UNIVERSITY OF NAIROBI.

PREVALENCE OF BACTERIAL VAGINOSIS AMONG WOMEN OF REPRODUCTVE AGE ATTENDING THIKA DISTRICT HOSPITAL, KENYA 2011

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DECLARATION

This dissertation is my original work and has not been presented for the award of a degree in any other university.

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To my wife Joyce and all the colleagues who encouraged me to pursue MSc. Clinical Cytology, University of Nairobi.

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LIST OF ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
BV	Bacteria Vaginosis
CI	Confidence interval
DNA	Deoxyribonucleic acid
FP	Family planning
GC	Gonorrhea
HIV	Human Immunodeficiency Virus
HSIL	High grade squamous intraepithelial lesion
IUCD	Intra-uterine contraceptive device
ID	Identification number
LSIL	Low grade squamous intraepithelial lesion
P.I.D	Pelvic Inflammatory Disease
PCR	Polymerase chain reaction
PR	Prevalence ratio
RNA	Ribonucleic acid
S.D	Standard deviation
SES	Socio-economic status
STIs	Sexually transmitted infections
UTIs	Urinary Tract Infections

ABSTRACT

Background: Bacterial vaginosis (BV) is the most common cause of abnormal vaginal discharge among women of child bearing age and is associated with adverse obstetric and gynecologic outcomes.

Objectives: The aim of the study was to determine the prevalence of BV by use of Amsel criteria and the risk associated factors in women of reproductive age attending Thika District Hospital.

Methodology: The study was descriptive cross sectional where vaginal specimens from 150 women of child bearing age attending Thika District Hospital were obtained. Bacterial morphotypes indicative of BV were identified at light microscopy. A precoded questionnaire was used to collect demographic and sexual behavioral characteristics of the study participants.

Data analysis: In bivariate analyses, prevalence ratios (PR) and 95% confidence intervals (CI) for the association between BV and demographic or behavioral characteristics was calculated using Poisson regression.

Results: A prevalence of 26.0%, (95% CI 34.2- 48.6) was obtained from the study population. Classical BV cases were 16%, (95% CI 64.2-78.7) and non-classical 24%, (95% CI 12.0-28.3). Single sexual relationships, low socioeconomic status and hormonal contraceptive use were associated with BV. In terms of cervical lesions, 2% had HSIL, 4% LSIL, 2.6% Candidiasis, 3% Trichomoniasis and 1.3% Actinomyces.

Conclusions: The prevalence of BV was 26.0% in this population. Risk factors for BV ought to be evaluated periodically for intervention strategies.

CHAPTER 1

1.0 Introduction

Bacterial vaginosis (BV) is the most common vaginal condition among women of child bearing age. It's an imbalance in the ecology of the normal vaginal flora that is characterized by the depletion of *lactobacilli* and the proliferation of anaerobic bacteria such as *Gardnerella vaginalis*, *Mobiluncus species*, *Prevotella spec*ies, *Mycoplasma hominis* and the recently identified *Atopobium vaginae.* [1, 2, 3]

It most often manifests clinically at a vaginal pH of > 4.5, the presence of thin whitish homogenous vaginal discharge, presence of "clue" cells and an amine odor following the addition of 10 percent potassium hydroxide. BV has been shown to increase the risk of gynecological morbidity and adverse obstetrical outcomes such as preterm delivery, pelvic inflammatory disease (PID) and upper genital tract infections. [4, 5, 6]

The magnitude of the association between BV and HIV is variable in epidemiological studies, ranging from the absence of any association to nearly four-fold odds of being HIV infected among BV-positive women compared to BV-negative women. It is estimated to be the most prevalent vaginal condition particularly in countries with high HIV prevalence. Therefore, treatment of BV could be a meaningful intervention to significantly reducing HIV acquisition. [7, 8, 9]

Previous studies have identified a number of risk factors and behavior associated with BV, including the number of lifetime male sexual partners, recent partner change, lower

age of first intercourse, lesbianism, being unmarried, working as a sex worker, smoking and failure to use condoms. [10] However, many previous studies have used small number of patients or highly selected patient populations. Therefore, there is a paucity of published literature in investigating risk variables for BV, particularly in Kenyan populations. [11]

CHAPTER 2

2.0 Literature review

2.1 Aetiology of Bacterial vaginosis

The normal vagina of a woman of child bearing age is colonized by *lactobacilli.* These micro-organisms produce bacteriocins, hydrogen peroxide and lactic acid, all of which are substances that lower the vaginal pH. The low pH creates a hostile environment for bacteria other than *lactobacilli.* Consequently, reduction of these bacteria results in increased pH which favours an overgrowth of anaerobic and facultative bacteria, thus creating conditions favourable for the development of BV.[12]

The predominant organisms that cause BV are *Gardnerella vaginalis*, *Mycoplasma hominis* and *Ureaplasma urealyticum*. Other anaerobes, such as *Prevotella, Mobiluncus, Bacteroides and Peptostreptococcus* have also been identified as flora associated with BV. [13]

A change in normal vaginal bacterial flora including the reduction of *lactobacilli* may also occur due to use of antibiotics. Low pH imbalance allows more resistant bacteria to gain a foothold and multiply. Women with BV have increase in bacteria of up to 1000 fold above usual levels.

