibly relates to the temporarily antagonizing effect of sexual contact on the persistence of hydrogen peroxide-producing lactobacilli. In other words, the ultimate conclusion is that the observations on the antagonizing effect of sexual contact on the persistence of hydrogen peroxide-producing lactobacilli and the concomitant observations on the acquisition of BV in relation to vaginal lactobacilli seems to fit rather well the infectious disease model of BV as a sexually enhanced disease, rather than that of an STD. (Verstraelen 2008: 576)

2.5.2.3 Contraceptive use

Studies looking at contraception use and bacterial vaginosis have so far demonstrated that hormonal and barrier contraceptives such as condoms, decrease the risk of acquiring bacterial vaginosis (Roy 1991, Shoubnikova et al. 1997, Calzolari et al. 2000, Baeten et al. 2001, Riggs et al. 2007), while intrauterine contraceptive devices (IUDs), increase the risk. (Moi 1990, Roy 1991, Calzolari et al. 2000, Georgijević et al. 2000, Go et al. 2000).

Longitudinal / cohort studies showing the protective effect of hormonal contraceptives and condoms against bacterial vaginosis, have been carried out in different countries, being Rome (Calzolari et al. 2000), Kenya (Baeten et al. 2001), United States of America (Riggs et al.2007), and Sweden (Moi 1990, Shoubnikova et al.1997). These studies also used large sample sizes, ranging from 956 to 3077 female participants, hence they provide the best evidence. In some of the studies (Baeten et al. 2001, Brabin et al. 2005, Riggs et al. 2007) both oral and injectable hormonal contraceptives were protective against bacterial vaginosis. Riggs et al.'s (2007) study, which had the largest sample size (3,077 American women of reproductive age), showed a decreased risk of overall bacterial vaginosis prevalence among oral contraceptive users (odds ratio (OR) 0.76; confidence interval (CI) 0.63-0.90) and among users of hormonal injection/implant (OR 0.64; CI 0.53-0.76). Furthermore, Roy (1991) found the same number and types of vaginal organisms in patients having a contraceptive vaginal ring and those using oral contraceptives. However, similar results showing the protective effects of oral contraceptives against bacterial vaginosis, have also been obtained using cross-sectional

study designs in the United Kingdom (Brabin et al. 2005), the United States of America (Yen et al. 2003), and Australia (Bradshaw et al. 2005).

According to Roy (1991), the impact of contraceptive steroids on cellular and humoral immunologic factors may explain these observations. Later in 1997, Shoubnikova et al. attributed these findings to speculations that oral contraceptive use increases the glycogen content of vaginal epithelial cells, in turn, inhibiting the in vitro growth of certain bacteria, presumably, including anaerobes found in BV.

As would be expected, a study by Moi (1990) showed higher prevalences of bacterial vaginosis in women who were not using contraceptives, and in another study (Smart et al. 2004), women with bacterial vaginosis were significantly less likely to have used hormonal contraception.

On the other hand, Go et al. (2000) reported higher prevalences of bacterial vaginosis in rural Vietnamese women (n = 1,163) using IUDs for birth control, (OR = 1.64, CI 0.84-3.22) and in those who reported vaginal itching or discharge (OR = 1.33, CI 0.66-2.68), although differences were not statistically significant. In Calzolari et al.'s (2000) study results on Roman women (n = 1,314), there was a significant increase of bacterial vaginosis among IUD users, both before and after adjustments, and this lead to a suggestion for systemic microscopic evaluations to be carried out in order to identify bacterial vaginosis for IUD users. A more recent prospective longitudinal study (Riggs et al. 2007) on American women (n = 3,077) also observed an increased risk for BV

incidence (OR 1.43; CI 1.02-2.07) among those subjects who had tubal ligation. However, greater remission of BV was found among women using hormonal injection or implant, (OR 1.67; CI 1.23-2.27) than among those subjects who had tubal ligation (OR 0.56; CI 0.39-0.80).

Despite the fact that many authors in the past years have investigated the relationship between contraceptives and bacterial vaginosis, Calzolari et al. (2000) argue that it is difficult to compare users with non-users of a specific contraceptive method because the risk factors may be very different.

2.5.2.4 Feminine hygiene practices

Several cross-sectional studies on American women found an association of BV with the feminine hygiene practice of vaginal douching. These included earlier studies by Go et al. (2000), who found vaginal cleansing agents to be associated with current BV in women (n=1,163) aged 18 – 49 and Ness et al. (2002) who identified a higher risk for BV in women (n=1200) who had douched within 7 days compared to those who had douched at least once a month. Interestingly, in another earlier study (Holzman et al. 2001), significant increases in BV prevalence was noted in women who reported using vaginal douches at least once a month in the past year, before the age of twenty years, as well as within the past two months (OR=2.9). Other studies found frequent use of scented soap (Georgijević et al. 2000), and bathing as opposed to showering (Holzman et al. 2001) to be also associated with BV. In addition, more recent studies found significant associations of BV with douching three or more times before or during pregnancy

(Trabert & Misra 2007), a higher BV prevalence of 45% compared to 24% (P<0.001) in women who reported douching in the past six months (Allsworth et al. 2007), and furthermore, a longitudinal study by Brotman et al. (2008), also on non-pregnant women, identified a higher relative risk for BV with regular douching compared with no douching practice.

On the other hand, however, some authors (Marrazzo et al. 2002, Demba et al. 2005, Bradshaw et al. 2005, Bukusi et al. 2006) found no association of douching with acquisition of BV.

2.5.2.5 Smoking

Several authors have demonstrated smoking as a risk factor for BV among Australian (Smart et al. 2004, Bradshaw et al. 2005), Swedish (Shoubnikova et al. 1997, Larsson et al. 2007), Yugoslavian (Georgijević et al. 2000), and American women (Koumans et al. 2007, Cherpes et al. 2008), using various study designs. A randomized controlled trial (RCT) on 9025 pregnant Swedish women was the first to indicate that BV was more common among former smokers than in women who had never smoked (Larsson et al. 2007). In this study, BV was found to be more than twice as common among smokers (OR=2.53, 95%CI=2.11-3.04), although a higher prevalence of BV in the younger age group was also revealed, this creating doubt on whether these two markers could be tools for screening. Similarly, a recent National Survey (Koumans et al. 2007) on 3739 American women, aged 14-49 years revealed a weighted BV prevalence of 35% in

current smokers, compared to 29% in former smokers and 25% in those who had never smoked.

However, a smaller cohort study on 438 American women found no association between smoking and BV.

2.5.3 Sexually transmitted infections (STIs)

Some studies (Morris et al. 2001, Bradshaw et al. 2005) but not others (Holzman et al. 2001, Bukusi et al. 2006), reported bacterial vaginosis more common in women with a history of STI(s). Some authors (Moi 1990, Morris et al. 2001, Myer et al. 2005) showed that bacterial vaginosis was positively associated with gonorrhoea and chlamydia infections, and others showed an association with prior (Georgijević et al. 2000, Myer et al. 2005, Bukusi et al. 2006) or concurrent (Madhivanan et al. 2008) trichomonas *vaginali*s infections. Very recently, Madhivanan et al. (2008) as well as Cherpes et al. (2008) also showed that bacterial vaginosis was related to concurrent infections with herpes simplex virus (HSV)-2 seropositivity. Cherpes et al. (2008) revealed through longitudinal analyses, that HSV-2 serum antibodies were independently associated with loss of H_2O_2-producing lactobacilli, and hence, the development of bacterial vaginosis. In addition, in a study on Kenyan women and their male partners, Bukusi et al. (2006) found that the odds of having bacterial vaginosis was 5.7 times higher if either partner was HIV seropositive. However, an earlier study by Demba et al. (2005) on West African women had found no association of bacterial vaginosis with HIV. On the other

hand, very recently an independent association of bacterial vaginosis with sex with an uncircumcised male partner was discovered. (Cherpes et al. 2008)

2.5.4 Other risk factors

Other risk factors for BV which have been identified through different studies include, having a high body mass index (BMI) (Koumans et al. 2007), and induced abortion (Calzolariet et al. 2000, Shoubnikova et al. 1997).

