COMPARISON OF PAP-SMEAR AND MODIFIED-AMSEL'S CRITERIA IN SCREENING FOR BACTERIAL VAGINOSIS AMONG WOMEN AT KIAMBU LEVEL-V HOSPITAL, KIAMBU COUNTY, KENYA

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FEBRUARY, 2025

DECLARATION

Student

I declare that this thesis is my original work and has not been presented in any other institution for a degree or any other award.

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I dedicate this work to my husband Dr. George Ochiri and our children Ivanka, Giana, Samuel and Noella.

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LIST OF ABBREVIATIONS AND ACRONYMS

ASCH	Atypical Squamous Cells Cannot Exclude High Grade Lesion
ASCUS	Atypical Squamous Cell of Undetermined Significance
AUC	Area under the Curve
BD	Two Times a Day
BV	Bacterial Vaginosis
CDC	Centre for Disease Control
CI	Confidence interval
CIN	Cervical Intraepithelial Neoplasia
CIS	Carcinoma In-situ
CST	Community State Type
DNA	Deoxyribonucleic Acid
DPX	Dibutylphthalate Polystyrene Xylene.
DUHS	Directorate of University Health Services
HCL	Hydrochloric acid
HIV	Human Immunodeficiency Virus
HPV	Human Papilloma Virus
HSIL	High-grade Squamous Intraepithelial lesion
HSV-2	Herpes Simplex Virus type 2
HVS	High Vaginal Swab
IUCD	Intrauterine Contraceptive Device
KOH	Potassium Hydroxide
L.M.P	Last Menstrual Period
LSIL	Low-grade Squamous Intraepithelial Lesion
MOH	Ministry of Health
NACOSTI	National Commission for Science, Technology and Innovation
NILM	Negative for Intraepithelial Lesion or Malignancy
NOS	Not Otherwise Specified
NPV	Negative Predictive Value
Pap	Papanicolaou

PCR	Polymerase Chain Reaction
pH	Hydrogen ion concentration
PI	Principal Investigator
POCT	Point of Care Test
PPV	Positive Predictive Value
ROC	Receiver Operating Characteristics
SOP	Standard Operating Procedure
SPSS	Statistical Package for Social Sciences
STD	Sexually Transmitted Disease
STI	Sexually Transmitted Infection
USA	United States of America
WHO	World Health Organization

ABSTRACT

Bacterial vaginosis is a commonly experienced vaginal infection in reproductive age group women between 15 and 44 years old. Globally, 5-70% of women are affected. In Kenya the prevalence is between 10-50%. Bacterial vaginosis is a public health concern implicated in premature rupture of membranes, low birth weight, pelvic inflammatory disease, infertility and urogenital infections. Several methods exist for BV screening, among them Gram staining, Amsel's criteria, Papanicolaou smear, and Polymerase Chain Reaction. These methods require highly skilled microscopists, are not easily accessible, and they are expensive. The study's' main objective was to compare performance of the Modified Amsel's criteria and Pap smear to the gold standard method, Gram stain, to determine if the former which is simple and readily available is more suitable for bacterial vaginosis screening. A cross-sectional study involving 196 females between 18-55 years old was undertaken at the gynaecology Clinic in Kiambu level-V hospital, Kenya. A Pap smear and two high vaginal swabs were obtained from each participant. The Pap smear was evaluated as per the Bethesda system 2014 and smears positive for BV reported. In modified Amsel's criteria, the vaginal discharge was evaluated for homogeneity, pH, existence of clue cells and a fishy odour. For Gram stain method, smears were made on a slide and Gram-stained. Ten microscopic fields were observed for *lactobacillus* and *Gardnerella* morphotypes and rounded Gram rods. The bacteria were counted, scored and summed to get a full score within the range of 0-10. Purposive sampling method was used to recruit participants. The inclusion criteria was females between 18-55 years old who signed consent to be involved in the study. Exclusion criteria was pregnancy, menstruation and failure to give consent. Nominal and categorical data of the two methods was compared using Chi-square test. Statistical significance was determined by a p-value less than 0.05 at 95 percent Confidence Interval. Cohen's Kappa statistics was used to determine the level of agreement of the two methods. Out of the 196 participants 46 (23.5%) were positive for BV by the Gram's standard method, 60 (30.6%) by Modified Amsel's criteria and 18 (9.2%) by Pap smear method. Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value of Pap smear was 32.6%, 98.0%, 83.3% and 82.5% respectively, while that of modified Amsel's criteria was 82.6%, 85.3%, 63.3% and 94.1% respectively. There was slight agreement between Pap smear and modified Amsel's criteria k=0.195 and p=0.063. Out of the analysed risk factors for Bacterial vaginosis, only age had a statistical association with BV (p=0.03). Prevalence of BV was 23.5%. Modified Amsel's criteria was more accurate in screening for BV than Pap smear. These findings will inform policy makers on the most reliable method for BV screening in the absence of the gold standard.

CHAPTER ONE: INTRODUCTION

1.1 Background of the Study

Bacterial vaginosis (BV) is the commonest vaginal infection experienced by women all over the world. It is frequently detected among women in sexually transmitted diseases clinics, out-patient departments and reproductive health clinics, with about 5-70 % of women affected (Javed *et al*., 2019). It occurs when there is replacement of the beneficial lactobacillus that usually regulate the vaginal pH, making it acidic by production of hydrogen peroxide that is vital in preventing anaerobic microbes in the vagina from over-growing (Russo *et al*., 2019). Absence of lactobacillus causes pH increase within the vagina. This leads to increased population of non-pathogenic bacteria such as *Atopobium vaginae*, *Gardnerella vaginalis*, *Mycoplasma hominis* and *Ureaplasma urealyticum*, leading to a foul-smelling vaginal discharge. (Saraf *et al*., 2021).

Burden of BV is greater in Sub-Saharan region at 38% prevalence according to Jespers *et al*. (2014) and at 55 % as found by Woodman, (2016). The prevalence in Kenya varies among various studies in diverse areas of the country. For example, Mutuku *et a*l. (2021), in their study amongst H.I.V infected women in Machakos County had a prevalence rate of 10.3%. Another study by Musyoki *et al. (*2015) amongst female commercial sex workers in Nairobi had prevalence of 15.1 % while Nzomo *et al. (*2013) found 43 % in a study carried out in Thika. Regardless of the variation in prevalence rates across the country, BV remains a public health concern. Several factors are linked to its occurrence, such as Intrauterine Coil Device use, having several sex partners, new sexual partner, intra-vaginal cleaning and unprotected sex. The symptoms of BV include; itchy vulva,

sore and painful vagina, a discharge that is thin, white, grey or greenish in colour with a 'fishy' odour (Coughling & Secor, 2013). However, many women with bacterial vaginosis experience no symptoms, which further complicates the diagnosis and management of cases (Coughling & Secor, 2010).

Centre for Disease Control (CDC) recommends that clinical BV screening should be done through the Amsel's criteria. This involves checking the pH of the vaginal discharge which is usually more than 4.5 in the presence of BV, examining a wet preparation for clue cells and noticing an amine-like smell after addition of 10% potassium hydroxide to the vaginal discharge. The recommended gold standard method for detecting BV is through staining vaginal fluid by the Gram's method and determining the population of *Lactobacillus, Gadnerella vaginalis, bacteroides* and *mobilincu*s species which are linked to BV (Workowski *et al*., 2021).

The modified Amsel's criteria relies on two features only to screen for bacterial vaginosis, with a couple of studies proving this to be sensitive, less cumbersome and relatively fast (Verstraelen *et al*., 2009). Occurrence of clue cells on a wet preparation of the vaginal discharge and pH more than 4.5 have been shown to have a good match with Nugent score as found in studies by (Bhujel *et al*., 2021; Mengistie *et al*.,2014). This study considered at least two factors in the modified Amsel's criteria and compared its results to that of Gram stain and Nugent scoring. Screening of BV still remains complicated due to the fact that it is attributed to host, social, epidemiologic and biological influences (Muzny & Schwebke, 2016; Muzny *et al*., 2020).

1.2 Statement of the Problem

Bacterial vaginosis is the main cause of abnormal vaginal discharge with foul smell occurring in a majority of women of reproductive age, with almost 50% having no symptoms. Globally its prevalence ranges between 5-70% (Javed *et al*., 2019). In Sub-Saharan Africa its prevalence is 38% according to Jespers *et al*., 2014 and 55% as found by Woodman, (2016). In Kenya the prevalence of BV ranges between 10-43% as found by various studies. Its burden is experienced more at gynaecological clinics, sexually transmitted disease clinics and out-patient units. Bacterial vaginosis was initially assumed not to have long-standing clinical implications, but is now proving to be a public health concern often implicated in adverse reproductive health outcomes such as premature membrane rupture, low birth weight, pelvic inflammatory disease, urogenital infections and infertility in women (Joshi *et a*l., 2020). Bacterial vaginosis causes low self-esteem, stress and discomfort among females and reduces their work productivity. It also drains their resources due to the frequent need for treatment particularly with recurrent BV.

Amsel‘s criteria and conventional Pap smear are some techniques used to detect bacterial vaginosis. Gram staining vaginal smears is the gold standard method used to diagnose BV. These methods are however tedious to perform and the Nugent system require a highly skilled microscopist to accomplish. Pap smear on the other hand is of limited access in most healthcare set ups in the developing countries, require technical skills that are rare, very expensive and it takes long to get results. Polymerase Chain Reaction (PCR) is a molecular method that has high sensitivity and specificity in BV diagnosis and is able to categorise the bacteria associated with BV (Rodelinghuys *et al*., 2017) However, it is very costly and absent in most healthcare facilities in Kenya. There is need for a cheaper,

simpler, readily available and fast method of detecting BV so as to facilitate rapid patient diagnosis and treatment.

1.3 Justification

Several studies have been conducted in Kenya and beyond to validate Pap smear in screening for BV. However, Pap smear is not available in most healthcare set ups in the country, require technical skills, and is very expensive and it takes long to get results. Amsel‘s clinical criteria detects BV by screening for vaginal discharge that is homogenous and has a pH that is more than 4.5, clue cells on direct saline preparation and amine odour after adding 10% Potassium hydroxide (KOH) to the discharge. Three out of the four criteria confirm BV. On the other hand, the modified Amsel‘s criteria checks for presence of only two out of the four criteria to confirm BV. It is thus simpler, cost-effective and does not require high level of skills to perform. It was therefore necessary to compare efficiency of the modified Amsel‘s criteria and Pap smear to the gold standard (Gram stain). This would provide a fast, cheaper and accessible screening method, thus preventing BV complications. There are no existing studies conducted at Kiambu level-5 hospital to determine the prevalence and risk factors for BV. It was thus necessary to conduct this study so as to know the prevalence and risk factors of BV in this population.

The main objective of this study was to compare the modified Amsel‘s and Pap smear methods to the gold standard, Gram staining and Nugent scoring to find out which one is a more efficient alternative in detecting bacterial vaginosis in health care set-ups. The findings will offer factual evidence to the scientific community about modified Amsel‘s

criteria‘s performance in screening for BV. It will also give insights for further research and inform policy makers on the need for cheaper and accessible screening methods for Bacterial vaginosis screening.

1.4 Research Questions

1. What is the accuracy of Pap smear in screening for Bacterial vaginosis?
2. What is the accuracy of modified Amsel‘s criteria in screening for Bacterial vaginosis?
3. What is the prevalence and risk factors for BV among reproductive age group women attending gynaecology clinic at Kiambu level-V hospital?

1.5 Study Objectives

1.5.1 General Objective

To compare Pap smear and modified Amsel‘s criteria in screening for bacterial vaginosis among women at Kiambu level-V hospital, Kiambu county, Kenya.

1.5.2 Specific Objectives

1. To determine the accuracy of Pap smear in screening for Bacterial vaginosis.
2. To determine the accuracy of modified Amsel‘s criteria in screening for Bacterial vaginosis.
3. To determine the prevalence and risk factors for BV among reproductive age group women attending gynaecology clinic at Kiambu level-V hospital.

1.6 Significance of the Study

The study will assist policy makers to better understand the performance of modified Amsel's criteria in screening for bacterial vaginosis and possibly consider it as a screening method in resource limited settings where Gram stain and microscopy is not attainable. This will allow for detection of BV among women in such areas and thereby enable treatment and improvement of the reproductive health of the women.

1.7 Limitations and Delimitations of the Study

1.7.1 Limitations of the Study

The participants were only recruited from one hospital, since logistics and time resources could not allow involvement of neighbouring hospitals within Kiambu County. This may not represent the magnitude of bacterial vaginosis in that population. On the other hand, only women visiting the gynaecology clinic were sampled and this could affect the generalization of the research findings to include women who were treated in other clinics or setups other than the gynaecology clinic

1.7.2 Delimitations of the Study

There were several delimitations to the study such as the time set by the researcher to begin the study and end the study, this was set to cover the months of April, May and June 2023. It also included restriction of the study to Kiambu level V hospital in Kiambu County only. The target population of females aged 18 to 55 years old locked out females not in that age range and may have been willing to take part in the study.