2.2 Clinical manifestation

A proportionate number of women with BV are asymptomatic. Among women with BV who report symptoms (an estimated 10% – 66%) [14], vaginal malodor is the most common

symptom. This condition is not associated with vaginal inflammation (such as finding excess leukocytes in the discharge or vaginal wall erythema) thus, the term "vaginosis" is used instead of "vaginitis. In clinical settings, finding of more than one leukocyte per epithelial cell on a microscopic evaluation of vaginal discharge should lead the cytologist to look for a diagnosis other than BV.[15]

Although a malodorous vaginal discharge, described as "fishy" suggests the presence of BV, it is not reliable enough to use as the only criterion for diagnosis. Some women with BV may report abnormal discharge, but this symptom is also unreliable.[16]

In a separate study involving women between the ages of 15 years and 44 years who attended twelve specific health departments for a routine annual health assessment, vaginal symptoms and tests for pH, wet mount, Gram stain, gonorrhea and chlamydia were evaluated. In addition, all participants underwent a pelvic examination.[17]

Reports of symptoms were compared between those who had a positive diagnosis of BV and those who were negative. Their results showed that 82% of women without BV reported never noticing any vaginal odor compared to 75% of women with confirmed BV who reported noticing an odor. The authors concluded that although more women with BV report vaginal odor than do women without BV, the difference was minimal and reliance on this symptom was not clinically significant. Some women may also experience pruritus, lower abdominal pain and pain during coitus.

2.3 Complications of Bacterial vaginosis

BV is associated with adverse obstetric and gynecologic outcomes, such as an increased risk in HIV acquisition, pelvic inflammatory disease (PID) following surgical procedures, hysterectomy or post abortion, an increased likelihood of other STIs, such as Chlamydia and gonorrhea. A substantial body of research has confirmed the association between BV and various gynecologic conditions, some of which have potentially serious implications for the pregnant and non pregnant woman.[17]

The obstetric risks associated with BV includes an increased likelihood of having ectopic (tubal) pregnancy following PID, pre term labour and delivery, post partum endometritis, low birth weight and premature infants as well as increased risk of acquisition (or reactivation) of HPV infection.[18]

2.4 Prevalence of Bacterial vaginosis

Vaginal inflammation is one of the most common condition for which women seek medical care. BV prevalence was reported to be between 25% and 36% in women attending sexually transmitted infection clinics. A study done by Temmermen among STIs patients in Nairobi (University of Nairobi) had a prevalence of 46% [19] and a similar study among sex workers in Mombasa had a prevalence of 32.0% [20]. BV also occurs in 11% among women attending reproductive health clinics and up to 25% of women attending gynecologic clinics. Thus, routine screening and prompt treatment of BV in these populations may be critical in certain clinical scenarios. A prevalence of 31.8% was reported in Havana, (Cuba) and 44%[21] had BV in a study conducted among women 18-45

years of age and their male partners from sexually transmitted infection clinics in Nairobi, Kenya. [22]

2.5 Risks factors for Bacterial vaginosis

Risk factors for BV have been identified which includes; douching, use of an intrauterine device, multiple sexual partners, no condom use and smoking.[23]

Klebanoff found an increased rate of BV in women who douched one or more times per week when compared to those who douched less frequently or not at all. He also studied douching and the prevalence of BV among adolescents who douched regularly. BV was found to be 44.8% among this population.[24]

Klebanoff found that douching in the previous week was positively associated with BV and that there was a strong association between douching after menses and the prevalence of BV. It is possible that douching decreases *lactobacilli*, thus facilitating the growth of BV causing microorganisms, but findings were inconsistent. Other factors which have been associated with BV are low socio-economic status, poor personal hygiene, marital status, HIV infection, STIs most commonly trichomoniasis, pregnancy and smoking. [25]

Uses of hormonal contraception and male condoms have been found to be inversely associated with BV. A reduced rate of BV is also seen among women in monogamous sexual relationships. Though, BV is rare in menopausal women and in adolescents prior to sexual onset, it can occur in female virgins.[25]

Sub-clinical iron deficiency (anemia) has been shown to be a strong predictor for BV among pregnant women. A link between psychosocial stress and BV has also been shown independent of other risk factors.

In Kenya, BV was significantly associated by univariate analysis with women's own risk factors: young age, being unmarried, early sexual debut, more than one life time sexual partner, HIV infection and Trichomoniasis.

Multivariate analysis including risk factors from both genders, the odds of having BV was 5.7 times higher if either partner was HIV seropositive, 13.2 times higher if the female had Trichomoniasis, 2.5 times higher if the female had more than one sex partner, but the odds decreased with increasing age of the female. [26]

2.6 Diagnosis of Bacterial vaginosis

BV has been shown to correlate well with Amsel clinical criteria and is an effective way to screen for asymptomatic cases. However, because Gram stain can be logically more difficult due to need of laboratory evaluation, the majority of practitioners use wet mount to detect presence of clue cells, pH and Whiff tests.

No single symptom has enough predictive power to accurately diagnose any of the common vaginal infections. Although signs and symptoms can assist in the diagnosis, the wet mount light microscopy is the best way to confirm diagnosis. Health care providers may miss the correct diagnosis by failing to perform laboratory tests.

Amsel's criterion has been used for making the diagnosis of BV for many years. The four diagnostic elements are: A vaginal fluid of pH greater than 4.5, Presence of epithelial

cells i.e "clue" cells (cells with unclear borders, dotted with bacteria), milky homogenous, adherent vaginal discharge and a positive "whiff" test, which is an amine or "fishy" odor noted after the addition of 10% potassium hydroxide. The presence of three out of four criteria is recommended by Amsel for diagnosis.[27]

An alternative diagnostic criterion utilizes Gram staining of vaginal secretions. This is usually scored using either Nugent or Spiegel's criteria. The loss of lactobacillus morphotypes and increase in Gardnerella and Bacteroides morphotypes, curved gram-variable rods, when combined with the pH, correlates well with Amsel's criteria for diagnosis of BV.