2.6 Problem statement

The body of evidence clearly points to multiple and diverse risk factors for bacterial vaginosis. Some of these risk factors are also discordant between studies, making it difficult to clearly define the etiology of bacterial vaginosis, and this in-turn, making it intricate to implement appropriate prevention mechanisms for bacterial vaginosis. It would therefore be most appropriate, to first determine possible risk factors for bacterial vaginosis in a population, before developing preventive interventions.

2.7 Study rationale

The literature reviewed has also made it evident that a lot of the studies on bacterial vaginosis were carried out in the United States of America, and fewer in less developed countries, especially African countries. It is therefore, most fitting to carry out this study on the risk factors for bacterial vaginosis, in a South African population in Cape Town,

more especially that black ethnicity has been found in many studies, to be one of the major risk factors for bacterial vaginosis. In addition, the need for more research on the inverse relationship between bacterial vaginosis and hormonal contraceptive use, among other things, has been identified. (Yen 2003). Hillier & Holmes (1999) also suggested further studies on the IUD, to, among other things, confirm or refute the association with bacterial vaginosis.

This study will therefore, be the first study aimed at assessing the risk factors associated with bacterial vaginosis in a peri-urban community in South Africa. Findings in the study will assist in developing mechanisms of preventing bacterial vaginosis, by addressing modifiable risk factors, in Cape Town communities residing in peri-urban areas. This in turn, may lead to the reduction of adverse health outcomes related to women's reproductive health, and may reduce susceptibility to human immunodeficiency virus (HIV) and sexually transmitted infections, which have been associated with bacterial vaginosis. This is more especially that bacterial vaginosis is more common than other STIs associated with enhancing HIV transmission, increasing the population attributable risk. (Morris et al. 2001). Furthermore, Hay (1998) contends that by understanding the etiology and epidemiology of bacterial vaginosis, treatment can be improved.

CHAPTER THREE

3.0 METHODS

3.1 Study Design

This study utilized a cross-sectional study design to analyze data collected at baseline (enrollment), from a prospective observational feasibility study, which was carried out over eighteen months, from June 2006 to December 2007, to determine the HIV incidence in preparation for a future preventive HIV vaccine efficacy trial.

3.2 Study Population

The study population comprised of healthy, sexually active, HIV negative women between the ages fifteen (15) and forty-one (41) years, who were enrolled in the Feasibility and HIV Incidence Study, in the Western Cape.

3.3 Sampling and Sample Size

Consecutive participants sampling was therefore carried out in this sub-study, using women who were enrolled in the Feasibility and HIV Incidence study. Recruitment had been from the fourteen existing Voluntary Counseling and Testing (VCT) sites in the Nyanga area near to the study site, with extension to the twenty-one VCT sites in the whole area, including Mitchell's Plain, in Cape Town, South Africa.

The sample size, therefore, comprised of 309 HIV negative women enrolled in the Feasibility and HIV Incidence Study.

3.4 Measurement and Data Collection

This study used baseline data from two sources, being questionnaires collecting demographic and risk factor information, and BV diagnostic results from gram-stained cervical smear slides. The data was collected at the screening and enrollment visits.

The demographic and risk factor information had been collected at baseline, by means of standardized interviewer-administered questionnaires. Past and current risk factor information was collected for selected HIV risk factors, which included, among other things, medical history, sexual behavior, drug use, history of smoking, and alcohol consumption. Demographic information collected, on the other hand, included age, marital status, ethnic group, education level, and indicators of socio-economic status.

Data on BV diagnoses was collected by means of microscopic examination of Gram-stained vaginal swab slides. Air-dried smear preparations on glass slides had been prepared from self-collected vaginal swabs. The vaginal smears were then heat-fixed and stained manually, using Gram's staining method (Appendix 1), at the University of Cape Town's (UCT's) Empilisweni Research Laboratory. The gram-stained slides were then examined microscopically by a qualified laboratory person, (myself) for BV diagnosis, using the Nugent's criteria scoring system (Appendix 2). The laboratory person was unaware of clinical findings on the participants, nor any other details, other than their study numbers, date of births, and specimen collection dates.

In the Nugent's method used, Gram-stained smears were evaluated under oil immersion (X1000 magnification; High-power field (hpf)), for three morphotypes: Lactobacilli (medium to large, gram positive rods), *Bacteroides* spp./*Gardnerella vaginalis* (small gram-variable rods, coccobacilli), and *Mobiluncus* spp. (thin curved, gram-variable rods). The numbers of morphotypes per hpf were first quantitated from 1 to 4+, by assigning 0 for no morphotypes, 1+ for less than 1 morphotype, 2+ for 1 to 4 morphotypes, 3+ for 5 to 30 morphotypes, and 4+ for 30 or more morphotypes. Re-screening of 19 of the 314 gram slides (i.e., 6%) was performed as an internal quality control method (to measure reliability).

The weighted quantitations (0, 1 to 4+) of the morphotypes were then summed up using Stata 10 statistical software, to yield a score on a 0- to 10-point scale, hence, providing BV diagnosis for each participant. A score of 0 to 3 was considered as normal vaginal flora (reported as negative for BV), 4 to 6, as intermediate (also reported as negative for BV), and 7 to 10 as positive for BV. The positive scores for BV were further divided to grade the severity of BV, by assigning Mild BV to a score of 7 to 8, and Severe BV to a score of 9 to10.

3.5 Data Management

Socio-demographic details and risk information on the participants were exported from the study database, into the statistical software, Stata 10, for the analysis.

The other data, were the participants' study numbers, date of births, specimen collection dates, and the weighted quantitations (0, 1 to 4+) of the morphotypes, obtained from the microscopic examination of the gram slides, which were first entered into Microsoft Excel. The specimen registration forms were then used to confirm the participants' study numbers and date of births which had not been clearly legible on the slides. The data was then exported from Microsoft Excel, into the statistical software, Stata 10, and Point Scores were computed from the weighted quantitations, to determine the BV diagnoses.

Finally, the two Stata files containing the BV diagnoses and the socio-demographic and risk information were then merged into one file, according to the study numbers and participants' date of births. The data was then edited to standardize the format, and all categorical data was coded into numerical values. This file then formed the source of data used for the analysis.

