RESISTANCE PATTERN OF ISOLATES FROM BACTERIAL VAGINOSIS IN FEMALE SEX WORKERS IN NAIROBI, KENYA

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A RESEARCH PROJECT SUBMITTED TO THE SCHOOL OF MEDICINE IN PARTIAL FULLFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE MASTER OF SCIENCE DEGREE IN MEDICAL MICROBIOLOGY AT THE UNIVERSITY OF NAIROBI.

DECEMBER, 2015

DECLARATION

I, Millicent Ogutu, hereby declare that this project is my original work and with affirmation that it has not been presented in any institution for examination or any other purpose. All sources of information have been acknowledged by means of references.

Signature……. Date……..

CERTIFICATION

This is to certify that this project has been submitted for examination with our approval as University Supervisors.

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DEDICATION

This work is dedicated to my family members; Jackim, Thaddius, Eune and Wesley

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LIST OF ABBREVIATIONS

BV- Bacterial vaginosis

CLSI – Clinical and Laboratory standards Institute

FSW- Female Sex Worker

HIV – Human Immunodeficiency Virus

HSV - Herpes Simplex Virus

NCCLS – National Committee for Clinical Laboratory Standards

STDs – Sexually Transmitted diseases

SWOP – Sex Workers Operating Point

ABSTRACT

Background

Bacterial vaginosis (BV) is a lower genital tract infection characterized by the presence of thin, white, homogeneous, fishy-smelling vaginal discharge. This discharge is present in the absence of signs of vaginal irritation. It is also characterized by a disruption of the normal vaginal equilibrium. BV accounts for 40% to 50% of all gynecologic conditions encountered by primary health care physicians. Its prevalence is reported to be generally high among women who are sexually active than those who are not. Some antimicrobials have been associated with marked evidence of resistance among vaginal anaerobic bacteria. Since the prevalence of BV in women of reproductive age is shown to be high in Kenya, there is need to investigate the antimicrobial susceptibility pattern of anaerobic bacteria isolated from BV in female sex workers in Nairobi, Kenya.

Broad objective

The goal of this study was to determine the susceptibility of vaginal anaerobic bacterial isolates from female sex workers with bacterial vaginosis in Nairobi, Kenya.

Study design

This was a cross-sectional study. High Vaginal Swab (HVS) specimen was obtained from Female Sex Workers (FSW) attending Sex Workers Operating Point (SWOP) clinics and socio-demographic data obtained using questionnaires.

Methodology

Structured questionnaires were used to collect the socio-demographic features of the participants. These included age, educational level and residence of the participants. It was also used to elicit

information on participants' knowledge and attitude towards their reproductive health. Vaginal secretions were taken using sterile swabs at the SWOP clinic and cultured for isolation of *Gardnerella vaginalis*, *Mobiluncus* species, *Ureaplasma urealyticum*, *Peptostreptococcus* species. and *Bacteroides* species in an anaerobic environment at the University of Nairobi, Institute of Tropical and Infectious diseases laboratory. Susceptibility test was done using the disc diffusion and agar dilution technique.

Data analysis

The data collected was edited, coded and analyzed using SPSS version 21. Descriptive statistics was used to analyze socio-demographic features while odds ratio was used to determine the risk factors associated with bacterial vaginosis. Chi square was used to determine the difference in response to the antimicrobials used to treat bacterial vaginosis.

Results

Out of 160 participants enrolled in to the study, 18.4% were confirmed to have Bacterial vaginosis. Contraceptive use was found to be common among the participants (68.5%), 51.6% practiced douching while only 18% indicated that they smoked. BV was found to be inversely related to number sexual partners and contraceptive use (Pearson correlation -0.033 and -0.045 respectively) while douching and smoking were directly related to BV condition (Pearson correlation 0.099 and 0.044 respectively). *Bacteroides* dominated the isolates at 16.3% while *Morbiluncus* was at 3.1%. There was a significant difference in the antimicrobials used to treat BV ($P<0.001$). Most of the isolates were sensitive to clindamycin while majority were resistant to metronidazole.

Conclusion

Hormonal contraceptives were negatively associated with BV while smoking and douching were directly associated with the condition in FSW in Nairobi. *Bacteroides* sp was the most commonly isolated organism from samples collected from FSW in Nairobi. Clindamycin was the most effective drug for treating BV in FSW in Nairobi, Kenya.