Gram stain may not be useful in determining eradication of the condition because of its high proportion of indeterminate results. Since the predictive value of a positive culture for *Gardnerella vaginalis* is less than 50 percent, culture is not recommended as a diagnostic tool. The higher the number of *Gardnerella, Bacteroides* and *Mobiluncus species*, the higher the score with a maximum of four and two respectively. Lactobacillus morphotypes comprises more than 95% vaginal normal flora, hence the lower the number of lactobacillus morphotypes, the higher the score of four.[28]

In absence of a microscope or if the skills of the practitioner are limited, the Affirm VPIII microbial identification system (Beckon Dickinson and Company, Sparks, MD) is a choice. This DNA probe system is used to identify BV, Trichomoniasis or Candidiasis. This assay detects clinically significant levels of *Gardnerella species*, *Trichomonas vaginalis* and *Candida species* from vaginal fluid.

Another test used is the QuickVue Advance pH and Amines Test card (Quidel Corporation, San Diego, CA). A drop of vaginal secretion is placed on the card for rapid identification of pH. The sensitivity of this test is 94% when compared to culture.

A newer, easy test for detecting BV is OSOM BVBlue (Genzyme Corporation, Cambridge, MA). The kit has sample swabs and reagent. The vagina wall is swabbed and the swab placed into a test tube with reagent. The reagent turns blue or green if positive for BV. This is a ten minute test that detects elevated sialidase activity in the vaginal fluid. The sialidase is produced by bacterial pathogens associated with BV, including *Gardnerella spp* and *Mobiluncus spp.*[29]

2.7 Treatment of Bacterial vaginosis

Treatment of BV includes an oral antibiotic or antibiotic vaginal gel or cream. Metronidazole or tinidazole is taken as a single dose or more often in resistant cases. Nausea and stomach complains may occur with these drugs and alcohol consumption is avoided. A bitter metallic taste is also associated with unacceptably low (50% – 80%) compliance.

Metronidazole is generally used to treat pregnant women, although most clinicians state that it should not be used in the first trimester. Clindamycin, an antibiotic and a vaginal cream is often used in treatment for pregnant women. However, the cream is expensive and is known to target vaginal *lactobacilli* which increase likelihood of BV. Pregnant women with BV are advised to consult their obstetrician about treatment options. In absence of symptoms, treatment is usually not necessary. Given the potential for BV, to greatly impacting a woman's reproductive and overall health, clinicians should place great emphasis on maintaining a health vaginal flora. Routine screening and treatment should be initiated.[30]

2.8 Problem statement

BV is a common problem among women of child bearing age. This condition has a high obstetric and gynecologic morbidity, often undiagnosed and untreated. Its symptoms and signs are inconsistent and unreliable for clinical diagnosis. Thus, for BV cases, there is increased risk for HIV and other STIs transmission. Appropriate diagnosis and treatment of BV could lower acquisition of these diseases.

However, since no study for BV has been previously done at Thika District Hospital, and considering BV has adverse obstetric and gynecologic morbidity outcomes, it was vital to conduct BV research in this population.

In Thika District Hospital, HIV/AIDS prevalence rate (2000) was reported as 34%, way above the national prevalence of 14% then reported in 2001 by National AIDS control programme. However, considering the association of BV and HIV, the impact of HIV has been felt at all levels of the District's economic and social circles.

2.9 Justification

Diagnosis of BV is critical among women of child bearing age. This is important since the condition has adverse obstetric and gynecologic outcomes in association as outlined earlier. BV increases acquisition of HIV if a woman is exposed to the virus. This is as a result of high accumulation of CD4 cells which have receptors to HIV in the vaginal epithelium. High vaginal pH with a range of 7.0 to 8.5 may be contributory.

There are several problems associated with syndromic management in women with vaginal discharge. Vaginal discharge syndromes may be as a result of STIs such as (vaginal yeast and Trichomonas infection) or cervicitis (chlamydia and gonorrhea). Therefore, syndromic approach is not an efficient method for detecting vaginal conditions and alternative approaches to evaluation and intervention are necessary.

Routinely, surveys have shown that majority of health care professionals do not investigate BV, yet the condition is responsible for as many as 50 percent of all vaginal symptoms and is also common among STI patients. Therefore, the present study investigated the current status of BV among women of reproductive age attending Thika District Hospital.

2.10 Research questions

a) What is the prevalence of BV in women of reproductive age attending Thika District Hospital?

b) What are the risk factors associated with BV?

2.11 General Objective

To determine prevalence of BV among women of reproductive age attending Thika District Hospital.

2.12 Specific Objectives

1. To screen women of reproductive age attending reproductive health and family planning clinics for BV
2. To score the vaginal smears using Amsel diagnostic criteria for BV
3 .To establish risk factors associated with BV among women of reproductive age attending Thika District Hospital

CHAPTER 3

3.0 Materials and methods

3.1 Study site

The study was conducted in Thika District Hospital. Thika District is one of the seven Districts in Central Province. It borders Nairobi City to the south, Kiambu District to the west, Maragua District to the north and Machakos District to the east. The District lies between latitudes 3°53′ and 1° 45′ south of Equator and longitudes 36° 35′ and 37° 25′ east. It is divided into six divisions namely; Ruiru, Gatundu South, Thika Municipality, Kakuzi, Gatanga and Kamwangi (Gatundu North), with 20 locations and 89 sub-locations.

The District was densely populated but with a diverse population distribution varying from one Division to the other. Gatundu, Thika Municipality and Gatanga Divisions are the most densely populated with Gatundu having the highest population density. The lower parts of Ruiru and Kakuzi Divisions were the least densely populated, drier and less agriculturally productive.

The most prevalent diseases were malaria, HIV/AIDS, tuberculosis and broncho-pneumonia while the childhood diseases included anemia, eye infections, pneumonia, malaria and nutritional disorders.[31]

3.2 Study design

This was a descriptive cross sectional survey.

3.3 Study population

The study was conducted among women of child bearing age attending Thika District Hospital (18-49years).