3.6 Statistical Analysis

The statistical software used for data analysis was Stata Special edition (SE) Version 10. Further data cleaning was performed to group observations which had few numbers, for each variable. That is, for the variable 'Birth control methods', observations for IUD and BTL were combined, as well as male and female condom; for 'Marital status', married and widowed were combined; for 'Education level', no education and primary were combined, as well as secondary and post-secondary; for 'past pregnancy(s)', 1 to 5 pregnancies were combined; for 'washing inside the vagina in the last seven days with water only', 1 to 7 times were combined and 'with antiseptic', 1 to 14 times were

combined; for 'washing inside the vagina with soap & water in the last seven days', the variable 'soap_more_daily' was generated, which was binary, grouping observations into vaginal washing at least once a day in the last seven days, or not; 'Frequency of sexual intercourse (i.e., sex with regular partner) in the last seven days' was grouped into no sex, once, and more than once; for 'Condom use in the last seven days', two variables were generated: 'Sex without a condom', with observations 0 to 7 times, and 'Sex always with condom', with binary observations no and yes.

Data exploration was performed on all variables at the initial stage, to produce simple descriptive statistics. Summary measures for continuous variables included means, medians, minimum and maximum values, inter-quartile ranges, standard deviations, and skewness. However, only the median, minimum/maximum values, and inter-quartile ranges were taken into consideration, since the variables did not follow normal distribution. For categorical variables, one-way frequency distribution tables were computed, producing percentages. This formed the basis of calculating the prevalence of BV at baseline.

The Chi-square test was used to test for crude associations, between BV and various independent variables, and then collinearity among the independent variables. This was followed by bivariate logistic regression analysis to test for independent associations between BV and each independent variable.

Finally, multivariate analysis was performed, using logistic regression to first assess for confounding relationships within the socio-demographic and behavioral risk factor variables (independent variables), and then subsequently use different combinations of these variables in a forward stepwise process, to build a model using maximum likelihood.

Selection of variables for entry into the logistic regression model for multivariate analysis was on the basis of a) P–values ≤ 0.1 in unadjusted bivariate analyses of associations with BV and b) factors found to be statistically non-significant in crude analyses but had been hypothesized to be associated with the development of BV in previous studies. In addition, only the variables with a comparable number of observations between the intra-variable categories (i.e. non-discriminatory observations), were selected.

Initially selected variables included: vaginal washing with soap and water, which was selected for its statistical significance (P=0.05), and unskilled manual labour employment, having a vaginal discharge, tobacco use, birth control methods, and having a past pregnancy, which were selected for their borderline statistical significance (P>0.05 & ≤0.1), and lastly, age, marital status, education level, unemployment, having a cell phone, having a history of sexually transmitted infection (STI), the number of regular sexual partners, frequency of sexual intercourse, sex with and without condom use, sex during menses, and sex under the influence of alcohol, who's associations with BV had been demonstrated in previous studies.

However, because the age variable showed collinearity with most of the other variables, it was excluded from the first model building exercise, along with other variables which

also showed collinearity, particularly with the variables of borderline statistical significance. This resulted in the following variables remaining for inclusion in model building: marital status, education level, unskilled manual labour employment, and having a cell phone, as the socio-demographic factors; tobacco use, birth control methods, and washing inside the vagina with soap and water, as the behavioral factors, and with condom use with regular partner, frequency of sex with regular partner, and number of sexual partners as the sexual behavior factors. Although the variable vaginal discharge was of borderline significance, it was not included in the model building exercises, because of having been cited as a possible symptom of BV infection.

Washing inside the vagina with soap and water was selected for the starting model, being the only variable showing statistical significance in its independent association with BV. However, because a possible confounding relationship was identified between this variable and always using a condom during sex, these two were included together as starting variables in the model. The other variables were then added one by one to this model, except for the socio-demographic factors, unskilled manual labour employment and education level, which were initially added together because they demonstrated a possible confounding relationship. In addition, the variables past pregnancy(s) and birth control methods were not added together to the same model as there was indication of a collinear relationship between the two.

Another model building exercise was also computed to include the age variable in multivariate analysis, since this factor had been shown to have an association with BV in

previous studies. Therefore, other variables modeled with age included tobacco use, history of STI, frequency of sex, and sex during menses, all of which had not shown collinearity with it, nor with each other.

3.7 Ethical Considerations

The main study, which was titled: A Prospective, Observational Feasibility Study to Determine HIV Incidence in Preparation for a Future Preventative HIV Vaccine Efficacy Trial, had obtained ethics approval from the University of Cape Town's Health Sciences Faculty, Research Ethics Committee, on the 09 May 2006, REC REF: 097 / 2006. This granted ethics approval was in compliance with the ICH Harmonized Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56, and 312.

In addition, however, the protocol for this sub-study was submitted to the University of Cape Town's Ethics Committee, together with a copy of the ethics approval letter for the main study. Furthermore, another copy of the protocol had also been submitted to, and approved by University of Cape Town's Public Health Department Research Committee and the Head of the Public Health Department.

Ethical considerations had been prioritized throughout the main study to ensure full protection of the participants. Anonymity continued to be maintained at all times in the sub-study, by use of study numbers as means of identity for the participants. The participants' BV results and other details were also treated confidentially throughout the study.

CHAPTER FOUR

4.0 RESULTS

4.1 Descriptive Analysis

4.1.1 Sample size

The number of women enrolled in the study and the exclusion and inclusion criteria used for gram stain analysis of the vaginal smear samples are presented in Figure 1.

A total of 309 HIV negative women enrolled in the Feasibility and HIV Incidence Study, each had a self-collected vaginal specimen analyzed for BV diagnosis at baseline. Of these specimens, 3 were unreadable, as there was no material on the slides, and they were therefore omitted, leaving 306 women for inclusion in the analysis. Eight of the slides were difficult to read as they were obscured by soot from the heat-fixing procedure for the Gram Stain method. Nonetheless, BV diagnosis was able to be carried out on these 8 slides. Furthermore, baseline questionnaire data was missing for 11 of the 306 women with BV results, and this further reduced the sample size for analysis to 295 women.