1.8 Conceptual Framework

The vaginal smears were subjected to two staining methods, Conventional Papanicolaou, and Gram stain to allow microscopic examination for clue cells. Presence of >20% clue cells indicated positive bacterial vaginosis test and absence of clue cells indicated a negative bacterial vaginosis test.

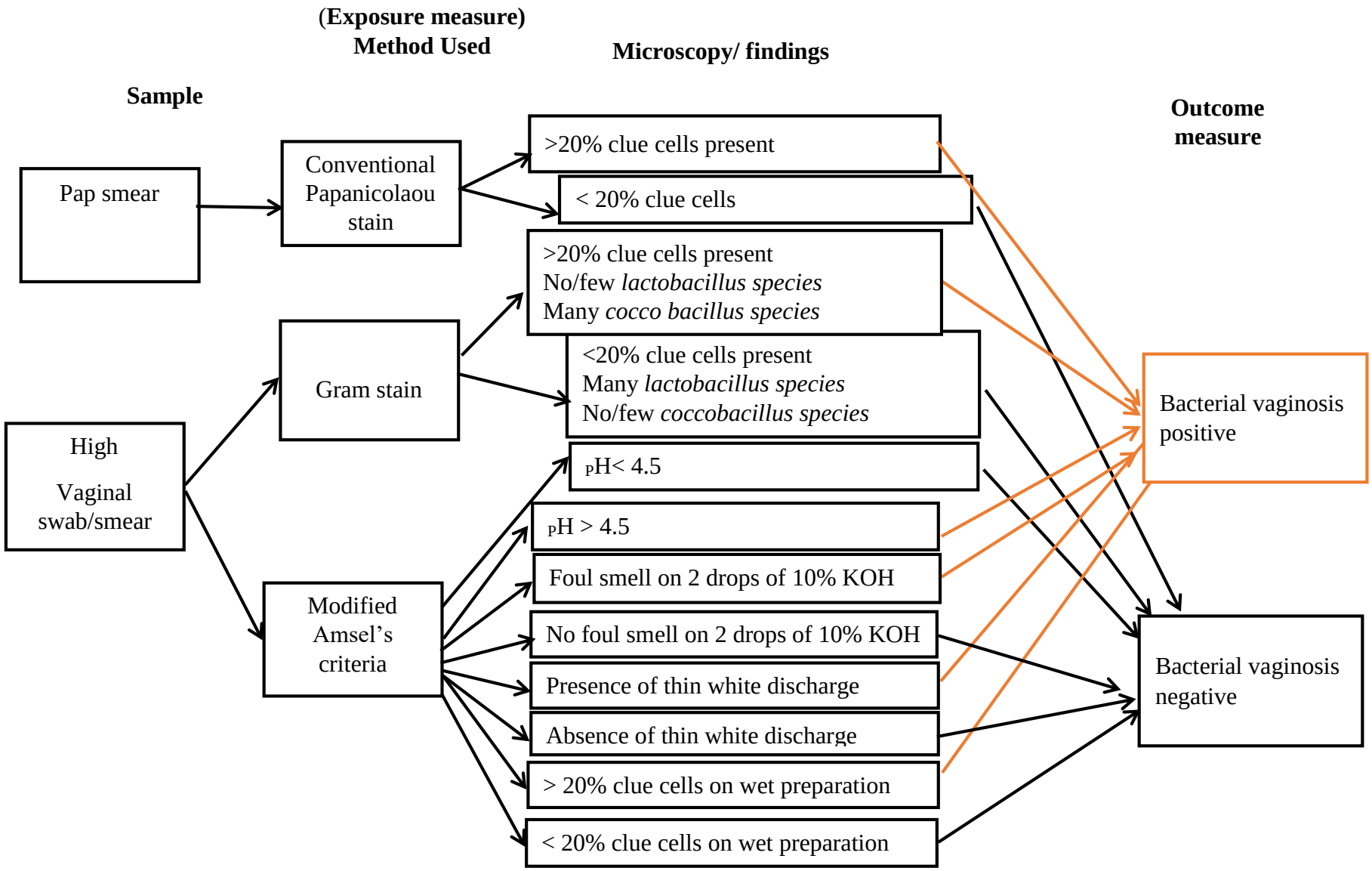


Figure 1.1: Conceptual Framework showing BV screening plan for the HVS and Pap smear (Mengistie et al., 2013)

CHAPTR TWO: LITERATURE REVIEW

2.1 History and Aetiology of Bacterial Vaginosis

In the year 1955 bacterial vaginosis got described primarily by Gardner and Dukes, and they named the causative agent as Hemophilus vaginalis (Gardner & Dukes, 1955). It was also referred to as 'non-specific vaginitis' (Amsel *et al.*, 1983), and later named *G. vaginalis* (Mendling, 2016). Microbiota in the vagina is dynamic and complex and consist of numerous *lactobacilli* species which change in the course of a woman's life, dependent on stage of life, environment, estrogen hormone level, sexual habits as well as other factors (Bilardi, *et al*., 2016).

Alteration of the vaginal microflora population and reduction of lactobacillus leads to overgrowth of obligate and facultative anaerobes which include *Gardnerella vaginalis*, *Atopobium vaginae, Ureaplasma urealyticum*, *Mycoplasma hominis*, *Prevotella Peptoniphilus*, *Megasphaera*, and *Mobilincus* species as well as other uncultured bacteria (Marrazzo & Hillier, 2013; Mardh, 1993). The factors which trigger this overgrowth of anaerobes remain unknown, but seem to be linked to an alkaline vaginal environment following loss of the protective benefits of lactobacilli (Kumar *et al.*, 2011).

2.2 Vaginal fluid, Microbiota and its Importance

The normal vaginal discharge is often white or clear and does not have a bad smell. Its evenness varies from stretchy, sticky and thick (Rao & Mahmood, 2020). On the other hand, the normal volume ranges from 1-3 ml in a day (Eschenbach *et al*., 2000).

Women have variations of what they consider as a normal vaginal discharge (Kasarz & Anderson, 2003). At times they may experience increased amount of discharge as a symptom and other times a 'normal' situation. In spite of that, more understanding and indicators of normalcy are required (Anderson *et al.,* 2004; Chaturvedi *et al.,* 1993).

Fluids within the vagina comprise those which are autogenous to the vagina, from the upper part of the genital tract, the cervix and those not generated by the woman (Owen & Katz, 1999). Subsequently, the vaginal fluid pH of a woman is determined by the combination of factors from the vagina, cervix and seminal fluid if she has recently had unprotected intercourse. Lubrication of the vagina is determined by the quality and amount of transudate from the main circulation. Its amount depends on pressure from the blood vessels and the epithelium. The interstitial fluid spreads and covers the whole vagina after getting into the vaginal cavity (Dabinskaya *et al.*, 2021).

The vaginal fluid contains lactic acid which is the end product of carbohydrate metabolism by *lactobacilli* (Haya *et al*., 2014). The lactic acid makes the vagina acidic and the presence of lactic acid odor denotes a normal and healthy vagina, but then lack of the odor does not mean lack of normalcy. In future it will be possible to differentiate vaginal fluid as normal or not normal based on the over one thousand proteins present in it (Kim *et al.,* 2021).

2.3 Vaginal Fluid Components

The substances which make up the normal vaginal fluid include host cells, microorganisms, mucus and soluble components.

2.3.1 Host Cells

This comprises leucocytes and epithelial cells. The vagina is lined by abundant coatings of stratified squamous epithelium. These are shed frequently inside the vaginal lumen (Thurman *et al*., 2015). Normal vaginal fluid mainly consists of the superficial layer of the vagina and the epithelium of the ectocervix. One layer of cells is lost every four hours, with the rate of loss being dependent on sexual intercourse, hormone state of the host and use of vaginal products (Thurman *et al*., 2015). Epithelial cell desquamation act as a main glycogen source which is a substrate to the vaginal lactobacilli (Thurman *et al.*, 2015).

A healthy vagina often has leucocytes, with the T Lymphocytes being the dominating granulocytes. Macrophages and B Lymphocytes have also been detected in smaller numbers (Givan *et al*., 1997). Natural Killer cells within the vagina mimic those in the blood circulation as opposed to those seen in upper part of the genital tract. They have a role in fighting infections from viruses (Monin *et al*., 2020). Cervical ectropion, a normal condition in which glandular cells from the endocervix are seen in the squamocolumnar junction of the ectocervix, may cause a discharge with leucocytes (Mancuso & Ryan 2015).

2.3.2 Mucus and Soluble Components

These are mainly secretions from glandular cells of the upper reproductive tract and cervix. They also include desquamated epithelial cells from the vagina, metabolites and microbes, in addition to several products released from the systemic circulation into the vagina. The surface of the vagina is coated with cervical mucus, thus forming a protective

barrier. The mucus has 2-5% mucin glycoproteins and 1% of antibodies, peptides and antibacterial protein (Moncla *et al*., 2016).

Mucins are also secreted which form a viscoelastic gel. Carbohydrates form more than 80% of the weight of mucin and the mucin mainly contains acetyl-glucosamine, galactose, sialic acid, fucose and N-acetyl-galactosamine (Moncla *et al.*, 2016).

Progesterone and oestrogens have an influence in pH of the vagina, its protein content and viscosity (Chappell *et al*., 2014). Glycogen released from the cells that are shed is broken down by vaginal amylase to tri-saccharides, disaccharides and mono-saccharides which are a main source of nutrients for lactobacilli metabolism (Nasioudis *et al*., 2015). The vaginal amylase is formed by the host as well as numerous bacteria such as *Lactobacillus crispatus*, *Lactobacillus iners*, *Bifidobacterium lacrimalis*, and *B. vaginale* (Nunn *et al*., 2020; Spear *et al.,* 2014). Epithelial cells of the vagina form part of the inborn immune system. They release antibacterial compounds and cytokines which trigger antigen specific immunity (Linhares *et al.,* 2019). Concentration of compounds and immune active cells found within a vagina differs depending on composition of microbiota within the vagina, levels being lower with dominance of *Lactobacillus crispatus* (Dabee *et al.,* 2019).

2.3.3 Microorganisms

Bacteria, fungi, viruses, protozoa and archaea, inhabit the vagina. Lactobacilli being the main bacteria. They breakdown macronutrients and release metabolites, produce lactic acid which controls pH, release bacteriocins and regulate immunity locally in a

phenomenon that has been described by Verstraelen *et al*. (2022) & Kovachev, (2018). Non-bacilli bacteria are also within the vagina and their prevalence vary with the stages of life. In most women, one among the four Lactobacilli species is dominant in the vagina. They include *Lactobacillus crispatus*, *Lactobacillus gasseri, Lactobacillus jensenii* and *Lactobacillus iners*. It remains unknown why only one of the Lactobacillus species predominates in a specific woman. Ravel *et al.* (2011) classified vaginal microbiota into five Community State Types (CSTs). Among the five, four are mainly lactobacilli i.e. CST 1, 11, 111 and V which are predominated respectively by *Lactobacillus crispatus*, *Lactobacillus gasseri, Lactobacillus iners* and *Lactobacillus jensenii*. CST-IV has highly diverse bacteria and is scientifically thought to be a "risky" community state (McKinnon *et al*., 2019; Verstraelen *et al*., 2022). Historically, dominance of *L. crispatus, L. gasseri* and *L. jensenii* has been connected with healthy vagina in women. However, in bacterial diversity, characteristic of *L. iners* vaginal dysbiosis often occurs (Han *et al*., 2021; Petrova *et al*., 2017).

Lactobacillus iners is highly resistant to hydrogen peroxide and is capable of tolerating fluctuations that occur due to changes in vaginal pH, menstruation, mucus concentrations, hormones and infections. It also contributes to development of BV by secreting inerolysin enzyme (Macklaim *et al.*, 2011; Petrova *et al*., 2017). The prevalence of non-lactobacillus species varies depending on the life stages, and ethnicity. The *Sneathia* and *Prevotella* species may be found commonly in new-borns, while the *Gardnerella species* and *Bifidobacterium species* may often be seen in post-menopausal women (Gliniewicz *et al*.,

2019; Dominguez *et al*., 2010). *Leptotrichia amnionii* and *Atopobium vaginae* are more prevalent in Americans of African origin (Srinivasan *et al.,* 2012).

Viruses too have been isolated in vaginas of women who are healthy. They are mainly eukaryotic viruses and bacteriophages (Madere &Monaco, 2022; Happel *et al*., 2021). The most commonly encountered bacteriophages are from the order *Caudovirales* and families *Podoviridae, Siphoviridae* and *myoviridae* (Madere & Monaco, 2022; Jacobsen *et al.,* 2020). Other phage families are *Herelleviridae, Ackermannviridae, Microviridae, Filoviridae, Tectiviridae, Lipothrixviridae* and *Plasmaviridae.* They play a vital role in inflammation of the vaginal mucosa by activating type-1inflammatory interferon response (Madere & Monaco, 2022). Candida species frequently occur in the vagina of healthy women. The most common is *Candida albicans* (Sobel, 2016). Components of the immune system present in the vaginas of healthy women avert change of Candida albicans from a non-pathogenic yeast form to the invasive hyphae form and reduce capability to reproduce (Verma *et al*., 2017). The vaginal flora of healthy women can change during each menstrual cycle or be stable, though menstruation often changes this microbial composition intensely (Krog *et al*., 2022; Chaban *et al*., 2014; Gajer *et al*., 2012).