3.4 Selection criteria

Inclusion criteria: Women

- Aged between 18 and 49 years
- Clients at the reproductive health and family planning clinics
- Able and willing to give informed consent

Exclusion criteria: Women

- Less than 18 years and above 49 years of age
- On treatment for STIs
- With abnormal uterine bleeding

3.5 Sample size calculation

Number of vaginal samples for the study participants was calculated using prevalence of 11% and the binomial formula for 95% confidence interval on a simple proportion mean + 1.96

$N = Z^2 Pq/d^2$ (32)

Where,

N = desired sample size (where population is greater than 10,000)

Z = the standard normal deviate set at 1.96 which corresponds to the 95% confidence level.

P = the proportion within the target population estimate assumed to be a low risk with a characteristic prevalence of 11%, similar to a study by Sweet *at al.*, (2000).[11]

$q = 1 - p$

d = degree of accuracy desired, usually set at 0.05

Thus $N = (1.96)^2 (0.11) (0.89)/ (0.05)^2$

$N = (3.8416 \times 0.0979) / (0.0025)$

$N = 150$

A total of one hundred and fifty one women were recruited into the study.

3.6 Sampling procedure

The women recruited and enrolled were those attending Family planning and Reproductive Health Clinics at Thika District hospital. The family planning and reproductive health clinics are within the hospital.

Those who consented were invited into the research room at the hospital for interviews and clinical examination. Once in the study room, they were given consent statement to read and/or explained. Convenient sampling method was used.

3.7 Data collection

Vaginal swabs for testing BV were collected by a trained Nurse/Doctor. Thin smear of the material was immediately smeared onto a clean microscope slide labelled with patient ID and fixed with polyethylene glycol. Thereafter, the thin smear was stained using Pap staining protocol (**Appendix iv**), while the residual material preserved in buffered normal saline was used to perform pH and Whiff tests. The results were recorded, those found to have BV and abnormal vaginal lesions were advised to consult clinicians for management. Pap smear results were added advantage to the study participants and were a secondary objective. Precoded questionnaires were used to collect demographic or

behavioral data from study respondents. Demographic characteristics were tallied as shown in the questionnaire (**Appendix i**) while level of income was based on those who earned more or less than Kshs.200 per day. Those who earned less than Kshs 200 per day were categorized as earners of less than Kshs 6,000 per month while those who earned more than Kshs 200 per day were computed to have earned more than Kshs 6,000 per month. Quality control measures were observed during recruitment, specimen collection, processing and dispatch as shown in quality control procedures. (**Appendix v**)

3.8 Data management

STATA software v 9.2 (StataCorp LP, Texas USA) was used to analyze data. The 95% confidence intervals were calculated using an online tool available at (confidence interval Calculator for a Completion Rate http://www.measuringusability.com/wald.htm by Saulo,2005). In bivariate analyses, prevalence ratios (PR) and 95% confidence intervals (CI) for the association between BV and demographic or behavioral characteristics was calculated using Poisson regression. Significant level was pegged at $p < 0.05$.

3.9 Ethical considerations

The approval to conduct the study was obtained from KNH/UON ethical review committee. The participants were carefully taken through a consent process that informed them about the study and what was required from them. They had the choice to take part in the study or not. Those who consented signed consent form prior to participation. Any information obtained from the participants was strictly confidential. Data was not processed in a way that could be directly linked to the individual for confidentiality. Results were dispatched to the clinicians for management.

CHAPTER 4

4.0 Results

A total of 150 vaginal smears were examined for the presence of BV by light microscopy. The Amsel clinical criterion was used in reporting the smears. Normal smears were those devoid of bacterial morphotypes consistent with BV while abnormal smears had clue cells. Normal smears accounted for (74.0%) among the study participants. The smears were scored on the basis of the four elements developed by Amsel. The classical BV cases met the four elements while the non-classical met three out of the four. There was testing for pH and odor among study respondents. Precoded questionnaires were used to obtain demographic and/or behavioral characteristics from study participants. Cervical lesions and pathogenic microorganisms were reported.

4.1 Socio-demographic characteristics of the study population

The mean age of 150 women enrolled into the study was 30.0 years, range (18 – 48 years) and median 29 years with a S.D of 8.19. The age group 21-30 years represented 44.4% with the least 11.3% being those aged 41-49 years.

Majority 53% of the respondents had secondary level education with 3.3% having no formal training; 17.2% had tertiary level education. Monthly income was selected as an indicator of socioeconomic status. Those who earned more than Kshs 6,000 per month were 59.3% and less than Kshs 6,000 per month constituted 40.2% of the study participants.

Almost half 44.4% of the women were married with 44.5% being single. The separated or divorced constituted 6.0% while 4.6% were widowed. A small proportion 3.9% of the women was nulliparous, 49.0% had one child and 47.1% had more than one child. (Table 4.0)

Table 4.0 **Socio-demographic characteristics of the study population**

Characteristics	Frequency	
	N	%
Age in years		
18-20	22	14.6
21-30	67	44.4
31-40	45	29.8
41-50	17	11.3
Education level		
None	5	3.3
Primary	40	26.5
Secondary	80	53.0
Tertiary	26	17.2
Monthly income		
Less than Kshs 6,000	61	40.2
More than Kshs 6,000	90	59.3
Marital status		
Single	68	45.0
Married	67	44.4
Widow	7	4.6
Separated/divorced	9	6.0
Parity		
None	6	3.9
1	74	49.0
> 1	71	47.1

N - Number; % - Percentage

There were various family planning methods used and reported by the study participants. These included condoms, IUCD and Depo provera. The oral pills and natural method were not reported among the respondents and none reported the use of more than one family planning method. The frequency of the family planning methods, reproductive characteristics and BV prevalence are as shown in Table 4.1.