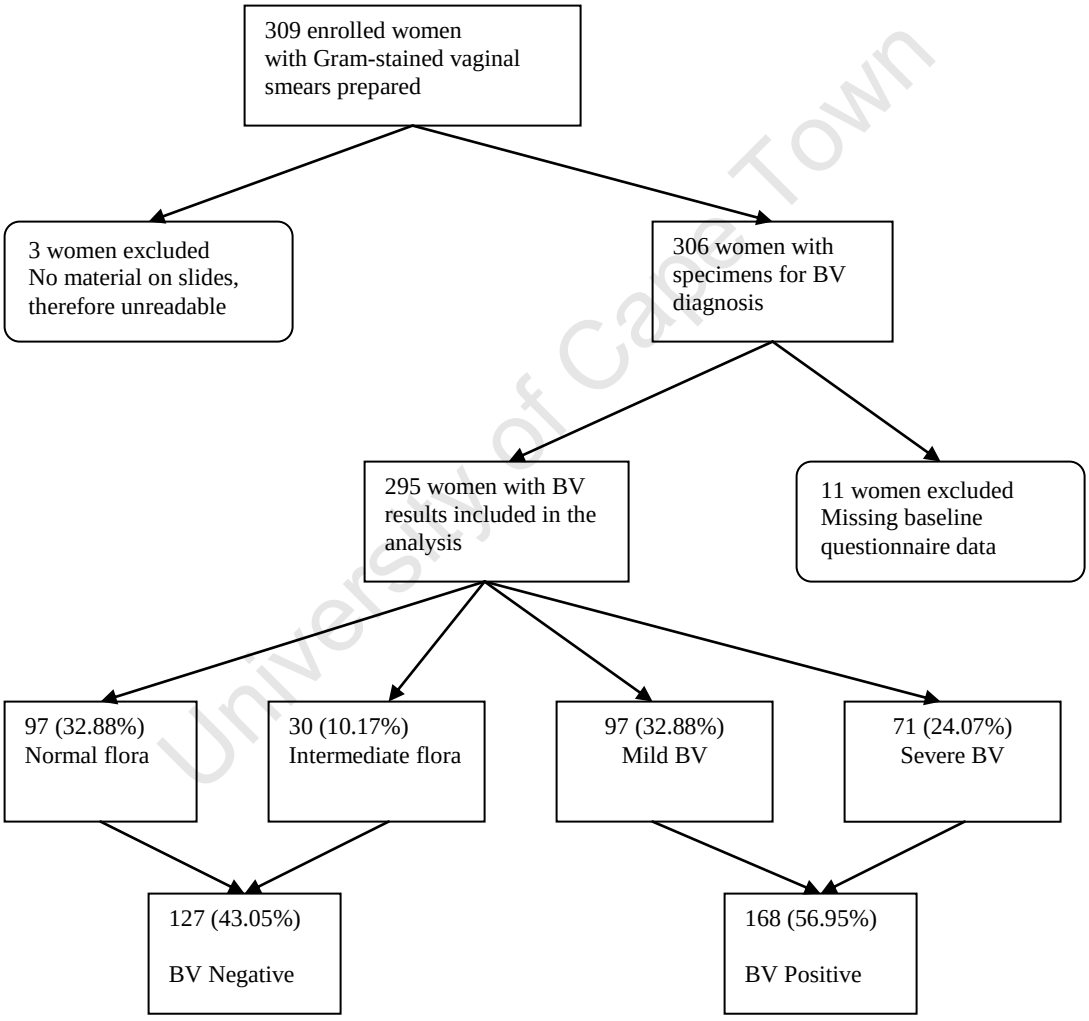
4.1.2 Socio-demographic characteristics

The socio-demographic characteristics of the study sample, stratified by the absence and/or presence of BV, are presented in Table 1.

The median age of the participants was 22 years (range 16 to 40 years). Figure 2 demonstrates the age distribution of women in the study. Thirty-three percent were ages 16 to 19, 27% ages 20 to 24, 13% ages 25 to 29, 10% ages 30 to 34, and 17%, ages 35 to

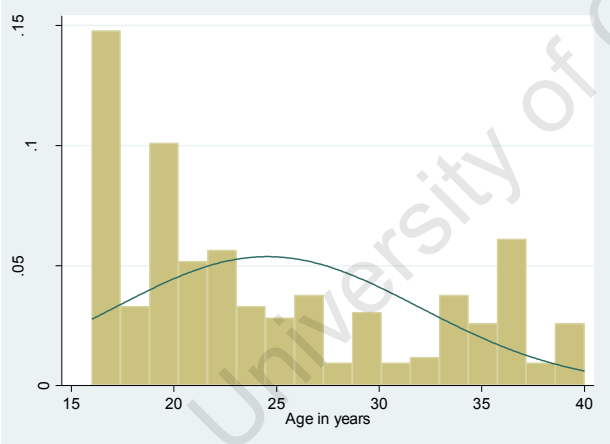
40 years. All women were of South African origin, mostly (291= 99%) being of black race, with the other few (1%) being of mixed race, also referred to as 'coloured'. The distribution of tribal groups was as follows: Xhosa (n=285), Sotho (n=5), Zulu (n=1), and Mixed (n=4).

Figure 1: Inclusion and exclusion criteria and Gram Stain results at enrollment (baseline) for women in the study.



Most women (95%) were single / never married, whereas, the other 5% were either widowed or in a monogamous marriage. A small proportion (4%) had either no formal education or up to primary school level, and 96%, secondary school level or up to tertiary education. Of 116 (39%) women who reported having a primary source of income in the past 12 months, 31 were formally employed, 83 scholars and 2 were students. However, more of the women (61%) were unemployed. The study population had been residing in the Nyanga area for an average of 18 years. Over 85% women had their own water, toilet, electricity, radio, and television, with a little less (74%) owning a mobile phone, and only 1% having none of the amenities.

Figure 2: Age distribution of study population



4.1.3 Behavioral characteristics

Behavioral characteristics and medical history of the women in the study, including sexual practices, feminine hygiene practices, and birth control methods are also described

in Table 1. The proportion of women who reported using tobacco in the past month was 21%. Reports for using marijuana were from 2% of women (data not shown), and almost half (49%) for alcohol use. One-fifth reported history of a sexually transmitted infection (STI), with less than 7% reporting having an open sore on the genital area, an excessive or foul smelling discharge, and/or dysuria in the last 3 months. Under half (45%) of the women reported not ever being pregnant, whereas, 55% reported having 1 to 5 past pregnancies. Thirty-three percent of the women reported washing inside the vagina with soap and water at least once a day in the past week, whereas, 23% reported washing with an antiseptic (e.g. Dettol), and 3% using water only.

With regards to sexual practices, 5 women (2%) reported having 2 regular sexual partners (which included their spouse), in the last 7 days. Sexual frequency reported was 38% having sexual intercourse once in the last seven days, and 48% more than once. Of the 254 women who reported having sexual intercourse in the last 7 days, 163 (64%) reported having used a condom all the time, whereas, 91 (36%) reported not using a condom always. On the other hand, three women reported having sexual intercourse seven times in the past seven days and never using a condom at any of those times. There were no reports of having sex with a new partner in the last 7 days.

The various birth control methods the participants reported using included the oral contraceptive pill (OCP) (3%), injectable hormones: Depo Provera, Petogen, and Nuresterate (13%), the intrauterine device (IUD) or had bilateral tubal ligation (BTL) (2%), and the male or female condoms (60%).

4.1.4 BV Prevalence

The prevalence of BV in the study population at enrollment was high at 57%, with 33% having mild BV and 24%, severe BV, whereas, 10% had intermediate flora (Figure 1).

4.2 Associations of BV with socio-demographic and behavioral factors

4.2.1 Crude analysis

Table 1 also presents the statistical significance of associations of BV with socio-demographic and behavioral factors. In *crude analyses*, P-values generated using Chi square statistics (X^2) showed a statistically significant association of BV with the variable washing inside the vagina with soap and water in the past week (P = 0.05). However, borderline statistical significance was found with the four variables: smoking tobacco in the last month (P = 0.07), having a past pregnancy(s) (P = 0.06), unskilled manual labour employment (P = 0.09), and having a vaginal discharge in the last three months (P = 0.06). Other variables (socio-demographic, medical, and behavior factors) were of statistical insignificance (P > 0.1) and these included history of sexually transmitted infections, open sore on genitals, dysuria, washing inside the vagina with water only, washing inside the vagina with antiseptic, level of education, marital status, unemployment, having certain amenities or not, age, and various sexual practices like having sex under the influence of alcohol, sex during menses, condom use, number of sexual partners, and frequency of sexual intercourse.