The first person to describe the normal vaginal flora composition was the German gynaecologist Albert Doderlein in 1892. Doderlein stated that Gram positive bacilli formed part of healthy women's vagina. He referred to them as Doderlein's bacilli but afterward they were recognized as the same group with the genus *Lactobacilli* by Beijerink in 1901 (Lepargneur & Rousseau, 2005).

70-90% of bacteria that inhabit the vagina of healthy women at pre-menopause in normal circumstances comprise lactobacilli (Africa *et al.,* 2014). Nevertheless, several other bacteria such as *Bacteroides, Corynebacterium, Peptococcus, Pepto streptococcus* and *Streptococcus* exist in lesser numbers in healthy vaginas (Kumar, *et al.,* 2011). This vaginal flora offers the most vital protection means for sustaining a healthy vaginal setting and reproductive function. When the population of normal flora in the vagina is stable, increase of commensals and colonization by pathogens is controlled, thus averting infections (Donders *et al.,* 2017).

The commensals release substances that act as antimicrobials including hydrogen peroxide that maintains the balance, after they have created a monolayer on the mucosa of the vagina (Sgibnev & Kremleva, 2015). The commensals also release lactic acid that is important in maintaining low pH of the vagina, ranging from 3.5-4.5 (Tachedjian *et al.,* 2017). Also produced are antibacterial substances called bacteriocins (Stoyanchera *et al.,* 2014) as well as the enzyme arginine deaminase which breaks down arginine to ammonia and citrulline denying the anaerobes the amino acids they require for development (Makarova *et al.,* 2006).

Cervical mucus which largely comprises of mucin, plays a vital function alongside that of the lactobacillus by protecting the vaginal mucosa, optimizing and acting as a barricade against colonization by microbes. Also contained in the cervical mucus are numerous proteins with bactericidal potency that are not dependent on the availability of antibodies,

such as calprotectin, cathelicidin LL-37, lactoferrin and lysozymes (Nasioudis *et al.,* 2017).

2.4 Risks and Prevalence of Bacterial Vaginosis

Bacterial vaginosis may become apparent at any age though it is mostly common in the ages between 15-44 years old. Its prevalence differs significantly through the worlds' geographical regions, among similar populations and nations subject to the cultural background and socioeconomic state. Globally, the prevalence of Bacterial vaginosis ranges from 4%-75% based on category of people who are studied (Bitew *et al.,* 2017; Onderdonk *et al*., 2016).

For instance, its prevalence is 29 % in USA, lower in Norway and Poland with a prevalence of 20% (Kenyon *et al*., 2013). In the African continent prevalence rates are lower in West Africa at 6-8% and 14.2% in Burkina Faso and Nigeria respectively. In the East and Southern Africa, the rates are higher with 38% in Botswana, 37% in Kenya, 68.3% in Mozambique and 32.5% in Zimbabwe (Afolabi *et a*l., 2016; Bitew *et al*., 2017).

It is hard to categorize bacterial vaginosis as STI because it lacks a documented causative agent (Watson & Reid, 2018). Though BV mechanically and chemically has some features of a sexually transmitted disease (STD) by interaction with seminal fluid which has high alkalinity (Lewis *et al.,* 2017).

Even though it is believed that bacterial vaginosis cannot arise in women who have never had intercourse, sexual activity is however still considered a big risk for BV, (Fethers *et al.,* 2009). Bacterial vaginosis has nevertheless been identified in young females who

have not had sex before albeit at a lower frequency compared to the sexually active females (Cherpes *et al.*, 2008). Some meta-analyses and systematic review studies have come to the deduction that frequency of BV is related in a direct way to the sexual partners that females have, both numerous and new (Forcey *et al*., 2015). Thus, implying that it can be considerably reduced through practicing safe and protected sex (Gallo *et al.*, 2011). Condom use by the male partner has been shown to reduce acquisition of BV and its recurrence (Verstraelen, *et a*l., 2010). Risk of BV occurrence is shown to reduce through male circumcision since the prepuce of some men may harbour BV associated bacteria (Margolis & Fredricks, 2015).

In women having sex with women, a symptomatic female partner, and usage as well as sharing sex toys that are not washed are risks attributed to BV (Cherpes, *et al*., 2008). Poor genital hygiene is also a risk for BV acquisition as it promotes alteration of equilibrium of microbiota within the vagina. A single study established that women who failed to clean the area around their vagina were highly prone to bacterial vaginosis than women who cleaned this area often, as were women who failed to regularly change their undergarments, as opposed to the ones who changed them often (Bitew *et al.*, 2017).

Intra-vaginal douching and washing on the other hand increases susceptibility to BV according to Aslan & Bechelaghem, (2018); Alcaide *et al.* (2016) Cigarette smoking, Nelson *et al*. (2018) IUCD use, Achilles, *et al.* (2018); Seth *et al.* (2017) stress, Marrazzo & Hillier, (2013) similarly increase BV infection. Being of African origin Akinajo *et al*. (2017); Madden *et al.* (2012), and also education levels Holzman *et al.* (2001) are risk factors for BV.

2.5 Pathogenicity of Bacterial Vaginosis

Bacterial vaginosis occurs due to disproportion of the naturally present normal flora within the vagina, leading to a decline of the *lactobacilli* as well as proliferation in the entire population of microbes existing in the vagina (Russo *et al.,* 2019). Decline in *lactobacilli* leads to increased pH of the vagina. This cause increased growth of anaerobes which then yield large proportions of the enzymes known as proteolytic carboxylases (Zetian *et al.,* 2015). Peptides in the vagina are broken down by these enzymes into several amines (Nelson *et al.,* 2015) that resemble those formed by microbes which lead to fish spoilage. (Yeoman *et al.,* 2013). The amines being unstable, cause production of more vaginal fluid and cause disintegration of squamous epithelial cells leading to symptoms of bacterial vaginosis (Biswal *et al.,* 2014; Dasari *et al.,* 2014).

Majority of bacterial vaginosis cases are believed to begin with the anaerobe *G. vaginalis,* causing proteolysis thus promoting the growth of *P. vibia. Prevotella. vibia* produces ammonia which influences proliferation of *Gardnerrela vaginalis*, leading to formation of biofilm in the epithelial mucosal surfaces of the vagina (Castro *et al.*, 2019; Gilbert *et al.,* 2019). The biofilm offers a favourable environment which promotes the growth of other commensal anaerobic microbes to develop in the vagina (Verstraelen *et al.,* 2018). This biofilm contains plentiful *Gardnerella vaginalis*, less *Atopobium vaginae*, *lactobacilli* and additional species of microbes. (Swidsinki *et al*., 2005). The BV biofilm can tolerate hydrogen peroxide and lactic acid better and permits microbes in it to resist antibacterial substances. This promotes biofilm persistence and hence the recurrent and chronic nature of BV (Soto, 2014; Stewart & Costeron, 2001).

Growing proof proposes that *Gardnerella vaginalis* is the main microbe in the development of bacterial vaginosis (Janulaitiene *et al.,* 2017; Younus *et al.,* 2017). Bacterial vaginosis is connected to several obstetric complications and contrary results during gestation. Various studies have associated BV to amplified danger of preterm delivery (Juliana *et al.,* 2020; Menard *et al.,* 2010), miscarriage (Leitich & Kiss, 2007), pelvic inflammatory disease (Ravel *et al.,* 2021; Elkafas *et al.,* 2022), Infertility Hong *et al*., (2020); Van *et al.,* (2013); Ravel *et al.,* (2021), urogenital infections and amplified risk of acquiring sexually transmitted diseases (Marrazzo & Hillier, 2013). BV increases the risk for type 2 Herpes simplex virus (HSV-2) Human papilloma virus (HPV), Human Immunodeficiency Virus (HIV), chlamydia trachomatis, gonorrhoea and *Trichomonas vaginalis* infections. Females whose Gram stain smears have been examined and scored by Nugent principle and have scores that range from 9-10 have a heightened risk while scores between 4 and 8 present reasonable danger of bacterial sexually transmitted infection (Allsworth & Peippert, 2011).

2.6 Differential Diagnosis

Presence of a fishy odour confirms BV but when absent, other infections and conditions such as Trichomoniasis, inflammatory vaginitis and atrophic vaginitis could be the problem. Bacterial vaginosis is however distinguished by lack of dyspareunia and inflammatory signs such as redness in the vulva among affected females. Another distinguishing characteristic is the non-increase in white blood cell and parabasal cell population as in the case of most bacterial infections (Krauss-Silva *et al*., 2014)

2.7 Screening and Diagnosis of Bacterial Vaginosis

2.7.1 Screening of Bacterial Vaginosis

2.7.1.1 Amsel's Clinical Criteria

This was established by Amsel *et al.,* (1983). In this technique, the existence of bacterial vaginosis is showed by three of four clinical characteristics, Malaguti *et al.* (2015). These include; vaginal discharge which is non-thick, consistent, whitish, and covers the vaginal walls evenly, Prasad *et al.,* (2016) unlike the usual vaginal discharge which is usually floccule (Money, 2005). Secondly, pH of vaginal fluid more than 4.5 when determined using a pH paper (Hoffman *et al.,* 2017).

Third, the occurrence of a fishy smell when 10% KOH is mixed with the discharge. Money. (2005) and finally the existence of clue cells when one drop normal saline is mixed with the discharge on a microscope slide, cover-slipped and microscopically examined with X40 objective. A positive result is designated by clue cells covering over 20% of total epithelial cell population in the sample (Frobenius & Bogdan, 2015). Various studies have been done to establish the accuracy of Amsel's criteria in screening for bacterial vaginosis. Mengistie *et al*. (2014) in their study, attained sensitivity of 35% and specificity of 99%. Another study by Bansal *et al*. (2019) had 75% sensitivity and 95% specificity. A higher sensitivity of 80.4% and a specificity of 94.2 % was documented by Adeniran *et al*., (2021).

2.7.1.2 The Modified Amsel's Criteria

The modified Amsel's criteria relies on two features only to screen for bacterial vaginosis, with a couple of studies proving this to be sensitive, less cumbersome and relatively fast (Verstraelen *et al*., 2009). Occurrence of clue cells on a wet preparation of the vaginal discharge and pH more than 4.5 have been shown to have a good match with Nugent score as found in studies by (Bhujel *et al*., 2021; Mengistie *et al*.,2014).

2.7.2 Diagnosis of Bacterial Vaginosis

2.7.2.1 Conventional Pap smear

Papanicolaou test is a cytological screening technique that was invented in 1941 by George Nicholas Papanicolaou (Ciardullo, 2017). It is used as the standard method of assessing cytomorphology of the cervix to detect cervical abnormalities such as cancers and pre-cancerous lesions (Narayankhedkar *et al.,* 2015).

The conventional Pap smear technique has gone through several changes aimed at reducing the time schedule, quantity and changes of ethanol used in the staining process so as to make it cheaper (Gachie *et al.,* 2011). Smears stained by Papanicolaou technique have widely been employed to screen for bacterial vaginosis. The Bethesda guidelines accommodate reporting BV as shift in vaginal flora indicative of Bacterial vaginosis (Bombase *et al*., 2014). This is when clue cells are seen covering discrete squamous epithelial cells and when there are small coccobacilli appearing in a filmy background as well as notable depletion of *Lactobacillus species* (Narayankhedkar *et al.,* 2015).

Numerous studies have reported clue cells to suggest BV (Sabu *et al*., 2017) whereby clue cells > 20% is the threshold (Discacciati *et al*., 2006; Sachdeva, 2006). On the other hand, others have considered the occurrence of coccobacilli only (Prey, 1999). Studies show that the Pap technique is reasonably sensitive and highly specific in comparison to microbiological tests, and positive results have a diagnostic importance especially when it attains 95% as its mean specificity in comparison to the standard method (Filho *et al*., 2010). A study conducted in Kenya by Karani *et al.,* (2007) had a sensitivity and specificity of 59.4% and 83.3% respectively while another in India had 70.9% sensitivity and 56.8% specificity in screening for BV (Anand *et al.,* 2020).

2.7.2.2 Gram Staining/Scoring According to Nugent

The Amsel‘s principles provide a readily available and non-complicated clinical approach when compared to the Nugent score; however, the latter is more favoured for the screening of BV, owing to its high reproducibility and sensitivity (Nugent *et al.,* 1991).