4.2 Prevalence of Bacterial vaginosis

One hundred and fifty (150) study participants provided suitable vaginal swabs which were used to diagnose BV. Vaginal specimens from 40 (26%) women had BV from which 4 (10.6%) had classical and 36 (89.4%) non-classical BV. Majority of study participants 110 (74.0%) were negative for BV. (Figure 4.0)

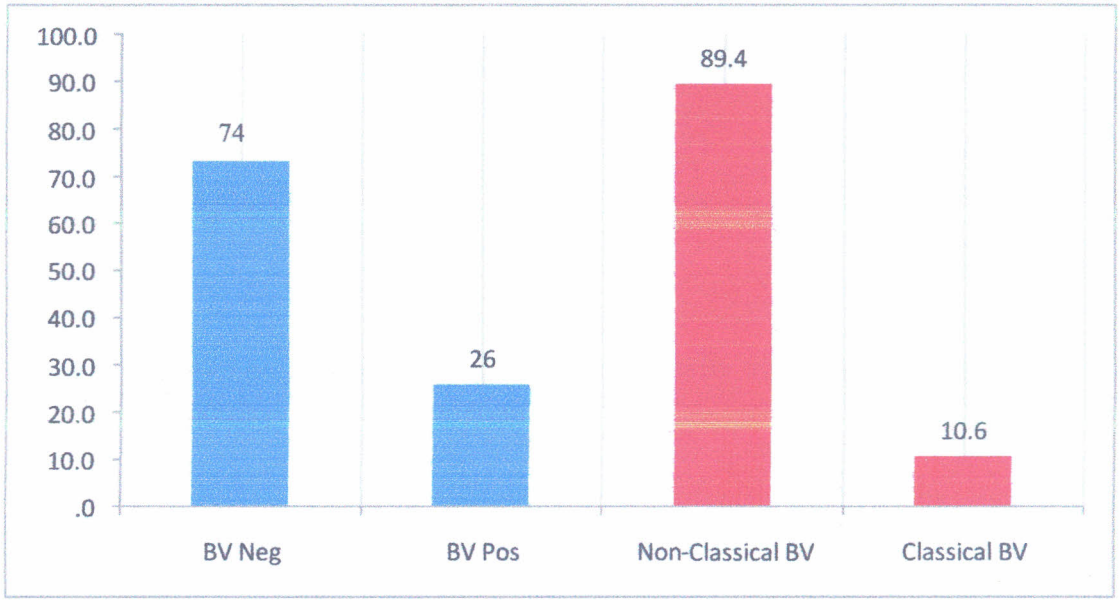

Figure 4.0 BV distribution/percentage of patient population

The socio-demographic profiles, reproductive characteristics, family planning methods use and BV prevalence rates are as shown in (Tables 4.1, 4.2).

Table 4.1: BV prevalence with socio-demographic characteristics

Demographic Characteristics	Frequency		BV Positive		BV Negative	
	N	%	N	%	N	%
Age in years						
18-20	22	14.6	4	18.2	18	81.8
21-30	67	44.4	12	17.9	55	82.1
31-40	45	29.8	18	40	27	60
41-50	17	11.3	6	35.3	11	64.7
Education level						
None	5	3.3	0	0	5	100
Primary	40	26.5	7	17.5	33	82.5
Secondary	80	53	24	30	56	70
Tertiary	26	17.2	9	34.6	17	65.4
Monthly income						
Less than Ksh 6,000	61	40.2	11	18.3	49	81.7
More than Ksh 6,000	90	59.3	29	32.2	61	67.8
Marital status						
Single	68	45	13	19.1	55	80.9
Married	67	44.4	24	35.8	43	64.2
Widow	7	4.6	1	14.3	6	85.7
Separated/divorced	9	6	2	22	7	78

N- Number, %- Percentage, BV- Bacterial vaginosis

Table 4.2: BV prevalence, reproductive profiles and family planning methods use

Reproductive Characteristics	Frequency		BV Positive		BV Negative	
	N	%	N	%	N	%
Parity						
None	7	4.6	0	0	7	100
1	73	48.3	18	24.7	55	75.3
> 1	71	47.1	22	31	49	69
Sexual Partners						
None	2	1.3	1	50	1	50
1	115	76.2	32	27.8	83	72.2
> 1	34	22.5	7	20.6	27	79.4
Condom Use						
None	83	54.9	26	31.3	57	68.7
At last sexual act	68	45.1	14	20.6	54	79.4
Use of IUCD						
Yes	141	93.4	38	27	103	73
No	10	6.6	2	20	8	80
Use of Depo Provera						
Yes	64	42.4	14	21.9	50	78.1
None	84	55.6	25	29.8	59	70.2

No - Number; % - Percentage; BV - Bacterial vaginosis

4.3 Vaginal smear score system

Smears were scored for BV using Amsels criteria. The classical cases were those who met the four elements developed by Amsel through evaluation for pH, odor, white vaginal discharge and detection of clue cells at microscopy. The frequency criteria and the report of pH, assessment of odor and vaginal discharge are as shown in (Table 4.3)

Table 4.3 BV diagnosis by Amsel criteria

Amsel Criteria Characteristics	Frequency	
	N = 40	%
Clue Cells		
Positive	40	100
Negative	0	0
Ph		
5	13	32.5
6	20	50
7	7	17.5
Vaginal Discharge		
Yes	4	10.6
No	36	89.4
Odor		
Fishy smell	37	92.8
No smell	3	7.2

N - Number; % - Percentage

A proportion of the women had vaginal smears with corresponding cervical lesions and pathogenic microorganisms as shown in (Table 4.4). These findings were an added advantage for the study participants. The cases were referred to clinicians for management.