Table 1. Descriptive Characteristics of Study Population and Prevalence of BV

	Enrolled women N = 295 n (%)	BV Positive n = 168 n (%)	BV Negative n = 127 n (%)	P - Value[†]
SOCIO-DEMOGRAPHIC FACTORS				
Age group (years)				0.68
16-19	98 (33)	57 (34)	41(32)	
20-24	79 (27)	44 (26)	35 (28)	
25-29	37 (13)	23 (14)	14 (11)	
30-34	30 (10)	19 (11)	11 (9)	
35-40	51(17)	25 (15)	26 (20)	
Marital status				0.77
Married (monogamous) or Widowed	15 (5)	8 (5)	7 (6)	
Single (never married)	280 (95)	160 (95)	120 (94)	
Race / Tribal group				0.55
Black	291 (99)	166 (99)	125 (98)	
Xhosa	285 (96.6)	162 (96)	123 (97)	
Sotho	5 (1.7)	3 (2)	2 (2)	
Zulu	1 (0.34)	1 (1)	0 (0)	
Mixed / Coloured	4 (1.36)	2 (1)	2 (2)	
Education level				0.92
Up to Primary	12 (4)	7 (4)	5 (4)	
More than Primary	283 (96)	161 (96)	122 (96)	
Employment				
Employed (includes students & scholars)[#]	116 (39)	68 (40)	48 (38)	
Domestic worker	6 (2.03)	2 (1)	4 (3)	0.24
Professional	1 (0.34)	1 (1)	0 (0)	0.38
Security	1 (0.34)	1 (1)	0 (0)	0.25
Manual labour	21 (7)	14 (8)	7 (6)	
Skilled	7 (2)	3 (2)	5 (4)	0.45
Unskilled	14 (5)	11 (7)	9 (7)	0.09
Waitress	1 (1.15)	1 (1)	0 (0)	
Foster home	1 (1.15)	1 (1)	0 (0)	
Scholar	83 (95.4)	49 (29)	34 (27)	
Student	2 (2.3)	0 (0)	2 (2)	
Unemployed	179 (61)	100 (60)	79 (62)	0.64
Amenities				
Has own water	255 (86)	141 (84)	114 (90)	0.15
Has own toilet	253 (86)	141 (84)	112 (88)	0.30
Has electricity	285 (97)	162 (96)	123 (97)	0.84
Has radio	284 (96)	160 (95)	124 (98)	0.28
Has television	271 (92)	154 (92)	117 (92)	0.89
Has cell phone	220 (74)	127 (76)	93 (73)	0.64
Has none of amenities	3 (1)	1 (1)	2 (2)	0.41
MEDICAL HISTORY				
Has past pregnancy(s)				0.06
None	132 (45)	83 (49)	49 (39)	
One or more	163 (55)	85 (51)	78 (61)	
History of STI (n=264)				0.34
No	212 (80)	119 (78)	93 (83)	
Yes	52 (20)	33 (22)	19 (17)	
Discharge in last 3 months				0.06
No	278 (94)	162 (96)	116 (91)	
Yes	17 (6)	6 (4)	11 (9)	
Open sore on genitals last 3 months				0.46
No	291 (99)	165 (98)	126 (99)	
Yes	4 (1)	3 (2)	1 (1)	
Dysuria in last 3 months				0.44
No	286 (97)	164 (98)	122 (96)	
Yes	9 (3)	4 (2)	5 (4)	
BEHAVIOURAL FACTORS				
Alcohol in last month				0.30
No	150 (51)	81 (48)	69 (54)	
Yes	145 (49)	87 (52)	58 (46)	
Tobacco in last month				0.07
No	234 (79)	127 (76)	107 (84)	
Yes	61 (21)	41 (24)	20 (16)	

Table 1. Descriptive Characteristics of Study Population and Prevalence of BV (Continued…)

	Enrolled women N = 295 n (%)	BV Positive n = 168 n (%)	BV Negative n = 127 n (%)	P - Value[†]
Feminine hygiene practices in past week*				
Water only				0.26
0	286 (97)	161 (96)	125 (98)	
1 to 7 times	9 (3)	7 (4)	2(2)	
Antiseptic (e.g. dettol)				0.78
0	228 (77)	133 (79)	95 (75)	
1 to 14 times	67 (23)	35 (21)	32 (25)	
Soap & water[#]				0.05
No	198 (67)	105 (62)	93 (73)	
Yes	97 (33)	63 (38)	34 (27)	
Birth control methods				0.18
None	28 (9)	19 (11)	9 (7)	
OCP	10 (3)	6 (4)	4 (3)	
Injectable hormones[§]	37 (13)	17 (10)	20 (16)	
IUD or BTL	5 (2)	4 (2)	1 (1)	
Male or female condom	178 (60)	106 (63)	72 (57)	
Missing data	37 (12)	16 (9)	21 (16)	
SEXUAL PRACTICES				
Sex with alcohol in last month				0.75
No	67 (23)	37 (22)	30 (24)	
Yes	228 (77)	131 (78)	97 (76)	
Sometimes	78 (26)	50 (30)	28 (22)	0.14
Frequently	150 (51)	81 (48)	69 (54)	0.30
No. of regular sexual partners last 7 days				0.90
0	40 (13)	24 (14)	16 (13)	
1	250 (85)	141 (84)	109 (86)	
>1	5 (2)	3 (2)	2 (1)	
Frequency of sexual intercourse in last 7 days				0.68
0	41 (14)	25 (15)	16 (13)	
1	113 (38)	61 (36)	52 (41)	
>1	141 (48)	82 (49)	59 (46)	
Condom use in last 7 days				
Sex without condom				0.45
0	204 (69)	117 (70)	87 (68)	
1	39 (13)	19 (11)	20 (16)	
2	34 (11)	18 (11)	16 (13)	
3	11 (4)	9 (5)	2 (1)	
4	4 (1)	3 (2)	1 (1)	
7	3 (1)	2 (1)	1 (1)	
Always used condom (n=254)		(n=143)	(n=111)	0.95
No	91 (36)	51 (36)	40 (36)	
Yes	163 (64)	92 (64)	71 (64)	
Sex during menses in past 3 months				0.46
No	270 (92)	152 (90)	118 (93)	
Yes	25 (8)	16 (10)	9 (7)	

[§]Depo provera, Petogen, Nuresterate
*Number of times washing inside the vagina with product
[†]Computed using Chi square (X^2) statistics
[#] Washing at least once a day

4.2.2 Unadjusted Logistic Regression

Table 2 demonstrates results of unadjusted logistic regression (bivariate) analyses, which revealed the potential independent risk factor for BV to be washing inside the vagina with

soap and water at least once a day for the past seven days (P=0.05, OR=1.64, 95%CI=0.99-2.71). Tobacco use (P=0.07, OR=1.73, 95%CI=0.95-3.12) and unskilled manual labour employment (P=0.08, OR=2.89, 95%CI=0.79-10.61) also showed as possible risk factors, but their P values indicated borderline statistical significance, and moreover, the confidence interval around the odds ratio for unskilled manual labour employment was wide.

In addition, other potential independent risk factors identified, but which were statistically non-significant (P>0.1), included being single (OR = 1.17), having a cell phone (OR = 1.13), alcohol use (OR = 1.28), sometimes having sex under the influence of alcohol in the last month (OR = 1.50), having an open genital sore in the last three months (OR = 2.29), having a history of sexually transmitted infection(s) (STIs) (OR = 1.36), having unprotected sex more than twice in the last seven days (OR = 3.34), having sex during menses in the past three months (OR = 1.38), having had a BTL or using an IUD (OR = 1.89), washing inside the vagina with water up to seven times in the past week (OR = 2.72), and belonging to age groups 25-29 (OR = 1.18) and 30-34 (OR = 1.24).