1.0 INTRODUCTION

Background to the study

Bacterial vaginosis (BV) is a lower genital tract infection characterized by the presence of thin, white, homogeneous, fishy-smelling vaginal discharge. This discharge is present in the absence of signs of vaginal irritation, such as pain, itching, burning, soreness, and dyspareunia. Bacterial vaginosis is characterized by a disruption of the normal vaginal equilibrium. The lactobacilli population decreases, which leads to an increase in vaginal pH (as high as 7.0) and overgrowth of and replacement by vaginosis-associated anaerobic microorganisms.

BV has been associated with a variety of adverse health outcomes, including preterm delivery, intrauterine infection, pelvic inflammatory disease (PID) and other gynecological complications (Cu-Uvin *et al*., 2001). BV may also increase susceptibility to Human Immunodeficiency Virus (HIV) and other sexually transmitted infections (STIs), and in prospective studies BV has been associated with the acquisition of HIV and herpes simplex virus (HSV) type 2 (Taha *et al*., 1998 and Van *et al*., 2001) . BV may also facilitate HIV and HSV-2 transmission by increasing the frequency of genital shedding of the virus.

Sexual activity and vaginal hygiene practices have been associated with BV (Fredricks *et al*., 2005). Reproductive hormones are also thought to play a role in the regulation of vaginal flora. A study conducted in United States by Koumans *et al.*, (2007) revealed 29.2% prevalence of BV in women. In sub-Saharan Africa, the prevalence of BV is very high, ranging from 30–51% in community based studies (Van *et al*., 2001). A high prevalence has also been reported among African–American women with estimates of up to 50% in population-based surveys (Peipert,

2008). In Kenya, the prevalence of BV in women of reproductive age was found to be 43.1% (Nzomo *et al.,* 2013). Some studies have found a relationship between BV and high-risk behaviors associated with sexually transmitted infections (STIs) such as early sexual debut and multiple sex partners (Bukusi et al., 2006 and Christopher et al., 2000). Prevention of BV will depend in part on identification of risk factors for BV that is susceptible to interventions and its susceptibility to the locally used antimicrobial drugs.

Several studies have demonstrated antimicrobial resistance to metronidazole and clindamycin that are commonly used in treatment of bacterial vaginosis (Bradshaw *et al.*, 2006 and Beigi *et al.*, 2004). This can act as a vaginal reservoir of macrolide-resistant bacteria. In this study, Nugent score, culture methods and susceptibility test were used to investigate BV and its correlates in female sex workers in Nairobi, Kenya.

2.0 LITERATURE REVIEW

2.1 BV epidemiology

Bacterial vaginosis (BV) is a condition characterized by the partial loss of the indigenous vaginal lactobacilli and polymicrobial anaerobic overgrowth of the vaginal mucosa. It is a common cause of malodorous vaginal discharge (Taha *et al*., 1998). It is also associated with sexually transmitted infections and adverse pregnancy outcomes (Van *et al*., 2000). BV was first described in 1955 by Gardner and Dukes who reported a strong correlation between BV and the presence of *Gardnerella vaginalis.* Bacterial vaginosis (BV) is a polymicrobial syndrome characterized by a change in vaginal flora away from predominantly *Lactobacillus* species (Rebecca *et al*., 2010; Holst *et al*., 1984). The resident *Lactobacillus* species are replaced by an overgrowth of vaginal anaerobes or Gram-negative bacteria including; *Gardnerella vaginalis*, *Atopobium vaginae*, *Mycoplasma hominis*, *Mobiluncus* species, *Ureaplasma urealyticum*, *Prevotella*, *Peptostreptococcus* species. Transmission is enhanced by penetrative sexual contact, non-penetrative digito-genital contact and oral sex. BV-associated bacteria have been shown to form a prolific polymicrobial biofilm (Swidsinki *et el.,* 2008), of which the main component was found to be *G. vaginalis* and *A. vaginae*, which adheres to the vaginal epithelium. Women who are sexually active run a high risk of developing the condition, as those with multiple partners or who have changed partners recently. Complications associated with bacterial vaginosis include preterm delivery (PTD), preterm premature rupture of membranes (PPROM), chorioamnionitis, post abortion pelvic inflammatory disease (PID), infections following invasive gynecologic procedures, urinary tract infections (UTIs), and susceptibility to human immunodeficiency virus (HIV).