Table 4.4: Distribution of cervical lesions from study population

Characteristics	Frequency	
	N	%
Cervical lesions		
HSIL	3	2
LSIL	6	4
ASCUS	3	2
None	139	92.1
Vaginal microorganisms		
Actinomyces	2	1.3
Candida	4	2.6
Trichomonas	3	2
None	142	94

No - Number; % - Percentage

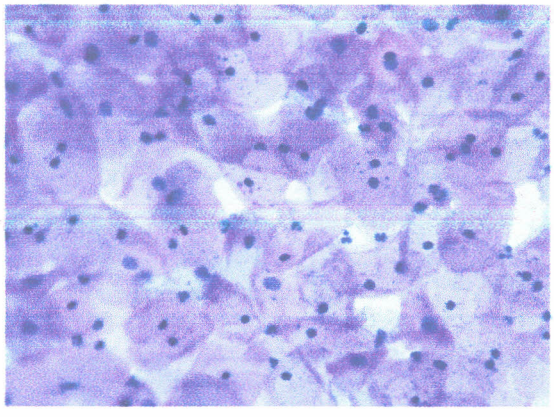

Figure 4.1: **Normal smear /Pap x40**

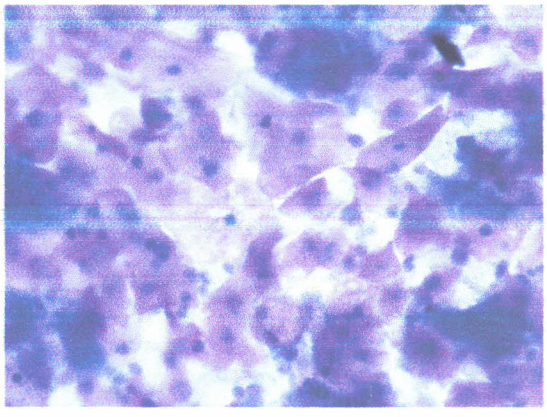

Figure 4.2: BV positive smear/Pap x40

4.4. Risk associated factors to Bacterial Vaginosis

In bivariate analysis, Single sexual partner relationships (p=0.023), low socioeconomic status (p=0.014) and non users of Depo provera (p=0.042) showed significant association with BV with a p value < 0.05. There was no significant association with the other variables. The confidence interval for income was skewed; thus, there was likelihood of a measurement error bias in reporting. (Tables 4.5, a, b)

Table 4.5 (a): Bivariate analysis of BV by socio-demographic characteristics

Characteristics	Frequency	Bacterial Vaginosis		P value	Bivariate
		N	%		PR (95% CI)
Age in years					
18-20	22	4	18.2	Referent	Referent
21-30	67	12	17.9	0.304	0.51 (0.14 - 1.82)
31-40	45	18	40	0.175	0.51 (0.19 - 1.37)
41-50	17	6	35.3	0.791	1.13 (0.44 - 2.85)
Education level					
None	5	0	0	0.999	
Primary	40	7	17.5	Referent	Referent
Secondary	80	24	30	0.714	0.86 (0.41 - 1.86)
Tertiary	26	9	34.6	0.176	0.51 (0.19 - 1.35)
Income					
Less than Ksh 6,000	61	11	18.3	0.014	1.3 (1.89 - 2.13)
More than Ksh 6,000	90	29	32.2	Referent	Referent
Marital status					
Single	68	13	19.1	1	1 (0.42 - 2.36)
Married	67	24	35.8	0.992	1 (0.42 - 2.39)
Widow	7	1	14.3	0.995	1 (0.29 - 3.67)
Separated/divorced	9	2	22	Referent	Referent

PR - Prevalence ratio; CI - Confidence interval; N - Number; % - Percentage

Table 4.5 (b): Bivariate analysis of BV by reproductive profiles and family planning Methods use

Characteristics	Frequency	Bacterial Vaginosis		P value	Bivariate
		N	%		PR (95% CI)
Parity					
None	8	1	12.5	Referent	Referent
1	94	40	42.6	0.986	0.27 (0.04 - 1.96)
> 1	91	43	47.3	0.445	0.92 (0.59 - 1.42)
Sexual partners					
None	7	0	0	1	
> 1	73	5	27.8	Referent	Referent
1	71	11	50	0.023	0.35 (0.23 - 0.54)
Condom use					
At least last sexual act	83	9	34.6	0.572	1.1 (0.74 - 1.74)
None	68	7	50	Referent	Referent
IUCD use					
Yes	141	15	40	0.205	0.67 (0.35 - 1.27)
No	10	1	50	Referent	0.67 (0.35 - 1.27)
Depo- Provera usage					
Yes	84	10	40	0.929	0.85 (0.53 - 1.35)
None	3	1	100	0.042	0.52 (0.27 - 0.96)

PR - Prevalent ratio; CI - Confidence interval; N - Number; % - Percentage

CHAPTER 5

5.0 Discussions

Bacterial vaginosis is characterized by a shift from predominant lactobacillus vaginal flora to an overgrowth of anaerobic bacteria. The mean age of 150 women enrolled into the study was 30.0 years range (18 – 48 years), median 29 years with a S.D of 8.19. The age distribution had a normal distribution curve. The socio-demographic correlates to BV were evaluated among the study participants.

The present study investigated BV prevalence, potentially modifiable behavioral and biological risk factors for BV. The prevalence of BV in this population at 26.0% was similar to previous findings from other populations. Previous studies have reported BV prevalence between 20%-50% such as 44.8% were reported in Havana (Cuba) and 28.0% in Butare University Hospital, Rwanda. In Kenya, a study done by Caleb *et al.*, (2008), among women of reproductive age in Kisumu had prevalence of 28% and Bukusi *et al.*, (2006) reported a prevalence of (44.3%) within Nairobi area. This shows that prevalence of BV varies widely among different areas and communities' globally.[5, 6, 19]

In the study, Amsel criteria were used for BV diagnosis. The four elements were correlated for each study respondent. The method of assessment was highly sensitive as it had several criteria for evaluation. Among the study respondents, those who had pH > 5.0 and white vaginal discharge were considered for correlation. These were essential elements established by Amsel.[26]