The technique depends on evaluation of Gram-stained vaginal discharge, and was first outlined by Spiegel *et al*., in 1983 and subsequently adjusted by Nugent *et al.,* in 1991. In these two methods, Gram-stained smears are scored by enumerating the different bacterial morphological types in the vagina. A minimum of ten microscopic fields are evaluated for the presence of; *Lactobacillus*, large Gram-positive rods, *Gardnerella vaginalis* which are small, Gram-variable bacilli.

Bacteroides species which are Gram-negative small rods and *Mobilincus* species, Gram-variable curved rods. Also noted in the smear is the presence of Gram-negative cocci and large, Gram-negative bacilli (Mahajan *et al*., 2017). Once identified, these morphotypes

are classified based on any of the following standard methods of Gram-stain interpretation systems;

2.7.2.2.1 Spiegel Classification System for Gram-Stained Smears

The identified Gardnerella and lactobacillus morphological types detected and counted in the Gram-stained smear, are scored as 1+, 2+, 3+ and 4+. Positive one to two is represented by presence of lactobacillus species and more prominent Gardnerella morphotypes. This represents a positive Bacterial vaginosis diagnosis. A negative BV diagnosis is made when only lactobacillus morphological types are detected in the smears from the vagina (Nugent *et al.,* 1991).

2.7.2.2.2 Nugent System of Classifying Bacteria in Gram-Stained Smears of Women at Kiambu Level V Hospital

This acts as the gold standard for classifying bacteria in Gram-stained smears whereby; More than 30 *lactobacilli* per high power film scores 0 points and its absence per high power film earns 4 points. When 30 small bacteria are seen per high power film, this garners 4 points and their absence 0 points. Curved rods if present per high power film earns extra 1 or 2 points (Table 2.1).

Table 2.1: Nugent Scores of Bacterial Vaginosis Associated Bacteria in Gram-stained Vaginal Smear

Lactobacillus species	**Gardnerella species**	**Curved rods**	**Score**
>30	0	0	0
5-30	<1	1-5	1
1-4	1-4	>5	2
<1	5-30		3
0	>30		4

Nugent scores interpretation (Nugent *et al*., 1991)

0-3 = Bacterial Vaginosis (Negative)

4-6 = Intermediate

7-10 = Bacterial Vaginosis (Positive)

2.7.2.2.3 Ison and Hay Classification of bacteria in Gram-stained vaginal smear

Assessment and scoring of smears by the Nugent technique is however very inclined and requires a highly skilled expert microscopist and lots of time to accomplish. Consequently, a simpler description was developed by Ison and Hay in the year 2002 (Antonucci *et al.*, 2017; Ison & Hay 2002). It comprises separating the vaginal microflora in three classes as; Normal, Intermediate, and Bacterial vaginosis, based on the amount of lactobacillus and Gardnerella morphological types (Table 2.2).

Table 2.2: Ison/Hay Classification of Vaginal Microflora in Women at Kiambu Level V Hospital

	Lactobacilli Morphotypes	**Gardnerella morphotypes**
Normal	Numerous	Few
Intermediate	Same number	Same number
Bacterial vaginosis	Few	Numerous

Source: Chawla *et al*., 2013

2.7.2.3 Culture of Vaginal Fluid

Culturing of Gardnerella vaginalis in the laboratory is also an efficient technique of diagnosing bacterial vaginosis (Gergova *et al*., 2013). Though its specificity is very low since *G. vaginalis* has often been found in about 50% to 60% of healthy females with no symptoms of bacterial vaginosis (Sha *et al*., 2005). It is not reliable since it does not indicate the true vaginal microbiota in bacterial vaginosis owing to the fact that *Gardnerella vaginalis* is not the only bacteria linked to bacterial vaginosis (Tokyol *et al*., 2004). Furthermore, several other microbes linked to bacterial vaginosis are obligate anaerobes which are difficult to isolate by normal culture methods (Ravel *et al*., 2011). The use of culture in BV diagnosis leads to overdiagnosis making it unsuitable in determining treatment plans and post-therapy assessment of a patient. Given that BV occurs due to fluctuations in vaginal normal microflora, culturing the discharge is not important in its detection (Balashov *et al.,* 2014).

2.7.2.4 Molecular Techniques for Diagnosis of Bacterial Vaginosis

Diagnosis of BV remains complicated and challenging due to the fact that it has several intrinsic microbiological characteristics and different clinical manifestations making its

detection problematic (Money, 2005). To overcome the challenges, numerous methods have been developed, among them molecular, enzymatic as well as chromatographic methods. The most commonly applied method in diagnosis of bacterial vaginosis is the quantitative Polymerase chain reaction (PCR) technique. It is a molecular method that has high sensitivity and specificity. It is widely employed in measurement of bacteria linked with BV including *A. vaginae, G. vaginalis, Megasphaera* species, Mobilincus species and *leptotrichia species* (Breding *et al.,* 2020). Several studies have projected impartial molecular cut-offs to foresee Bacterial vaginosis from bacterial loads (Redelinghuys *et al.,* 2017).

Among the numerous available commercial molecular analysers that have been assessed for BV detection among females include NuSwab quantitative Polymerase chain reaction (Cartwright *et al.,* 2018), SureSwab bacterial vaginosis DNA quantitative assay, bacterial vaginosis multiplex assay (Hilbert *et al.,* 2016) and BD Max vaginal panel (Gaydos *et al.,* 2017). Quantification through PCR allows the accurate diagnosis of bacterial vaginosis associated bacteria. These methods have a specificity range from 85.8% to 95% and sensitivity range between 90.5 to 96.7% when compared to Gram stain method and Amsel‘s criteria (Coleman & Gaydos, 2018).

These techniques are however, costly and are not meant for point of care diagnosis even if their specificities and sensitivities are higher than for the commonly available tools for BV diagnosis. Dessai and colleagues, nevertheless, documented performance of the first BD Affirm TM VPIII assay as a point of care test during pregnancy care. However, the method is an invaluable tool for screening to detect infections of the vagina among

pregnant females (Dessai *et al.,* 2020). It is thus recommended that cost analysis be considered vital in the production and assessment of new diagnostic tools.

2.7.2.5 Emerging Strategies for Diagnosis of Bacterial Vaginosis

An enzymatic approach has been developed founded on the enzyme sialidase activity as an indicator for bacterial vaginosis. OSOM BV Blue Rapid kit is a chromogenic point of care test manufactured by Genzyme Diagnostics. It qualitatively detects high sialidase enzyme levels generated by anaerobic microbes such as *Gardnerella* and *Bacteroides species* in samples of vaginal discharge. It has proven to be more reliable than Amsel‘s clinical principles and Nugent scoring system. It has sensitivity range between 88% to 94% and specificity between 91 to 98% (Madhivanan *et al.,* 2014; Shujatullah *et al.,* 2010).

A current study conducted by Liu and colleagues demonstrated the possibility of a sialic acid-coated tetraphenylethene luminogen (TPE4S) as a means of detecting bacterial vaginosis based on high-level fluorescence. The study used intensity of light signals to sense and analyse the comparative levels of sialidase enzyme in vaginal fluid samples. It is a single-step test since its reagents are confined to a sample buffer and reagent bead. It is a quantitative method that is highly sensitive at 95.4% with a specificity of 94%, when compared to the Amsel‘s clinical criteria which has sensitivity of 92.5% and a specificity of 91.8%. This method has potential to be utilized as a tool for BV diagnosis, risk valuation of females with bacterial vaginosis according to the levels of sialidase enzyme activity and monitoring therapy by antimicrobial drugs (Liu *et al*., 2018).

2.8 The Burden of Bacterial Vaginosis

Globally, the prevalence of BV ranges from 23% to 29%. Central Asia and Europe have a prevalence of 23%, Latin America and the Carribean 24%, North Africa and Middle East 25%, Sub-Saharan Africa 25%, North America 27% and South Asia 29%. Racially the black and Hispanic women have the highest prevalence at 33% and 31% respectively. It is lower among Asian women, 11% and white women 23% (Peebles, 2019).

Bacterial vaginosis has a high global economic burden to treat its symptoms. In the United States, this burden is estimated to be $ 4.8 and ranges between 3.7-6.1 billion dollars. The Sub-Sahara has lower economic burden of BV than other regions at $ 11.73 per treated case. The economic burden of BV is high as a result of BV related conditions such as Pelvic inflammatory disease, sexually transmitted infections and preterm births, where treatment can cost three times more than treating BV alone. The healthcare costs are also high as a result of the recurrent nature of BV, there is also constant requirement to understand the causes, develop preventive measures and treatment options (Watkins *et al*., 2024).

2.9 Treatment and Management of Bacterial Vaginosis

2.9.1 Antibiotics and Nitroimidazoles

Current treatment goals focus on bringing to an end multiplication of bacterial vaginosis associated bacteria and recovering the normal flora population within the vagina (Marrazzo & Hillier, 2013). The treatments employed are broad spectrum antibiotics with greater action in eliminating anaerobic bacteria and protozoa with nitroimidazoles and clindamycin commonly used, with or without probiotics (Bacterial vaginosis 2015;

Bradshaw & Sobel, 2016). The treatment of choice recommended by WHO is metronidazole 500 mg orally BD for seven days (Bacterial vaginosis 2015; World health organization 2021). Oral 300 mg clindamycin given two times a day for seven days. 100 mg ovules of clindamycin can also be given for five days intravaginally. Metronidazole 0.75% gel applied into the vagina for 5 days or alternatively intravaginal application of 2% clindamycin cream when going to bed for seven days (Donders *et al.,* 2014; WHO, 2021). Nevertheless, local clindamycin can cause damage to latex-based condoms and could also cause inflammation of the colon 'pseudomembranous colitis' (Machando *et al.*, 2016).

Tinidazole, similar to metronidazole is an alternative oral therapy given as 2g once daily for a period of 2 days or 1g daily for a period of 5 days in the event that metronidazole and clindamycin fail to be tolerated by the patient (Bacterial vaginosis, 2015; Dickey *et al.,* 2009). Efficacy of secnidazole has been researched and it displayed same action to the suggested nitroimidazoles and it has the benefit of not altering lactobacilli population. Bacterial vaginosis is also sensitive to rifaximin which acts to restore *lactobacillus* and increase lactic acid in the affected, which is a beneficial factor in BV treatment.

Even though the above medications act effectively in eliminating bacteria which are associated with bacterial vaginosis by relieving the signs, this relief is short-lived and re-infection is seen in most patients following therapy (Mayer *et al.,* 2015) Recurrent BV occurs within 12 months in 58-76% of females treated using metronidazole (Lee *et al.,*2020). The high recurrence rate among patients may be due to failure of medications to get rid of bacteria associated with bacterial vaginosis biofilm within the vagina

(Ahren‘s *et al.*, 2020; Javed *et al.*, 2019; Swidsinki *et al.*, 2008). Females who experience recurrent bacterial vaginosis also suffer from low self-esteem, discomfort, embarrassment and constant worry about BV (Bilardi, *et al.*, 2013).

2.9.2 Probiotic Therapies

Lactobacillus probiotics are gaining popularity as a substitute treatment or complementary therapy to antibiotics. They assist in restoration and maintenance of a well-balanced normal flora of the vagina (Borges *et al.*, 2014; Bradshaw *et al.*, 2012). Several studies have tested the effectiveness of oral and vaginal lactobacilli probiotics among them (Hemalatha *et al*., 2012; Vicariotto *et al.*, 2014). The outcomes indicated that probiotics when used intravaginally or orally were successful in getting rid of BV.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Site of the Study

This research was conducted at the gynaecology clinic of Kiambu level V hospital, which offers services for women's' reproductive health and wellbeing. The study site was chosen because it is a referral hospital that attends to patients from all over Kiambu county and neighbouring counties such as Nairobi and would give a clear reflection of the magnitude of bacterial vaginosis among women in that county.

3.2 Study Design

A descriptive cross-sectional design was employed in the study-whereby data was obtained from the study population at a specific point and time through analysing their vaginal swabs and Pap smears. This was done between April and June 2023.

3.3 Target Population

The study involved women whose ages ranged between 18 years and 55 years, attending gynaecology clinic at the Kiambu level V hospital. This population of interest was determined and selected because it caters for the reproductive health needs of all females who seek such services at the hospital. It is in this clinic where routine Pap smear tests and several other reproductive and sexual health problems, are undertaken, making this clinic suitable for the study.

3.3.1 Study Population

One hundred and ninety-six females seeking services at the gynaecology clinic of Kiambu level V hospital, were sampled.

3.3.2 Inclusion Criteria

Females aged between 18-55 years old attending gynaecology clinic at Kiambu level V hospital who agreed to be involved in the research, and signed a consent form, were enrolled into the study.

3.3.3 Exclusion Criteria

Pregnant and menstruating females as well as those who declined to give a signed consent did not take part in the research.

3.3.4 Calculating Sample Size

Studies conducted in Kenya have yielded BV prevalence rates ranging from 10-50% according to the literature. The sample size calculation was based on research conducted by Musyoki *et a*l., 2015, where bacterial vaginosis prevalence was 15.1%. The Fisher *et al.*, 1998 formula was used to determine sample size whereby:

$$n = \frac{Z^2PQ}{E^2}$$

n= Desired sample size.