On the other hand, bivariate analysis showed negative associations with BV for having had a past pregnancy(s) (OR=0.64, 95%CI=0.40-1.03) having a discharge in the last three months (OR=0.39, 95%CI=0.14-1.09), and using injectable hormones for birth control (OR = 0.40, 95%CI = 0.14-1.12), but these were of borderline statistical significance (P = 0.06, P = 0.07, and P = 0.08 respectively).

Table 2: Unadjusted associations of socio-demographic, medical and behavioral factors with BV

	Unadjusted Logistic regression Odds Ratio (95% Confidence Interval)	P-value
SOCIO-DEMOGRAPHIC FACTORS		
Age group (years)		
16-19	1.0	Reference
20-24	0.90 (0.50 - 1.64)	0.74
25-29	1.18 (0.54 - 2.57)	0.67
30-34	1.24 (0.53 - 2.89)	0.61
35-40	0.69 (0.35 - 1.36)	0.28
Marital status		
Married (monogamous) or Widowed	1.0	Reference
Single (never married)	1.17 (0.41-3.31)	0.77
Education level		
Up to Primary	1.0	Reference
More than Primary	0.94 (0.29-3.04)	0.92
Employment		
Manual labour		
Skilled	0.56 (0.12-2.54)	0.45
Unskilled	2.89 (0.79-10.61)	0.11
Unemployed	0.89 (0.56-1.43)	0.64
Amenities		
Has own water	0.59 (0.29-1.21)	0.15
Has own toilet	0.70 (0.35-1.38)	0.30
Has electricity	0.88 (0.24-3.18)	0.84
Has radio	0.48 (0.12-1.86)	0.29
Has television	0.94 (0.40-2.19)	0.89
Has cell phone	1.13 (0.67-1.92)	0.64
Has none of amenities	0.37 (0.03-4.17)	0.42
MEDICAL HISTORY		
Has past pregnancy(s)	0.64 (0.40-1.03)	0.06
History of STI (n=264)	1.36 (0.72-2.54)	0.34
Discharge in last 3 months	0.39 (0.14-1.09)	0.07
Open sore on genitals last 3 months	2.29 (0.23-22.29)	0.47
Dysuria in last 3 months	0.59 (0.16-2.26)	0.45
BEHAVIOURAL FACTORS		
Alcohol in last month	1.28 (0.80-2.03)	0.30
Tobacco in last month	1.73 (0.95-3.12)	0.07
Feminine hygiene practices in past week*		
Water only		
0	1.0	Reference
1 to 7 times	2.72 (0.55-13.31)	0.22
Antiseptic (e.g. dettol)		
0	1.0	Reference
1 to 14 times	0.78 (0.45-1.35)	0.38
Soap & water[†]		
No	1.0	Reference
Yes	1.64 (0.99-2.71)	0.05
Birth control methods		
None	1.0	Reference
OCP	0.71 (0.16-3.16)	0.65
Injectable hormones[§]	0.40 (0.14-1.12)	0.08
IUD or BTL	1.89 (0.18-19.48)	0.59
Male or female condom	0.70 (0.30-1.63)	0.40
Missing data	0.36 (0.13-1.01)	0.05
SEXUAL PRACTICES		
Sex with alcohol in last month		
No	0.91 (0.53-1.58)	0.75
Yes	1.09 (0.63-1.89)	0.75
Sometimes	1.50 (0.88-2.56)	0.14
Frequently	0.78 (0.49-1.24)	0.30
No. of regular sexual partners last 7 days		
0	1.0	Reference
1	0.86 (0.44-1.70)	0.67
>1	1.00 (0.15-6.67)	1.00
Frequency of sexual intercourse in last 7 days		
0	1.0	Reference
1	0.75 (0.36-1.55)	0.44
>1	0.89 (0.44-1.81)	0.75
Condom use in last 7 days		
Sex without condom		
0	1.0	Reference
1	0.71 (0.35-1.40)	0.32
2	0.84 (0.40-1.73)	0.63
3	3.34 (0.70-15.88)	0.13
4	2.23 (0.23-21.81)	0.49
7	1.49 (0.13-16.66)	0.75
Always used condom (n=254)		
No	1.0	Reference
Yes	1.02 (0.61-1.70)	0.95
Sex during menses in past 3 months	1.38 (0.59-3.23)	0.46

[§]Depo provera, Petogen, Nuresterate

*Number of times washing inside the vagina with product

[†]Washing inside the vagina at least daily

4.2.3 Adjusted Logistic Regression

Table 3 presents results of the adjusted logistic regression (multivariate) analysis.

Including the variables showing a possible confounding relationship, washing inside the vagina with soap and water and always using a condom during sexual intercourse, together in the initial model, resulted in an increase in the statistical significance of the positive association of BV with vaginal washing with soap and water (P=0.04, OR=1.81), but always using a condom during sex remained non-significant (P=0.67, OR=1.12), and moreover, the overall model was of borderline significance (P=0.11). Vaginal washing with soap and water was therefore used independently as the starting model.

Adding the variable tobacco use to the starting model resulted in no change in the significance of the positive associations of BV with both vaginal washing with soap and water and tobacco use, other than an increase in the level of significance of the overall model. With addition of the third variable, having a past pregnancy(s), however, the positive associations of BV with tobacco use and vaginal washing with soap and water increased (P=0.07, AOR=1.73, 95%CI=0.95-3.16 and P=0.03, AOR=1.79, 95%CI=1.07-2.99 respectively), although tobacco use remained borderline statistically significant, whereas, the protective effect of having a past pregnancy(s) was also increased, and moreover, it became statistically significance (P=0.03, AOR=0.59, 95%CI=0.36-0.96).

Alternatively, when birth control methods was added as the third variable instead of past pregnancy(s), it maintained its borderline statistical significance (P=0.09, AOR=0.41,

95%CI=0.14-1.16). The risk effects of tobacco use, vaginal washing with soap and water, and unskilled manual labour employment were lessened (AOR=1.74, 1.46, and 3.07 respectively), with unskilled manual labour employment and tobacco use also becoming borderline statistically significant, and vaginal washing with soap and water becoming statistically non-significant.

Another alternative, removing the variable vaginal washing with soap and water and adding unskilled manual labour employment to tobacco use and past pregnancy(s) increased the risk effect of unskilled manual labour employment (P=0.05, AOR=3.63, 95%CI=0.97-13.55), although the 95% confidence interval around the odds ratio was wide. In addition, tobacco use also gained statistical significance (P=0.05, AOR=1.82, 95%CI=1.0-3.32).

Other selected variables did not show any change in the level of significance of their association with BV when adjusted for possible confounding factors.

The model building exercise which included the age variable revealed no statistically significant associations between BV and any of the other variables, neither independently, nor adjusted for possible confounding factors.