There are several methods that are used to diagnose BV. This includes culture, DNA hybridization (PCR), Nugent or Spiegel and Amsel criteria. Conventional laboratory culture techniques take a minimum of three days for patient results to be completed for clinical management. The DNA ploidy analysis takes more time and is expensive thus not applicable in poor resource setting and due to the special technical expertise required. [27]

The results confirm that women of child bearing age are similarly affected by BV in various parts of the world and especially in sub-Saharan Africa. The proportion of women with BV among the age group of 31-40 years had the highest prevalence of 40.0% which was in contrast to a study done by Zhang *et al.*, (2004) of the same age group among African American women. The prevalence of BV for women from low socioeconomic stratum in USA was slightly lower than that in other reports from sub-Saharan Africa which ranged between 11-30%. [2, 11]

In addition, the magnitude of BV was slightly higher among women with tertiary level of education. This requires further research since the higher the level of education, the lower incidences of BV as documented in other studies. For example, in the study done by Bukusi *et al.*, (2006) among STI clients within Nairobi area, women of low level education had high prevalence rates. [19]

Among married women, BV prevalence of 35.0% was reported. This was far much less than the reported prevalence noted by Klebanoff *et al.*, (2004) among women in sub-Saharan Africa of 48.0%. It has been observed that the prevalence of BV has a range

between 50% and 60% in low resource settings. This might have added to the reasons for increased morbidity in such zones.[6]

In the study by Bukusi *et al.*, (2006), among STIs clients within Nairobi area, found that the prevalence of BV was high among the married women more than the single, widowed, divorced or separated. Thus, there is need for further inquiry into the prevalence of BV in this relationships.[10, 19]

Elsewhere studies have found BV to be associated with sexual activity in both communities based rural settings and among women at high risk of STIs. However, a prospective study in rural women in Gambia found no association between any sexual behavior and BV. A recent Meta-analysis found the epidemiological data to be consistent with BV having a sexual mode of transmission, but additional factors were likely to contribute to the risk.[7, 15]

Asymptomatic women are less likely seek treatment for the morbidity and thus are more likely to acquire other STIs. In view of this, it is suggested that women attending various health care facilities including ante-natal clinics, gynecologic clinics, genitor-urinary clinics or family planning clinics should be screened and treated for BV to reduce the risk of acquisition of other more serious STIs.[14]

The family planning methods use had varied prevalence rates. As indicated, more women who used IUCD had BV compared to those who used Depo provera and male condoms.

When inappropriately inserted, IUCD is not protective for BV since it provides a suitable environment for the growth of BV causing bacteria.[21]

In sub-Saharan Africa, the association between BV and Depo provera is inconsistent; perhaps reflecting the heterogeneity of the formulations. In a prospective study of sex workers in Burkina Faso, Depo provera was not associated with BV (Christopher *et al.*, 2000). Also, among family planning attendees in Zimbabwe and Uganda, Depo provera was not associated with BV. Although not statistically significant, the study found some evidence of a protective effect of hormonal contraception for BV which is consistent with other studies.[15]

In the study, BV prevalence was high among women who used IUCD although this was not statistically significant (p=0.205). However, a study in Uganda found no association between BV and IUCD. Furthermore, a cross over trial in Gambia STI clinics, found BV to be slightly more frequent among women who used IUCD. It was also found that male condom use was not protective against BV. This is probably because male condom use is often irregular and is indicative of increased risky sexual behavior.[25] (Table 4.2)

In bivariate analyses, although BV was not significantly associated with age, some researchers have previously reported that the condition is more common among younger women; while others have found that the risk for BV increases with age. It is suggested that, in this population, age was a proxy for cumulative sexual activity which was found to be associated with BV. Interestingly, BV prevalence varied with age were the older

women 41-50 years had a prevalence rate of (35.3%) and those aged 31-40 years had a prevalence of (40%). This seems counterintuitive because it would be assumed that a higher number of women aged 21-30 years would reflect elevated levels of BV among the same age group. Thus, BV prevalence was high among the sexually active women of reproductive age. (Table 4.2)

In the present study, there was no statistical association between prevalence of BV with age. However, other studies showed a strong association between the prevalence of BV and age greater than 25 years. [6]

Single sexual partner relationships, (P= 0.023; PR, 0.35, 95% CI 0.23-0.54), hormonal contraception (P= 0.042, PR, 0.52, 95% CI 0.27- 0.96) and low socioeconomic status (P= 0.014, PR, 1.3, 95% CI 1.89-2.13) were associated with BV in this population.
(Tables 4.5, a, b)

In multivariate analysis, none of the tested demographic and sexual behavior characteristics of the respondents were independently associated with BV.

5.5 Conclusions

Bacterial vaginosis prevalence was relatively high in this study population (26.0%). Factors; Single sexual partner relationships, low socioeconomic status and hormonal contraceptive were associated with increased BV in this population. Thus, this condition being a common problem among women of reproductive age and its prevalence also being similar to that in many treatment seeking populations, further studies are needed to disentangle the interactions between BV and its correlates.

5.6 Recommendations

Routine screening for BV, treatment and subsequent follow up should be done among women of reproductive age in order to prevent its obstetrics and gynecological outcomes. Women with tertiary level of education need further exploration to ascertain why they had a high prevalence of BV. Risk factors to BV such as single sexual partners, low socioeconomic status and contraceptive use are dynamic. Better understanding and evaluation on regular basis is warranted in order to optimize intervention strategies.

5.7 Study limitations

Since the analyses were cross-sectional in nature, it was not possible to infer any causal association between risk factors and BV. In addition, risk factors were collected at face to face interviews, thus there was a possibility of under-reporting and misclassification of risky behaviors. The survey involved collection of sensitive sexual behavior as well as information on women's sexual partners, so there was a likelihood of measurement error that may have led to residual confounding obscuring the relationships. There was also the potential for bias since the study lacked a quality control measure for reporting whiff test. The findings were based on the usual fish odor.