Z= CI at 95% (standard value of 1.96)

P= Projected occurrence of bacterial vaginosis (15%).

E= Possible random error range (5%).

Q= 1-P or projected failure proportion.

Therefore $n = \frac{1.96^2 \text{ X } 0.15 (1-0.15)}{0.05^2}$

$= \frac{3.8416 \text{ x } 0.15 \text{ X } 0.85}{0.05 \text{ x} 0.05}$

= 196 participants.

3.4 Method of Sampling

Purposive sampling technique was employed in selecting participants to take part in the study. Patients who presented at the clinic during the period of study and met all the required criteria for inclusion and were willing to take part, were considered in the study till attainment of the desired sample.

3.5 Enrolment of Study Subjects and Collection of Data

Recruitment of research participants was conducted at the gynaecology clinic of Kiambu level V hospital. After obtaining approval of the study from the ethics review committee, the principal investigator (PI) contacted the nursing officer in-charge of the clinic who assigned a trained and registered nurse to assist with the collection of Pap smears and

HVS from the study participants. A refresher training on Pap smear collection was done by the PI before the procedures were conducted.

The PI recruited a total of 196 participants who fulfilled the inclusion criteria for taking part in the study and given a signed consent (appendix 1) willingly. The PI conducted a short education session on bacterial vaginosis that included; the importance of the study to the participants, a description of BV in terms of aetiology, symptoms, risk factors, importance of screening and treatment. The procedures to be conducted were explained to the participants in English and Swahili for them to understand well. A questionnaire (Appendix 11) which included age, marital status, parity, L.M.P, recent use of antibiotic, contraceptive method, presence of a discharge and other demographic data was given to the participants to complete. The assigned nurse gathered all materials needed for the procedures and then obtained Pap smears and HVS swabs from the participants, after briefly explaining the procedure, reassuring them and setting them in the required position for samples to be obtained.

3.6 Collection of Pap Smear Samples and High Vaginal Swabs

With the patient lying on a couch, legs apart, the vaginal wall was opened up using a sterile single use, non-lubricated speculum to allow visualization of the cervix aided by a source of light. A cyto-brush was used to sample the endocervix, ectocervix and the squamocolumnar junction. The obtained material was smeared onto a clean labelled slide and immediately immersed into the fixative solution (95% ethanol). Papanicolaou technique was used to stain the fixed smears after which they were evaluated using the Bethesda system 2014 by the PI and confirmed by a cytologist.

After obtaining the Pap smear, two high vaginal swabs were labelled and used to collect discharge from the vagina of the participant. The nature of the discharge was noted and recorded as positive if characteristic for bacterial vaginosis. One vaginal swab was utilized in the measurement of pH using a pH colour indicator and for conducting whiff test, by addition of 2 drops 10% KOH on a slide and mixing with the discharge after which the presence or absence of a 'fishy' odour was noted. The second swab was stored in ice pack to be used for wet preparation for clue cell identification, Gram staining and Nugent scoring.

3.7 Laboratory Methods

In the laboratory, a direct preparation of normal saline and the discharge was made on a slide then observed under x 40 magnification for clue cells. A smear was made from the discharge by rolling the swab over a drop of normal saline on a slide and leaving to air dry.

3.7.1 Gram Staining Method for the Vaginal Smear

Gram stain is based on the principle that the cell wall of Gram-positive bacteria retains crystal violet and are not decolourized by acetone and therefore appear purple-blue under a microscope. The cell wall of Gram-negative bacteria does not retain crystal violet stain, they are decolourized by acetone and take up the counterstain, which is neutral red and they appear red under a microscope (Tripathi & Sapra, 2023).

The smear was put on a staining rack and heat fixed by passing a burner flame underneath the slide three times. This was followed by flooding the smear with crystal violet stain for I minute, washing with tap water, adding iodine to the smear for 1 minute, washing

with tap water and decolourizing with acetone for 1 minute followed by counterstaining with neutral red for 1 minute. The neutral red was washed up with tap water. The slide was blotted and air dried on a slide rack. The Gram-positive bacteria appeared blue-violet while Gram negative bacteria appeared red. This was followed by examination of the smear for clue cells, *Lactobacillus species* and *coccobacilli* that are Gram-variable. These were enumerated according to Nugent *et al*. (1991)

3.7.2 Papanicolaou Staining Procedure for Cervical Smears

Papanicolaou staining method is composed of both acidic dyes and basic dyes. The acidic dyes stain the basic cellular components while the basic dyes stain the acidic parts of the cell according to Sathawale *et al.* (2022)

The Conventional Papanicolaou staining procedure involved hydrating the smear using descending grades of alcohol starting with 95% alcohol, 80% alcohol, 70% alcohol and then 50% alcohol for 2 minutes in each solution. The smear was then dipped in distilled water for 1 minute. This was followed by dipping the smear in Harris Hematoxyline staining solution for 5 minutes. The smear was dipped in distilled water for 2 minutes to remove excess hematoxyline stain. The smear was put in 0.5% aqueous HCL to decolourize. It was washed in Scott's tap water for 2 minutes followed by distilled water for another 2 minutes. The smear was dipped in 70% alcohol followed by 95% alcohol for 2 minutes in each solution to dehydrate. The smear was dipped in O-G-6 stain for 2 minutes followed by two changes of 95% alcohol. It was stained in EA-50 solution for 3 minutes. This was followed by 10 dips in two changes of 95% alcohol and 10 dips in three changes of absolute alcohol. This was followed by dipping the smear 10 times in

three changes of xylene. The smear was mounted with DPX mountant to make a permanent preparation. The nucleus of the cell stained blue, Keratin orange-red, Eosinophil orange-red, Intermediate and parabasal cells blue-green, Superficial cells pink and basophilic cells blue-green. Bacterial vaginosis positive smears were seen as numerous coccobacilli covering squamous epithelial cells against a filmy background. The bacterial vaginosis positive cases with Papanicolaou smear and with modified Amsel‘s criteria were compared to those positive with the Gram‘s Method as the gold standard for determining performance of the two methods. To avoid reporting bias, the positive results were reviewed by peers, and communicated to the participants.

3.8 Quality Assurance

The Pap smears and HVS were collected by qualified and knowledgeable nurses who conduct the procedures every day.

The Standard Operating Procedure (SOP) for staining with Papanicolaou method and Gram stain were followed. All the staining solutions were prepared according to the SOPs and filtered before use. Old stains were discarded and replaced with new stains. Known positive and negative slides were stained together with the samples to assess the quality of the stains. All the slides were examined by the PI and a cytologist and all the examined slides stored for future reference.

3.9 Data Management and Analysis

3.9.1 Data management

The data and obtained results were entered into excel spreadsheet. The data was imported from Excel into Statistical package for social sciences (SPSS) which analysed the dependent and independent variables. Nominal and categorical variables were also analysed and presented as tables, charts and graphs. The outcomes were expressed in form of percentages. Statistical significance was represented by p-value not exceeding 0.05 at 95% confidence interval. Categorical and nominal data of the two methods was compared using chi-square test and the agreement levels between modified Amsel's criteria and Pap smear methods measured using the Cohen's Kappa test. The sensitivity, specificity, positive predictive value and negative predictive value of modified Amsel's criteria and Pap smear was obtained by the following formulae:

Sensitivity = True positive/ True Positive + False Negative x100

Specificity = True Negative/ True Negative + False Positive x100

Positive Predictive Value = True Positive/ True Positive + False Positive x100

Negative Predictive Value = True Negative/ True Negative +False Negative x100

3.9.2 Data analysis

The data was analysed using S.P.S.S version 25 and a Confidence Interval of 95% was used to determine the p-values. A p-value less than 0.05 was considered statistically significant in the determination of all the three objectives. For the first and second objectives Chi-square test was used to analyse the 2 X 2 contingency tables. Cohen's Kappa statistics was used to measure the level of agreement between Papanicolaou

method and the modified Amsel's criteria method. Linear regression was used for the third objective that focused on determining the relationship between the prevalence of bacterial vaginosis and the risk factors analysed for bacterial vaginosis.

Receiver Operating Characteristic (ROC) curve was used to determine the accuracies of Papanicolaou method and the modified Amsel's criteria in screening for bacterial vaginosis. The ROC curve illustrated the false positive rate and the true positive rate of the two methods. The Area under the Curve (AUC) close to 1 indicated a strong classifier and 0.5 was suggestive of a random guess.

3.10 Ethical Considerations and Handling of Research Participants

Approval for the study was obtained from Kenyatta University Ethics Review Committee (KUERC), approval number PKU/2657/E1781 and authorisation attained from the National Commission for Science Technology & Innovation (NACOSTI). A voluntary permission was obtained from all the willing participants before any procedure was undertaken. All procedures to be done were clearly explained to the study participants. Confidentiality was observed and strictly adhered to throughout the study period and the abnormal findings were only shared with the nurse at the clinic.

The samples were collected by the PI together with qualified and experienced nurses and were only identified by serial numbers rather than names to maintain patient privacy and confidentiality of the results.

CHAPTER FOUR: RESULTS

4.1 Socio-demographic Features of Study Participants

Mean age of the participants was 40 years. Majority were of the age group 46-50 years old while the age 16-20 years had only one participant. Most of the participants were married 112/196 (57.1%) and only 75 (38.3%) were not married. The illiteracy level was very low since only 4/196 (2.0%) had no formal education and 44.9% had primary education whereas only 15.3% had college level education. Majority of the enrolled participants were small scale traders (42.9%), 14.8% were farmers and 12.3% were housewives (Table 4.1).

Table 4.1: Sociodemographic features of the study participants

	Count	Percentage (%)
Age Group (years)		
16-20	**1**	0.5
21-25	12	6.1
26-30	23	11.7
31-35	20	10.2
36-40	38	19.4
41-45	40	20.4
46-50	56	28.6
51-55	6	3.1
Total	**196**	**100%**
Marital Status		
Single	75	38.3
Married	112	57.1
Divorced	3	1.5
Widowed	6	3.1
Total	**196**	**100%**
Education		
None	4	2.0
Primary	88	44.9
Secondary	74	37.8
College	30	15.3
Total	**196**	**100%**
Occupation		
None	7	3.6
Trader	84	42.9
Teacher	7	3.6
Marketing	3	1.5
Farmer	29	14.8
Secretary	2	1.0
Casual	11	5.6
Housewife	24	12.3
Tailor	3	1.5
Student	7	3.6
Hair dresser	3	1.5
Social work	1	0.5
Hospitality	9	4.6
IT	2	1.0
Nanny	3	1.5
Cashier	1	0.5
Total	**196**	**100**

4.2 Accuracy of Pap-smear in the Screening for Bacterial Vaginosis

A total of 46/196 (23.5%) patients had positive BV results by the Gram stain standard method. Four Pap smears were unsatisfactory for evaluation and a repeat was done and the smears evaluated. The number of patients positive for BV by the Pap smear method were 18/196 (9.2%) while 178/196 (90.8%) had negative Bacterial vaginosis results (Table 4.2). The sensitivity, specificity, PPV and NPV of Pap Smear test in detecting BV was 32.6%, 98%, 83.3% and 82.5% respectively (Table 4.4)

Table 4.2: Comparison of Pap smear and Gram stain results of Women at Kiambu Level V Hospital

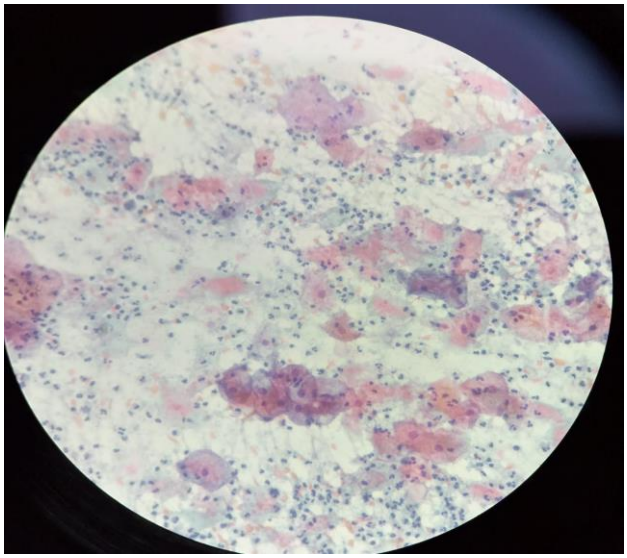
	Bacterial Vaginosis	
	Gram stain Total Number (%)	Pap smear Total Number (%)
Positive	46(23.5)	18(9.2)
Negative	150(76.5)	178(90.8)
Total	196(100)	196(100)

Table 4.3: Two by two Contingency table of Gram stain and Pap smear results among women at Kiambu Level V hospital

		Diagnostic Test		
		Positive	Negative	Total
BV IN PAP	Positive	15	3	18
	Negative	31	147	178
	Total	46	150	196

Table 4.4: Sensitivity, specificity, Positive Predictive Value and Negative predictive Value of Pap-smear in screening for Bacterial vaginosis.