Table 3: Adjusted associations of socio-demographic and behavioral factors with BV

	Unadjusted Logistic Regression (Bivariate Analysis)		Adjusted Logistic Regression (Multivariate Analysis)	
	OR (95% CI)	**P-value**	**AOR (95% CI)**	**P-value**
SOCIO-DEMOGRAPHIC FACTOR				
Unskilled manual labour employment	2.90 (0.79 - 10.61)	0.11	3.63 (0.97-13.55)	0.05
BEHAVIOURAL FACTORS				
Vaginal washing with soap and water in the last 7 days*	1.64 (0.99 – 2.71)	0.05	1.79 (1.07 – 2.99)	0.03
Tobacco use in the last month	1.73 (0.95 - 3.12)	0.07	1.81 (0.99-3.32)	0.05
Past pregnancy(s) (1 to 5)	0.64 (0.40 – 1.03)	0.06	0.59 (0.36 – 0.96)	0.03
Birth control Methods				
None	1.0	Reference	1.0	Reference
OCP	0.71 (0.16 – 3.16)	0.65	0.53 (0.11 – 2.59)	0.44
Injectable hormones§	0.40 (0.14 – 1.12)	0.08	0.41 (0.14 – 1.16)	0.09
IUD or BTL	1.89 (0.18 – 19.48)	0.59	2.08 (0.20 – 21.84)	0.54
Male or female condom	0.70 (0.30 – 1.63)	0.40	0.72 (0.30 – 1.72)	0.47
Missing data/unknown	0.36 (0.13 – 1.01)	0.05	0.41 (0.14 – 1.18)	0.10

*Washing inside the vagina with soap and water at least once a day

§Depo provera, Petogen, Nuresterate

4.3 Results Summary

The prevalence of BV among HIV negative women at baseline (enrollment) in this study was found to be high, at 56.9%.

The behavioral factor, washing inside the vagina with soap and water at least once a day for the past seven days was the only identified risk factor of statistical significance, found to be independently associated with women in the study being diagnosed with BV infection at baseline. The other behavioral risk factor, tobacco use (P=0.05, AOR=1.82, 95%CI=1.0-3.32), became statistically significant when adjusted for unskilled manual labour and past pregnancy(s). In addition, unskilled manual labour employment was the socio-demographic risk factor identified (P=0.05, AOR=3.63, 95%CI=0.97-13.55), when controlled for tobacco use and past pregnancy(s), but the confidence interval around its odds ratio was too wide, rather indicating a small sample size and a statistically non-significant association. No sexual behavior factor was found to be a statistically significant risk factor for diagnosing BV.

On the other hand, behavioral factors found to be independently protective of BV were having had a past pregnancy(s) and using the birth control method, injectable hormones, and the latter remained borderline statistically significant when adjusted for possible confounding factors (P=0.09, AOR=0.41, 95%CI=0.14-1.16), whereas, past pregnancy(s) became significant (P=0.02, AOR=0.56, 95%CI=0.35-0.92)). Neither socio-demographic factors nor sexual behavior factors were found to be protective of BV diagnosis.

CHAPTER FIVE

5.0 DISCUSSION

This study examined a cross-sectional overview of factors that determined the diagnosis of BV in HIV negative women living in a peri-urban area in Cape Town, South Africa.

5.1 Prevalence of BV

The prevalence of BV in the study population was 57%. This high prevalence is not surprising, as almost all women in the study belonged to the black ethnic group, and several studies (Jancin 2000, Holzman et al. 2001, Yen et al. 2003, Allsworth et al. 2007) have correlated the factor, black race, with a higher BV prevalence when compared to other racial groups, for example, white women and Hispanic women. Yen et al. (2003) postulated that this positive association of BV with black race could possibly be attributed to differences in douching practices or foreign body use affecting the ecosystem, or biological factors which vary by ethnicity. However, I support their argument that more detailed studies on behavioral and biological factors associated with BV among diverse racial or ethnic groups are warranted, in order to explain this association.

5.2 Positive associations with BV

The study results revealed two behavioral factors significantly associated with the risk of developing BV in the study population as being washing inside the vagina with soap and water at least once a day in the last seven days and using tobacco in the last four weeks,

whereas, the socio-demographic risk factor was unskilled manual labour employment. Vaginal washing with soap and water (that is, vaginal douching) had an independent association with BV, whereas, tobacco use and unskilled manual labour showed significance when controlled for possible confounding factors.

However, the 95% confidence interval for unskilled manual labour employment was wide around the odds ratio, thus, indicating a small sample size and imprecision. And true enough, only 5% of the study population was employed in unskilled manual labour. Despite this finding, statistically significant positive associations of BV with low socio-economic status have been previously demonstrated in other cross-sectional studies (Morris et al. 2001, Bukusi et al. 2006, Allsworth et al. 2007), and unskilled manual labour employment can be equated to this factor.

Similarly, the findings with regards to the two behavioral risk factors are also consistent with other cross-sectional studies (Koumans et al. 2007, Trabert & Misra 2007) which also examined the prevalence of and risk factors for developing BV, although Trabert & Misra's (2007) study was on pregnant women. However, there are studies which demonstrated the feminine hygiene practice of douching as a risk factor for BV in non-pregnant women. For example, Ness et al.'s study (2002) on women in the United States, found that douching at least once a month was independently associated with an increased frequency of BV (also diagnosed via gram staining of self-collected vaginal swabs), with those who had douched within seven days being at highest risk (OR=2.1, 95%CI=1.3-3.1). Similarly, the current study demonstrated that women who reported

washing inside the vagina with soap and water at least once a day in the last seven days had a higher prevalence of BV compare to those who had not washed daily. In addition, two other cross-sectional studies on non-pregnant American women (Holzman et al. 2001, Allsworth et al. 2007) also linked douching in the past two to six months to an increase in prevalence of BV (OR=2.9, 95%CI=1.5-5.6 and OR=1.93, 95%CI=1.54-2.40 respectively).

To explain these findings, Ness et al. (2002) related this association to the impact douching has on the vaginal microecology, where repeated douching may prevent the vaginal microbiology from rebalancing over time, and hence, disrupting the predominance of hydrogen peroxide producing lactobacilli, and in turn, this shift in vaginal flora, resulting in overgrowth of BV-related microflora. Similarly, findings from a large (n=3620)12 month longitudinal study (Brotman et al. 2008) indicated that douching confers an increased risk for disruption of vaginal flora, and these findings were believed to provide the best evidence to date for the risk of douching on BV, in the absence of a large randomized controlled trial (RCT).

With regards to smoking being associated with BV, other studies which had similarly demonstrated the risk effects included cohort studies (Shoubnikova et al. 1997, Cherpes et al. 2008) and a randomized controlled trial (Larsson et al. 2007), although the RCT was on pregnant Swedish women. Three possible explanations for a higher prevalence of BV in smokers as opposed to non-smokers are that 1) smoking has a causal connection to

BV, 2) women who smoke may have a risk behavior predisposing to BV, and 3) women who smoke may not notice the malodor caused by BV (Larsson et al. 2007).

No sexual behavior factors were found to be significantly associated with the risk of developing BV in the study population.

5.3 Negative associations with BV

On the other hand, the study demonstrated having a past pregnancy(s) as being protective of the study population developing BV. In addition, it also suggested using injectable hormonal birth control methods (i.e. Depo provera, Petogen, or Nuresterate) to have a protective effect, although this association was of borderline significance, even when controlled for confounders, and moreover, the 95% confidence interval around the odds ratio was wide (CI=0.14-1.12).

The protective effect of injectable hormonal contraceptives seen in this study (although it was approaching statistical significance) is consistent with findings from several studies (Baeten et al. 2001, Brabin et al. 2005, Riggs et al. 2007), carried out in other African, as well as non-African countries. A possible explanation for this association, which was cited by Brabin et al. (2005), is that Depo-Provera, for example, produces amenorrhoea, which decreases the risk of abnormal (raised) vaginal pH, which may in turn, prevent overgrowth of abnormal flora, hence, protecting against BV, since BV is associated with dysfunctional menstrual bleeding. However, since Brabin et al.'s (2005) study was cross-sectional, they were unable to determine whether the high pH levels associated with BV were a cause or product of the syndrome.