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Appendix 1

BV Questionnaire

This form was to be filled by the interviewer prior to collecting vaginal smear.

Study identity number……

Date dd/mm/yy……

1.1 Which year were you born? 19……

1.2 Age is? ----------.

2.1 What level of education have you attained?

- [0] Primary
- [1] Secondary
- [2] Tertiary

3. What is your marital status?

- [0] Single
- [1] Separated
- [2] Married
- [3] Divorced
- [4] Widowed

4. How often do you take a bath?

- [0] Less than 7 times a week
- [1] More than 7 times a week

5. Have you, in the last six months had a vaginal discharge?

- [0] No
- [1] Yes

6. If yes, what colour was it?

- [0] Yellow
- [1] Brown
- [2] White
- [3] Others ----------

7. Was the vaginal discharge accompanied by itching or burning in your genitals?

0	Yes
1	No

8. For the last 3 months, probably how many sexual partners have you ever had?

0	None
1	One
2	More than two

9. Does your partner use protective condoms during sexual intercourse?

0	No
1	Yes

10. How many children do you have?

0	None
1	One
2	More than one

11. What is the family income per month?

0	Less than Kshs 6000
1	More than Kshs 6000

12. What is the common economic activity in your family?

0	Small business enterprise
1	Farming
2	Employment (Driver, Teacher, Masonry)
3	Unemployment

13. Do you practice family planning?

0	No
1	Yes

14. If yes, what method of family planning do you prefer?

0	None
1	Barrier method (condoms)
2	Depo-provera
3	IUCD
4	Others ----------

Appendix 11

KNH/UON ethical review letter

KENYATTA NATIONAL HOSPITAL
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Ref: KNH-ERC/ A/519

12th July 2010

Nzomo J. Wambua
Dept.of Human Pathology
School of Medicine
University of Nairobi

Dear Mr. Wambua

RESEARCH PROPOSAL: "PREVALENCE OF BACTERIAL VAGINOSIS AMONG WOMEN OF REPRODUCTIVE AGE ATTENDING THIKA DISTRICT HOSPITAL- KENYA" (P129/4/2010)

This is to inform you that the KNH/UON-Ethics & Research Committee has reviewed and **approved** your above revised proposal for the period 12th July 2010 to 11th July 2011.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimens must also be obtained from KNH/UON-Ethics & Research Committee for each batch.

On behalf of the Committee, I wish you a fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of the data base that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely

PROF A N GUANTAI
SECRETARY, KNH/UON-ERC

c.c. Prof. K. M. Bhatt, Chairperson, KNH/UON-ERC
The Deputy Director CS, KNH
The Dean, School of Medicine, UON
The Chairman, Dept.of Human Pathology, UON
The HOD, Records, KNH
Supervisors: Dr. Muchiri L, Dept.of Human Pathology, UON
Dr. Okemwa M. P, Anatomic Pathology, UON

Appendix 111

Specimen collection

Vaginal swabs for testing BV were collected by a trained Nurse/Doctor. The Clinician welcomed the patient to the clinic. Under a private screened setting, briefly and kindly, the clinical procedure was explained on how to collect vaginal specimen aseptically.

A speculum was used to visualize the vagina; a cervex brush was inserted and rotated at 360^0 to ensure adequate material was collected from the wall of the vaginal epithelium.

Thin smear of the vaginal material was made onto a clean microscope slide labelled with patient ID and preserved with polyethylene glycol. Later, the BV smear was stained using Pap staining protocol, while the residual material preserved in buffered normal saline to carry other tests as per Amsel criteria (pH and Whiff tests). The clients were advised on when to collect results and the medical procedures to follow.

Appendix 1V

Laboratory methods:

Pap staining protocol

1. Thin smear made onto a clean slide was fixed with 95% ethanol for 15 minutes.
2. It was brought into water through absolute and 95% alcohol respectively.
3. The smear was stained in Harris haematoxylin for 4 minutes.
4. It was then rinsed in tap water, differentiated in 1% acid alcohol for a few seconds and blued in ammoniated water for 4 minutes.
5. Subsequently, it was stained in Orange G6 for 2 minutes, rinsed in 95% ethanol and then stained in Eosin Azure for another 2 minutes.
6. Finally, it was dehydrated in ascending grades of alcohol to absolute, cleared in xylene and mounted with DPX.
7. The Stained slides were allowed to air dry, examined with X10 and X40 to determine presence or absence of clue cells.
8. Results were recorded for analysis.

Appendix V

Amsel protocol

Amsel's criterion was used for making the diagnosis of BV. The four diagnostic elements were:

a) A vaginal fluid pH > 4.5

b) Presence of epithelial cells "clue" cells (cells with unclear borders, dotted with bacteria)

c) Milky homogenous, adherent vaginal discharge

d) A positive "whiff" test, which is an amine or "fishy" odor liberated after the addition of 10% potassium hydroxide. The presence of three out of the four criteria was recommended by Amsel for diagnosis. [3, 16]

Amsel score sheet

Indicator	**pH > 4.5**	**White vaginal Discharge**	**Fishy Odor**	**Clue cells**	**Total**
0					
1					

Appendix V1

Quality control measures (QC)

i) Specimens were collected aseptically by use of a cervex brush.

ii) Each specimen was smeared onto a clean microscope slide labelled with patient ID.

iii) It was immediately fixed by use of polyethylene glycol.

iv) The residue was preserved in buffered normal saline for further tests: (Whiff test and pH)

v) Patient's bio data was recorded in the research register.

vi) BV smears were stained by use of pap staining protocol.

vii) Smears were examined, results recorded and finally dispatched to clinicians for management.

viii) Whiff test was based on the usual smell of fishy odor.

ix) pH testing was determined by use litmus paper (range 4-8).