Sensitivity (%)	Specificity (%)	PPV (%)	NPV %)
32.6%	98.0%	83.3%	82.5%



X 400 Mg

Figure 4.1: Pap smear microscopy showing squamous epithelial cells coated with coccobacilli

4.3 Accuracy of Modified Amsel's Criteria in Screening for Bacterial Vaginosis

Positive BV results by the Modified Amsel's criteria was recorded in 30.6% of the patients (Table 4.5). The sensitivity, Specificity, Positive Predictive value and Negative predictive value of Modified Amsel's criteria was 82.6%, 85.3%, 63.3% and 94.1% respectively as shown in Table 4.7

Table 4.5: Gram stain of HVS and Modified Amsel's criteria results of women at Kiambu level V hospital

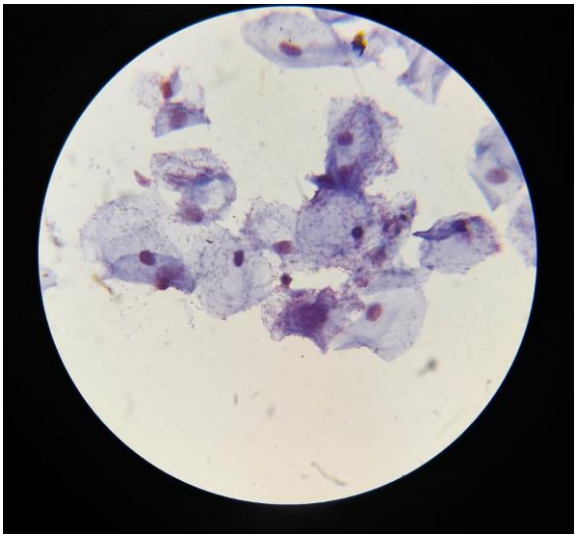
Bacterial Vaginosis		
	Gram stain Total Number (%)	Modified Amsel Total Number (%)
Positive	46(23.5)	60(30.6)
Negative	150(76.5)	136(69.4)
Total	196(100)	196(100)

Table 4.6: Two by two contingency table of HVS Gram stain and Modified Amsel's Criteria results of women at Kiambu Level V hospital

		Diagnostic Test		
		Positive	Negative	Total
MODIFIED AMSEL	Positive	38	22	60
	Negative	8	128	136
	Total	46	150	196

Table 4.7: Sensitivity, Specificity, Positive predictive value and Negative predictive value of Modified Amsel's criteria in screening for Bacterial Vaginosis

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Modified AMSEL'S	82.6	85.3	63.3	94.1



X 1000 Mg

Figure 4.2: Gram-stained High Vaginal Swab smear showing 'clue cells'

4.3.1 Receiver Operating Characteristic Curve of Papanicolaou Smear method and Modified Amsel's Criteria

Considering the gold standard method, which is Gram stain. The Sensitivity and Specificity of Pap and Modified Amsel's Criteria was equated using a ROC curve, by considering 95% as the confidence interval (Figure 4.3). Area under the Curve (AUC) of Modified Amsel's Criteria was 0.709 and a P- value of (P=0.003) AUC for Pap smear was 0.545 at a P- value (p=0.519). These results indicated both curves for Pap smear and Modified Amsel's criteria lie above the reference line. This shows that the two methods

have the ability to be utilized as screening methods for BV in the absence of the gold standard test, with the Modified

Amsel's Criteria having a better performance.

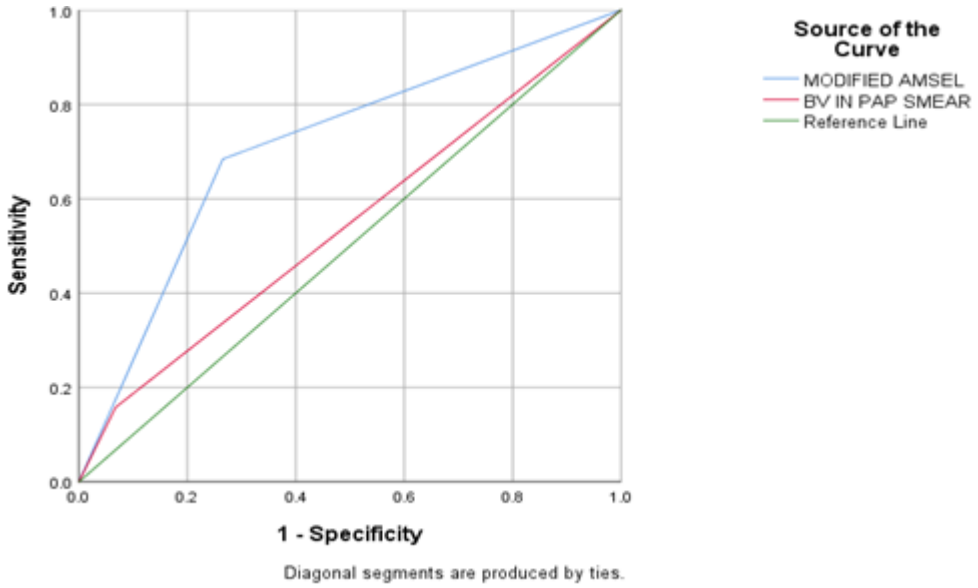


Figure 4.3: Receiver Operating Characteristic (ROC) curve for Pap smear and Modified Amsel's Criteria

Table 4.8: Area under the Curve of the ROC Curve for Pap smear and Modified Amsel's Criteria

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
MODIFIED AMSEL	.709	.065	.003	.582	.836
BV IN PAP SMEAR	.545	.073	.519	.401	.689

4.3.2 Cohen's Kappa Statistics

The measure of agreement for modified Amsel's criteria and Papanicolaou smear method was 0.195 at a P-value of 0.063. This indicated a slight agreement in diagnostic efficiency of the two methods (Table 4.9).

Table 4.9: Cohen's Kappa Measure for Pap smear and Modified Amsel's criteria

		Value	Std Error[a]	Approximate T[b]	Approximate Significance
Measure of Agreement	Kappa	.195	.063	3.736	.000
N of Valid Cases		196			

4.4 Prevalence and Risk factors for Bacterial Vaginosis

4.4.1 Prevalence of Bacterial Vaginosis among Women at Kiambu Level V Hospital

Overall, 196 women were tested for BV. Majority were from the age range 46-50 years old (28.6%), while the age range of 18-20 years had only one participant tested (0.5%) (Table 4.10). The prevalence of Bacterial vaginosis was 23.5 % by the Gram stain method (Figure 4.4).

The highest bacterial vaginosis prevalence was recorded by age group 41-45 years 15 (32.6%) followed by the age group 36-40 years 10 (21.7%). It was noted that those aged 51-55 years old had the lowest prevalence of BV 1(2.2%) and age had a significant statistical association with BV (P=0.03) as shown in Table 4.10.

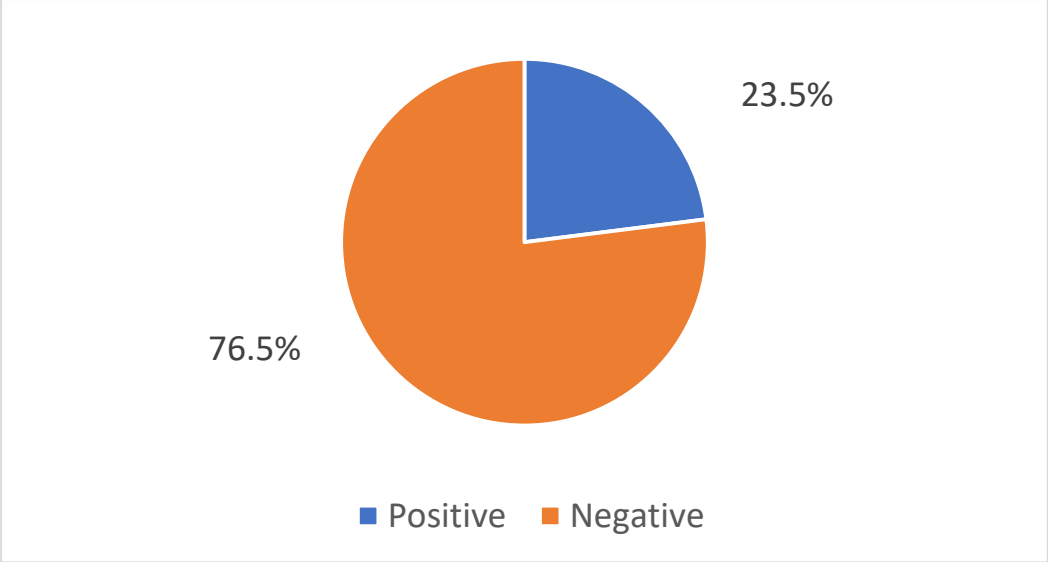


Figure 4.4: Prevalence of Bacterial Vaginosis in women attending gynaecology Clinic at Kiambu Level V Hospital

4.4.2 Risk Factors Analysed for Bacterial Vaginosis

Out of the analysed risk factors for bacterial vaginosis, only age had a significant association (p=0.03) with bacterial vaginosis. Prevalence of bacterial vaginosis was found to be higher in married women 26(56.5%) followed by single women 17(37%) Marital status had no statistically significant association with BV (p=0.427) Level of education had no significant statistical association with BV (p=0.149). The highest bacterial vaginosis prevalence was seen in participants with primary level education 20(43.5).

Family planning method had no statistical association with bacterial vaginosis (p=0.111). The highest prevalence of BV was among women who did not use any family planning method 33(71.7%) followed by those who used implants for family planning 4(8.7%).

Majority of the participants 194(98%) did not smoke cigarette and only 2(1%) smoked. Smoking did not have significant statistical association with bacterial vaginosis (p=0.763)

Douching was only practiced by 4(2%) of participants, while 192(98%) did not engage in douching. 43(93.5%) of the women who did not practice douching had bacterial vaginosis while 3(6.5%) of the women who engaged in douching had Bacterial vaginosis. Douching had no significant statistical association with bacterial vaginosis (p=0.850). Number of sexual partners is another independent variable that was analysed for BV and was found to lack significant statistical association with BV (p=0.873). The prevalence of bacterial vaginosis was highest in women who had only one sexual partner 35(76.1%) and lowest among those who had two sexual partners 1(2.2%) and four sexual partners 1(2.2%) as shown in Table 4.10.

Table 4.10: Risk factors associated with BV among women at Kiambu Level V Hospital

	Bacterial Vaginosis			
Age (years)	Total number (%)	Positive number (%)	Negative number (%)	*P-value*
18-20	1(0.5)	0(0.0)	1 (0.7)	P= 0.030
21-25	12 (6.1)	6 (13.0)	6(4.0)	
26-30	23(11.7)	5(10.9)	18 (12.0)	
31-35	20 (10.2)	2 (4.3)	18(12.0)	
36-40	38 (19.4)	10(21.7)	28(18.7)	
41-45	40(20.4)	15(32.6)	25(16.7)	
46-50	56(28.6)	7(15.2)	49(32.7)	
51-55	6(3.1)	1(2.2	5(3.3)	
Total number	196(100)	46(100)	150(100)	
	Bacterial Vaginosis			
Marital Status	Total number (%)	Positive number (%)	Negative number (%)	*P-value*
Single	75 (38.3)	17 (37.0)	58(38.7)	P=0.427
Married	112 (57.1)	26 (56.5)	86 (57.3)	
Divorced	3 (1.5)	2 (4.3)	1 (0.7)	
Widowed	6(3.1)	1(2.2)	5(3.3)	
Total number	196(100.0)	46 (100.0)	150 (100.0)	
Education	Total no (%)	Positive no (%)	Negative no (%)	P= 0.149
None	4 (2.0)	1 (2.2)	3(2.0)	
Primary	88 (44.9)	20(43.5)	68(45.3)	
Secondary	74 (37.8)	16(34.8)	58(38.7)	
College	30 (15.3)	9(19.6)	21(14.0)	
Total number	196(100.0)	46 (100.0)	150(100.0)	
Family Planning Method	Total no (%)	Positive no (%)	Negative no (%)	
None	137(69.9)	33(71.7)	104(69.3)	
Pills	19 (9.7)	3(6.5)	16(10.7)	
Coil	2(1.0)	2(4.3)	0(0.0)	
Implant	11 (5.6)	4 (8.7)	7 (4.7)	
Condom	8(4.1)	0 (0.0)	8(5.3)	
Injection	10 (5.1)	2(4.3)	8 (5.3)	
TL	6(3.0)	1(2.2)	5(3.3)	
IUCD	3(1.5)	1(2.2)	2(1.3)	
Total number	196(100.0)	46 (100.0)	150(100.0)	P=0.111
Smoking				
No	194(99.0)	45(97.8)	149(99.3)	
Yes	2(1.0)	1(2.2)	1(0.7)	P=0.763
Total number	196(100)	46(100)	150(100)	
Douching				
No	192(98.0)	43(93.5)	149(99.3)	
Yes	4(2.0)	3(6.5)	1(0.7)	P=0.850
Total number	196(100)	46(100)	150(100)	
Number of Sex partners	23(11.7)	6(13.0)	17(11.3)	
0	147(75.0)	35(76.1)	112(74.7)	
1	8(4.1)	1(2.2)	7(4.7)	P=0.873
2	10(5.1)	3(6.5)	7(4.7)	
3	5(2.6)	1(2.2)	4(2.6)	
4	3(1.5)	0(0.0)	3(2.0)	
5				
Total number	196(100)	46(100)	150(100)	

CHAPTER FIVE: DISCUSSION

5.1 Introduction

An efficient diagnostic test should have the ability to assign an individual with disease as Positive and individual who does not have disease as negative. The test needs to be highly Sensitive and specific

5.1.1 Accuracy of Pap smear in Screening for Bacterial Vaginosis

Studies have been conducted in Kenya and beyond, to assess the performance of conventional Pap smear method to screen for BV as provided for in the Bethesda system 2014 of reporting cytological smears. They have yielded varying levels of sensitivity. The sensitivity of Pap smear method in this study was 32.8%. This is close to 31.8% obtained by Santos *et al*. (2023). Nevertheless, a study by Karani *et al*. (2007) in Mombasa found a slightly higher sensitivity of 59.4%, while Vandana *et al.* (2018) had a sensitivity of 61.0%, differing with this study's findings. This difference in sensitivity could have occurred as a result of interobserver variability.