With regards to the moderate protective effect of having a past pregnancy(s) found in this study (AOR=0.59), however, these results are surprising, since they contradict findings from a case control study on Australian women (OR=1.5, P<0.0006) (Smart et al. 2004) and a cross-sectional study on American women (Koumans et al. 2007) which demonstrated prior pregnancy(s) as a risk factor for developing BV. On the other hand, the 95% confidence interval around the odds ratio in the current study findings was close to including 1, which showed an inclination towards a relationship showing no association.

5.4 Strengths and Limitations

The strength of this study lies within the BV diagnosis methodology followed. Nugent's Gram Stain method was used for diagnosing BV, with microscopic examination of the slides carried out by the same laboratory person, hence, standardizing the diagnostic procedure, thus, avoiding variations that would occur if more than one person made the diagnosis.

On the other hand, the study is limited by the fact that consecutive sampling was used and all HIV negative women enrolled in the main Prospective, Observational Feasibility Study to Determine HIV incidence in Preparation for a Future Preventative HIV Vaccine Efficacy Trial were included in the sub-study. Selection bias can therefore not be ruled out. Hence, the study population was most likely homogeneous, having resided in Cape Town Nyanga area for an average of 18 years. Moreover, 99% of the study population belonged to the black ethnic group, this factor being commonly demonstrated to have an

influence on increased BV prevalence in studies involving populations comprising different ethnic groups. In addition, observations for variables such as education level, marital status, amenities owned, and sexual behavior factors were non-discriminatory, which resulted in statistically non-significant associations with BV being computed in the analyses.

Another limitation is in the cross-sectional nature of the study, meaning that a causal relationship cannot be inferred regarding vaginal washing with soap and water, the only variable found to be independently associated with BV in the study.

5.5 Adding to current knowledge

The current study is the first aimed at determining the risk factors associated with BV in a peri-urban community in South Africa. Few studies of this nature have been conducted in less developed countries, especially African countries.

5.6 Recommendations

The two factors found to be significantly associated with the South African women in the current study developing BV infection were tobacco use and vaginal washing with soap and water (i.e. douching). These factors are modifiable, since they are behavioral factors, therefore, mechanisms aimed at encouraging behavior change can be embarked upon, to reduce smoking and douching among the Cape Town community, which in turn, may have the advantage of reducing the prevalence of BV. A decrease in BV prevalence

could thus, lead to a reduction in adverse health outcomes related to women's reproductive health, and may also reduce susceptibility to HIV and sexually transmitted infections.

However, more studies are required to unravel the causal mechanism involved with the factor, black race increasing the risk of developing BV infection.

CHAPTER SIX

6.0 CONCLUSION

This study revealed a high prevalence of BV (57%) among HIV negative South African women living in a peri-urban area in Cape Town, which was not surprising, considering the generally low SES of the study population, where risk factors tend to be prevalent.

Behavioral factors which were found to be significantly associated with determining the diagnosis of BV in the study population were, using tobacco in the last month and washing inside the vagina with soap and water at least once a day in the last seven days before being enrolled in the study.

The study failed to demonstrate any socio-demographic factor or sexual behavior factor of statistical significance, as possible determinants of BV diagnosis at enrollment.

On the other hand, women having had a past pregnancy(s) in the past four weeks were likely to be protected from being diagnosed with BV at enrollment. This was an unusual finding which was in contrast with findings from other studies, which demonstrated past pregnancy(s) as a risk factor for developing BV.

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APPENDICES

Appendix 1

Gram Stain Method for vaginal smears

- Heat-fix the slide by briefly passing the bottom-side (back) over a flame, 2 to 3 times
- Place the slide on the staining wrack
- Flood the slide with Gram's Crystal Violet (1 minute)
- Gently wash off excess Crystal Violet with cold tap-water
- Flood the slide with Gram's Iodine (1 minute)
- Gently wash off Iodine with cold tap water
- Flood the slide with Gram's Decolourizer until no more purple dye runs off (between 30 to 60 seconds)
- Immediately wash the slide gently and thoroughly with cold tap water
- Counterstain with Gram's Safranin (1 minute)
- Wash off excess stain with cold tap water
- Shake off the excess water from the slide and wipe the back of the slide with a paper towel
- Stand the slide (vertically) on a piece of paper towel and allow the smear to dry completely
- The slide is then ready to be viewed under the light microscope

Appendix 2

Nugent's Gram Stain Interpretation For Bacterial Vaginosis (Nugent et al.1991)

Nugent's criteria for diagnosing bacterial vaginosis on gram stained slides uses a scoring system, which awards points for Gram-negative/variable rods, coccobacilli (G. *vaginalis*, *Bacteroides* spp.) and Gram-variable curved rods (*Mobiluncus* spp.), and deducts points for Gram-positive rods (Lactobacilli spp.) However, less weight is given to curved gram-variable rods.

The Gram-stained smears are evaluated microscopically under oil immersion (X1000 magnification) as follows:-

- View 5 fields to obtain the average number of organisms to be reported for Nugent's criteria evaluation
- Evaluate 3 morphotypes of bacteria:-
    - Medium to large gram-positive rods (Lactobacillus morphotypes)
    - Small gram-variable rods (Gardnerella *vaginalis*) and small gram-negative rods (*Bacteroides* spp. morphotypes)
    - Curved gram variable rods (*Mobiluncus* spp. morphotypes)
- Count the total number of organisms in each field
- Sum up the total number of organisms in 5 fields then divide the sum by 5
- To obtain the +Number Evaluation Score correlate the dividend against Table a
- Assign a point score according to the +Number Evaluation Score by referring to Table b (*this was calculated electronically using Stata statistical software*)
- Add up all point scores to get the final Total Score: refer to Table c (*This was calculated electronically using Stata statistical software*)
- Report on spermatozoa if present
- Report on yeast cells and/or pseudohyphae if present
- Report on any other comments on the slide e.g. problems affecting evaluation

Table a

Weighted quantitation of morphotypes from 1 to 4+ with regard to the number of morphotypes per high power field (hpf) (i.e. oil immersion, X1000 magnification)

Number of morphotypes	**+ Number Evaluation Score**
0 per hpf	= 0
< 1 per hpf (i.e. 2 in 5 fields)	= 1+
1 – 4 per hpf	= 2+
5 – 30 per hpf	= 3+
> 30 per hpf	= 4+

Table b

Scoring system for Gram-stained vaginal smears

Large Gram-positive rods (Lactobacillus morphotype)	**Small Gram-negative to -variable rods (G. *vaginalis* and *Bacteroides* spp. morphotypes)**	**Curved gram-negative to –variable rods (*Mobiluncus* spp.)**	***Points Scored per morphotype**
4+	0	0	0
3+	1+	1+	1
2+	2+	1+	2
1+	3+	2+	3
0	4+	2+	4

Table c

The weighted quantitation of morphotypes (*points scored), are summed up to yield a final score of 0 to 10 for each person.

Final Points Scored	**Nugent's Interpretation**
0 - 3 points	Normal (report as negative for BV)
4 – 6 points	Intermediate (report as negative for BV)
7 – 10 points 7 – 8 9 - 10	Bacterial vaginosis positive Mild BV Severe BV