The Papanicolaou smear processing and staining procedures involve passing the smear through several staining solutions and grades of alcohol before examination and this could have led to the loss of material that is vital in BV diagnosis, thus leading to the lower sensitivity achieved. However, the high specificity registered by this study strongly agrees with that of the studies mentioned above. This low sensitivity is reinforced by the ROC curve which had an AUC of 0.545 (p= 0.519) which is statistically non-significant.

5.1.2 Accuracy of Modified Amsel's Criteria in the Screening of Bacterial Vaginosis

The Modified Amsel's criteria considered at least any two positive criteria out of the four clinical criteria. This study recorded a sensitivity of 82.6% and a specificity of 85.3%. This is agreeable with what was achieved by Mengistie *et al*. (2013) whereby, the sensitivity was 85.7% and specificity 91.3%. This sensitivity is also not very different from 71.8% which was recorded by Challa *et al*. (2021). It however differs greatly with the 50% sensitivity achieved by Bhujel *et al*. (2021) and 37% by Sha *et al*. (2005) which were very low. This could have been due to the differences in characteristics of the studied population.

The high sensitivity obtained in this study can be accredited to the simple nature of the modified Amsel's criteria, since it only involves use of basic observational and microscopy techniques (Mohammadzadeh *et al*., 2014). This is also backed by a study which found that modified Amsel's criteria had specificity between 99% to 100%, with the existence of clue cells alone being 89.9% sensitive and 98% specific in screening for BV (Mengistie *et al*., 2013).

5.1.3 Prevalence and Risk factors for Bacterial Vaginosis

Bacterial vaginosis prevalence was 23.5% by the gold standard method. This agrees with a study done in Taiwan by Huang *et al*. (2023) which had a prevalence of 22.8% and 20.3% by Ndiaye and colleagues in 2023 which was also very close (Ndiaye *et al*., 2023). A study conducted in the year 2013 in Ethiopia had a prevalence of 19.4% and not very different from this study (Mengistie *et al*., 2013). Another study conducted by Ambike

and his colleagues had a prevalence of 23%, which exactly agrees with this study, even though it was done among pregnant women visiting ante-natal clinic (Ambike *et al.*, 2020). Bacterial vaginosis prevalence in this study however, differed considerably with that obtained by Mutuku *et al*. (2021) which was 10.3% and 15.1% by Musyoki *et al*. (2015). However, Lokken and his colleagues had higher prevalence of 35% (Lokken *et al.*, 2022). The BV prevalence was also different from that registered by Majigo and colleagues in Tanzania which was 33.2%. (Majigo *et al.*, 2021). These differences could have been as a result of inter-observer variability from the microscopists or difference in characteristics of the studied population.

This study demonstrated that age had a significant association with BV (p= 0.03). The age group 41-45 years was the most affected by BV followed by age group 36-40. These findings correspond to those obtained by Mutuku *et al*., in their study in which BV prevalence was higher among women aged 30-49 years, and age was statistically associated with BV (Mutuku *et al.*, 2021). Other studies by Ibrahim *et al*. (2014) and Bitew *et al*. (2017) had similar findings. This can be explained by the fact that the age group 18-45 years is the reproductive age for women and is characterized by heightened sexual activity. However, Yalew *et al*. (2022) did not find statistical significance of age and BV, though prevalence of bacterial vaginosis was high in women 30 years and above, agreeing with the findings of this study.

The study did not demonstrate any statistical significance relating bacterial vaginosis and marital status (p=0.427). This study revealed that BV was more prevalent in the married females at 56.5% and lowest among the widowed and divorced at 2.2% and 4.3%

respectively. Similar findings were registered by Mutuku *et al*. (2021). However, this outcome contradicts a study by Oparaugo *et a*l., in Nigeria who found that BV prevalence was higher among divorced and widowed women (Oparaugo *et al*., 2021). This high BV prevalence recorded in married women could be explained by the fact that married women engage in sex frequently as opposed to divorced and widowed women and research has shown that sexual activity has a link with BV acquisition (Fethers *et al*., 2009). Despite this fact, cases of BV have been reported among virgin girls and sexually inactive women, proving that other factors such as poor menstrual hygiene, tight undergarments, and diet together with lifestyle changes can also alter the vaginal flora (Vaca *et al*., 2010).

Education had no statistical association with bacterial vaginosis (p=0.149). This study established that women with low education level had the highest BV prevalence compared to those with college education. This agrees with the findings of Bonneton *et al.* (*2*021) and Bitew *et al*. (2017). Illiteracy has been associated with bacterial vaginosis, because the uneducated lack information about preventive measures (Donders *et al*., 2010).

Family planning did not demonstrate any statistical relationship with bacterial vaginosis occurrence (p=0.111). In this study, women who did not use any family planning method had the highest BV prevalence followed by those who used implants, while those who used condom had the lowest prevalence of BV. Studies by Gallo *et al*. (2011) and Guedou *et al.* (2013) have reported that condom use reduced BV acquisition by preventing

alteration of vaginal flora as a result of seminal fluid exposure. The use of IUCD acts as a risk for BV acquisition according to Achhilles *et al*. (2018).

This study revealed no significant statistical association between BV and cigarette smoking (p=0.763). Cigarette smoking was only reported by two participants out of 196 (1.0%) and only one tested positive for bacterial vaginosis while the other one tested negative. This outcome contradicts studies by Tuzil *et al*. (2021) and Brotman *et al*. (2014) who found a relationship between cigarette smoking and BV mainly through promoting growth of *Gardnerella* and *Mobilincus* species. The low number of smokers could have possibly resulted to the lack of statistical significance of cigarette smoking and BV in this study. A large sample size of women who smoke however, needs to be studied to bring out the actual association between smoking and BV.

This study did not find a statistical association between vaginal douching and BV (p=0.850). Many researchers have investigated douching and its effects on vaginal health, with some indicating a significant association, while others did not show any correlation. This study's findings resonate with those by Newton *et al*. (2001) but contradicts an outcome by Aslan &Bechelaghem, (2018). Douching is thought to alter vaginal microflora according to Cotrell, (2010) & Hilber *et al*., (2010). Nevertheless, this study failed to prove that, and this could have been due to the small number of participants who reported engaging in the practice, thus rendering insignificant its statistical importance. Douching is a practice that was reported by only 4/196 (2%) of participants, with 98% reporting not douching. This was a small number out of which only 2.2% had a positive BV result.

The number of sexual partners that the participants had did not have statistical significance with BV (p=0.873). This outcome is consistent with those of a study by Forcey and colleagues which detailed that the number of sexual partners whether numerous or few can lead to BV infection (Forcey *et al*., 2015). Smart *et al*., (2004) also recorded a statistical relationship of BV and the number of sexual partners.

Most of the study participants had only one sexual partner, that is 147/196 (75%) and the highest number of BV positive cases was reported among this group 35/46(76.1%). This outcome could have been as a result of most participants not giving true information about their sexual partners, thus leading to under reporting. Participants with the highest number of sexual partners were 3(1.5%) and none of them tested positive for Bacterial vaginosis.

5.2 Conclusions

1 Pap smear registered sensitivity of 32.6% and specificity of 98%. This sensitivity was very low, indicating that it was not accurate in detecting BV and cannot be relied on in the absence of Gram stain which is the gold standard.

2 The modified Amsel's criteria had a sensitivity of 82.6% and specificity of 85.3% proving to be dependable in detection of BV in the absence of the gold standard.

3 Prevalence of bacterial vaginosis amongst reproductive age women attending gynaecology clinic at Kiambu level V hospital was 23.5%. Out of all the risk factors analysed for Bacterial vaginosis, only age had a significant association (p=0.03). Therefore, the null hypothesis was rejected and the alternative hypothesis accepted.

5.3 Recommendations

5.3.1 Recommendations from the study

1. A larger study is necessary to give a broader magnitude of BV in this population since this prevalence may not be representative, considering that only women visiting the gynaecology clinic of Kiambu Level-V hospital were studied.
2. Educating women on how to avoid BV and empowering them to recognize the signs and symptoms, in order to seek treatment early.

5.3.2 Recommendation for policy

1. Empowering healthcare practitioners in remote settings where Gram stain and microscopy is not attainable, on the Amsel's clinical criteria which are helpful in detecting Bacterial vaginosis.
2. Formulating and reviewing policies to accommodate the modified Amsel's criteria in remote settings.

5.3.3 Recommendation for further studies

Invention of a simple and effective way to detect presence of amines in vaginal samples rather than sniffing the KOH preparation.

5.4 Economic Importance of Modified Amsel's Criteria

The Modified Amsel's criteria is a fast and cheaper method of screening for bacterial vaginosis. It will therefore lower the economic burden of BV diagnosis to Kenyan families and reduce the budgetary allocation towards BV diagnosis, thus lowering the national economic burden.

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APPENDICES

Appendix I: Consent Information Form

COMPARISON OF PAP-SMEAR AND MODIFIED-AMSEL'S CRITERIA IN SCREENING FOR BACTERIAL VAGINOSIS AMONG WOMEN AT KIAMBU LEVEL-V HOSPITAL, KIAMBU COUNTY, KENYA

My name is Susan Akinyi Omwono, a postgraduate student pursuing a Master's degree in Histocytopathology at Kenyatta University. I am doing a study on a disease called bacterial vaginosis, which affects females and is emerging as a public health concern in Kenya. I humbly request you to participate in this research. The aim of this consent form is to equip you with important information regarding this study so that you can make an informed choice whether to take part in this research or not. Do not hesitate to ask any questions or clarifications on possible risks, benefits, your role in the study or any other questions or concerns about the study.

Your participation is voluntary and declining participation will not affect your accessibility to services in this hospital or anywhere. You shall not pay anything to participate in this study and you shall not be paid.

What to expect

The research will involve collecting vaginal samples from you.

Procedure involved

It is simple and painless with no complications.

You will position yourself and lie comfortably on a couch. A nurse will gently introduce a speculum into the vaginal opening to enable her see the cervix. The cervix will be sampled using a cyto-brush, a smear made and fixed immediately. An additional sample will be collected at the same time using a sterile swab. The speculum will be removed gently and you will be released. The samples will be delivered to the laboratory for processing and evaluation.

Benefits

You will find out if you have Bacterial vaginosis and cervical abnormalities, and if you have your doctor will prescribe medication.

The study will help in understanding the performance of the two methods under comparison, in relation to their efficiency in testing for bacterial vaginosis.

The study will help the MOH to make informed decisions on the best diagnostic methods and tools to recommend to healthcare providers for use in diagnosis of BV.

Confidentiality

All the information given and the test results will be kept private and the results will be communicated by your doctor at this clinic during your next visit.

In case you have any concern or queries about this research and your participation, kindly contact the following people:

i. Susan Akinyi Omwono (The principal investigator)

Telephone number: **0721 832505**

ii. Dr. Onesmus Muia Mutuku

Telephone number**: 0712 652085**

iii. Kenyatta University Ethics Review Committee

P.O BOX 43844-00100

Telephone: **0208710901/12** or **kuerc.secretary.ku.ac.ke Declaration**

I……. after reading and understanding the information, give informed consent to be involved in this investigative research.

All procedures have been explained and I have been given a chance to raise any queries and be given answers. I have also been informed that I am free to pull out from the research without my rights as a patient being affected.

Participants signature……. Date……

Principal investigator‘s signature…… Witness…….

Appendix II: Questionnaires

COMPARISON OF PAP-SMEAR AND MODIFIED-AMSEL'S CRITERIA IN SCREENING FOR BACTERIAL VAGINOSIS AMONG WOMEN AT KIAMBU LEVEL-V HOSPITAL, KIAMBU COUNTY, KENYA

All participants who have signed the consent form will fill the questionnaire before proceeding for specimen collection.

Section A: Information on demographics

Participants number……...
Date……
Age…….
Residence……...

1. Marital Status

Single ( )

Married ( )

Divorced ( )

Widowed ( )

2. Education level

None ( )

Primary ( )

Secondary ( )

College ( )

3. Family planning method

None ( )

Condom ( )

Depo ( )

Pills ( )

IUCD/coil ( )

1. Last Menstrual Period……..

2. How many children do you have?.....
3. Occupation?.....
4. Do you smoke cigarettes?..... If yes, for how long?.....
5. Age of sexual debut.....
6. Number of sex partners……
7. Antibiotic use in the last one week? Yes ( ) No ( )
8. Do you engage in Intravaginal cleaning/douching? Yes ( ) No ( )

Section B: Clinical history

1. Any discharge?

Yes ( )

No ( )

Section B: For investigators use only

1. Specimen adequacy

Satisfactory ( )

Unsatisfactory ( )

2. Pap smear results

NILM ( )

Shift in flora suggestive of bacterial vaginosis ( )

Others, specify……

3. Amsel‘s criteria

pH ( )

Clue cells present ( )

Absent ( )

4. Nugent score

0-3 ( )

4-6 ( )

7-10 ( )

5. Comments

Principal investigator……..…… Signature……..

Cytologist……...… Signature…….……..…

Pathologist……. Signature….……..….

Appendix III: Bethesda System of Reporting Cervical Cytology (2014)

Specimen type

-Indicated as conventional Pap smear

Specimen adequacy

Indicate whether the specimen was adequate for evaluation, shown by the presence of endocervical and transformation zone component or

Unsatisfactory for evaluation due to;

Absence of endocervical cells and the transformation zone component.

Partially obscuring blood.

Inflammation.

Specify why the specimen is unsatisfactory for evaluation and give a recommendation.

Interpretation

Results are reported as;

1. **Negative for intraepithelial lesion or malignancy (NILM). This is when there is no cellular evidence of neoplasia.**

State other non-neoplastic findings such as;

- **Changes associated with pregnancy.**
- **Tubal metaplasia.**
- **Atrophy.**
- **Keratotic changes.**
- **Squamous metaplasia.**

State presence of organisms such as;

- *Trichomonas vaginalis*
- Fungal organisms morphologically consistent with *Candida species*.
- Shift in flora suggestive of bacterial vaginosis.
- Bacteria morphologically consistent with actinomyces species.
- Cellular changes consistent with *Herpes simplex* virus.
- Cellular changes consistent with *Cytomegalovirus.*

Others

Endometrial cells, if present in a woman over 45 years old (Specify if NILM).

Epithelial cell abnormalities

Squamous cell

1. **Atypical squamous cells**

Of undetermined significance (ASCUS)

Cannot exclude HSIL (ASCH)

2. **Low grade squamous intraepithelial lesion (LSIL)**

(Encompassing HPV/Mild dysplasia/CIN)

3. **High grade squamous intraepithelial lesion (HSIL)**

(Encompassing moderate and severe dysplasia. CIS, CIN 2, CIN 3)

With features suspicious for invasion (If invasion is suspected).

4. **Squamous cell carcinoma**

Glandular cell

Atypical

Endocervical cells (NOS or specify in comments)

Endometrial cells (NOS or specify in comments)

Glandular cells (NOS or specify in comments)

Endocervical cells, favour neoplastic.

Glandular cells, favour neoplastic.

Endocervical adenocarcinoma in-situ

Adenocarcinoma;

- Endocervical
- Endometrial
- Extrauterine
- Not otherwise specified

Other malignant neoplasms (specify)

Appendix IV: Publication

Asian Research Journal of Gynaecology and Obstetrics

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Bacterial Vaginosis Prevalence and Its Associated Risk Factors among Women at Kiambu Level-5 Hospital, Kenya

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SAO and OMM designed the study and performed the statistical analysis and wrote the protocol and the first draft of the manuscript. Author AW managed the analyses of the study, while authors JNR and MM managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: Bacterial vaginosis (BV) is a commonly experienced vaginal disorder in women. It occurs when the beneficial lactobacillus species are replaced by anaerobic and facultative bacteria, leading to a foul-smelling vaginal discharge. Its diagnosis remains a big challenge in developing countries such as Kenya. Gram stain and Nugent scoring of the bacterial morphotypes is the recommended method of diagnosis, but is tedious to undertake and require highly skilled microscopists. The objective of this study was to determine the prevalence of BV among women at Kiambu Level-5 Hospital and to establish the risk factors associated with it.

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Methodology: The Cross-sectional study was carried out at Kiambu Level-5 hospital, department of Pathology between April 2023 and June 2023. We included 196 women between the ages 18-55 who were enrolled by convenience sampling and screened for bacterial vaginosis using Gram staining and microscopy for bacterial morphotypes.
Results: Out of the 196 participants 46 were positive for BV (23.0%) while 150 (77.0%) were negative. Yeast cells (candidiasis) was found in 23 participants (11.7%) while 1 participant (0.5%) had gonococci. Out of the risk factors analyzed, only age had a significant association with BV (P=0.03)
Conclusion: The relatively high prevalence of bacterial vaginosis attained in this study shows that it is a health concern among women in Kiambu, Kenya particularly those aged between 41-45 years. There is need to educate women on how to avoid BV and to empower them to recognize the signs and symptoms, in order to seek treatment.

Keywords: Bacterial vaginosis; prevalence; risk factors.

1. INTRODUCTION

Bacterial vaginosis (BV) is the most common vaginal infection experienced by women all over the world. It is frequently encountered in Sexually Transmitted Diseases clinics, out-patient departments and reproductive health clinics with about 5-70 % of women affected [1]. It occurs when there is replacement of the beneficial Lactobacillus that usually regulate the vaginal pH, making it acidic, by production of hydrogen peroxide that is vital in preventing anaerobic microbes in the vagina from growing extremely [2]. Absence of Lactobacillus causes pH increase within the vagina. This leads to increased population of non-pathogenic bacteria such as *Atopobium vaginae Gardnerella vaginalis*, *Mycoplasma hominis* and *Ureaplasma urealyticum,* among other bacteria, leading to a foul-smelling vaginal discharge [3,4].

Burden of Bacterial vaginosis is greater in Sub-Saharan region at 38% prevalence according to Jespers et al. [5], and at 55 % as found by Woodman [6]. The prevalence in Kenya varies between several studies. For instance, Muia et al. [7] conducted a study among HIV infected women in Machakos County that had a prevalence rate of 10.3%. Musyoki et al. [8] conducted a study in Nairobi amongst female commercial sex workers which had a prevalence of 15.1 %. Nzomo et al. [9] found a prevalence of 43 % in a study conducted in Thika. Regardless of the variation in prevalence rates across the country, BV remains a concern in public health and research. Several factors are linked to its occurrence such as contraceptive IUCD use, having many sex partners, new sex partner, intravaginal cleaning and unprotected sex. The symptoms of BV include; itching, sore and painful vagina, a discharge that is thin, white, grey or greenish in colour with a 'fishy 'odour [10]. However, many women with bacterial vaginosis experience no symptoms, which further complicates the diagnosis and management of cases [11].

Centers for Disease Control (CDC) recommends that clinical bacterial vaginosis screening should be done using the Amsel's criteria. This involves checking the pH of the vaginal discharge which is usually more than 4.5 in the presence of BV, examining a wet preparation for clue cells and noticing an amine-like smell after adding 10% potassium hydroxide to the vaginal discharge. The other method is through Gram staining the vaginal discharge, as the gold standard method and determining the population of *Lactobacilli, Gadnerella vaginalis*, *Bacteroides* and *Mobilincu*s species which are linked to BV [12]. Even though Gram staining and Nugent scoring is the gold standard for screening BV, it takes a lot of time to accomplish and requires an expert in microscopy.

The modified Amsel's criteria rely on two features only to screen for bacterial vaginosis with a couple of studies proving this to be sensitive, less cumbersome and relatively fast [13]. Presence of clue cells on a wet preparation of the discharge and pH more than 4.5 have been shown to have a good match with Nugent score as found in studies by Mengistie et al. [14]; Bhujel et al. [15]. Screening of BV still remains complicated due to the fact that it is attributed to host, social, epidemiologic and biological influences [16,17,18].

2. MATERIALS AND METHODS

2.1 Study Design

We conducted a cross-sectional study at Kiambu level-5 hospital comprehensive care centre

Appendix V: Kenyatta University Ethics Review Committee Letter

KENYATTA UNIVERSITY
CENTRE FOR RESEARCH ETHICS AND SAFETY

Fax: 8711242/8711575 **P. O. Box 43844,**
Email: chairman.kuerc@ku.ac.ke
Nairobi, 00100

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Website: www.ku.ac.ke
Our Ref: **KU/ERC/APPROVAL/VOL.1** Date: 21st /02/2023

Susan Akinyi Omwono
P.O Box 43844, 00100
Nairobi.

Dear Ms. Omwono,

APPLICATION NUMBER: PKU/2657/E1781- COMPARISON OF PAP-SMEAR AND MODIFIED AMSELS CRITERIA IN SCREENING FOR BACTERIAL VAGIONISIS AMONG WOMEN AT KIAMBU LEVEL 5 HOSPITAL, KIAMBU COUNTY, KENYA

This is to inform you that ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** has reviewed and approved your above research proposal. Your application approval number is **PKU/2657/E1781.** The approval period is **21st /02/*2023 to 21st /02/2024***

This approval is subject to compliance with the following requirements;

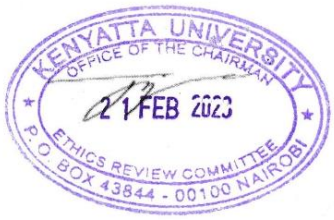
i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE***
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.

vii. Submission of an executive summary report within 90 days upon completion of the study to ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE***

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed.

To serve you better, researchers are kindly requested to access and complete a customer feedback form and sent it back online as you continue with research and upon completion of data collection found on the following website link; ;(https://docs.google.com/forms/d/1ytWefDwvyz5h1oz_VIn0xbxg3uGdlDzMXFWNDsMrRPQ/edit?usp=sharing

Yours sincerely



Prof. Judith Kimiywe

Director: Centre for Research Ethics and Safety

Appendix VI: Kiambu Level V Hospital Authorization

COUNTY GOVERNMENT OF KIAMBU
DEPARTMENT OF HEALTH SERVICES

All correspondence should be addressed to HEAD
HRDU – HEALTH DEPARTMENT
Email address: mndiritu@gmail.com
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Tel. Nos: 0721641516
0721974633

HEALTH RESEARCH AND DEVELOPMENT
UNIT
P. O. BOX 2344 – 00900
KIAMBU

Ref. No.: KIAMBU/HRDU/23/04/04/RA_OMWONO Date: 4th April 2023

TO WHOM IT MAY CONCERN

RE: CLEARANCE TO CONDUCT RESEARCH IN KIAMBU COUNTY

Kindly note that we have received a request by Susan Akinyi Omwono of Kenyatta University to carry out research in Kiambu County, the research topic being on "**Comparison Of Pap Smear And Modified Amsels Criteria In Screening For Bacterial Vaginosis Among Women At Kiambu Level 5 Hospital, Kiambu County, Kenya**"

We have duly inspected her documents and found that she has been cleared by the KU ERC to carry out the research for a period ending **21st February 2024**. As she has received approval from a NACOSTI licenced ERC, we hereby give her a provisional clearance to begin collecting her data immediately to avoid any delays in the research process. However, she is required to submit the NACOSTI license within 2 months of receiving this letter.

It is incumbent upon the institution where she is carrying out research to ensure that she receives adequate supervision during the process of conducting the research. This note also accords her the duty to provide a feedback on her research to the county at the conclusion of her research.

DR. MWANCHA KWASA
COUNTY CLINICAL RESEARCH OFFICER
KIAMBU COUNTY

Appendix VII: NACOSTI Research Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **912281**

Date of Issue: **05/April/2023**

RESEARCH LICENSE

This is to Certify that Miss.. SUSAN AKINYI OMWONO of Kenyatta University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Kiambu on the topic: COMPARISON OF PAP-SMEAR AND MODIFIED AMSEL'S CRITERIA IN SCREENING FOR BACTERIAL VAGINOSIS AMONG WOMEN AT KIAMBU LEVEL 5 HOSPITAL, KIAMBU COUNTY, KENYA for the period ending : 05/April/2024.

License No: **NACOSTI/P/23/24582**

912281

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

See overleaf for conditions

Appendix VIII: A Map of Kiambu County