Bacterial vaginosis accounts for 40% to 50% of all gynecologic conditions encountered by primary care physicians (Koumans *et al*., 2007). It is not considered a sexually transmitted infection (STI); though, its prevalence is generally higher among women who are sexually active than those who are not (Cohen *et al.*, 1992). Immunocompromised women (CD4+ cell count <200 cells/μL) and those infected with HIV have a higher prevalence and higher severity Taha *et al.*, 1998). In the general population, it is diagnosed in 30% to 52% of African-American women, 32% of Hispanic women, and 10% to 23% of white, non-Hispanic women (Peipert *et al.*, 2008). Reported prevalence of bacterial vaginosis among women of reproductive age ranges from 24% to 31.4% (Koumans *et al*., 2007). Among women from low socio-economic stratum in sub-Saharan Africa, BV prevalence ranged from 11% - 30% (Kouman *et al*., 2007). Bornstein *et al*., (2001) reported a prevalence of 35.0% among women attending STI clinic in Nairobi-Kenya.

2.2 BV and family planning

Hormonal contraceptive use is associated with a decreased risk of BV. Kouman *et al.,* (2007) in their study found that there is reduced risk of BV among oral contraceptive users and among those using hormonal injection/implant as compared to tubal ligation. Consistent condom use was associated with a decrease in the risk for bacterial vaginosis and associated vaginal microflora.

2.3 BV and intraviginal practices

The cause of disruption of vaginal microflora and increase the risk of bacterial vaginosis include previous history of bacterial vaginosis, douching, and smoking. Several studies have suggested that intravaginal practices may be associated with bacterial vaginosis and other shifts in dominant flora of the vagina. In sub- Saharan Africa, a handful of studies have shown that

women's intravaginal practices may be associated with an increased prevalence of bacterial vaginosis, including among Kenyan sex workers and Ivorian antenatal clinic attenders (Bukusi et al., 2006 and Nzomo *et al.*, 2013). Similarly, a prospective study in Zimbabwe found that intravaginal practices were inversely associated with normal vaginal lactobacilli (Van et al., 2000). There is widespread habit of douching among African female sex workers. The association between vaginal douching and BV is of concern, due to the increased risk of HIV infection with BV, which has now been shown in several studies (Cu-Uvin *et al.*, 2001). Douching can increase the risk of acquiring BV.

2.4 BV diagnosis

Up to 95% of women with bacterial vaginosis harbor *Gardnerella vaginalis* (Bradshaw *et al*., 2006). Other associated microbial populations identified include *Prevotella bivia*, *Mobiluncus* species, Gram-positive cocci, *Bacteroides*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Megasphaera*, and *Leptotrichia*. They also include *Atopobium vaginae*, *Chloroflexi*, *Streptococcus*, and *Veillonella* the metabolic by-products of these bacteria (amines and organic acids) produce the characteristic fishy odor. Koumans *et al*., (2007) cultivated BV organisms anaerobically using the GasPak anaerobic envelope system at 37°C on Trypticase + 5 % sheep blood. Beigi (2004) used Epsilometer tests to determine the minimal inhibitory concentrations (MIC) of the drugs used to treat BV. Spiegel *et al*., (1983) in their study concluded that a microscopically detectable change in vaginal microflora from the Lactobacillus morphotype, with or without the Gardnerella morphotype (normal), to a mixed flora with few or no Lactobacillus morphotypes (BV) can be used in the diagnosis of BV (Spiegel *et al*., 1983). Diagnosis can also be made on the basis of the foul smelling discharge, on the liberation of

amines with a fishy odor when 10% Potassium hydroxide is added to it. In this study, Nugent score, culture methods and susceptibility test will be used to investigate BV and its correlates in female sex workers in Nairobi, Kenya.

2.5 BV and antimicrobial agents

Metronidazole is a nitroimidazole antimicrobial agent used to manage protozoal infections such as trichomoniasis and anaerobic infections. Clindamycin is a second antimicrobial agent for the treatment of BV. Tinidazole is a nitroimidazole antibiotic and an antiprotozoal agent that was first reported in Europe, Asia, and Latin America for its use in BV treatment (Swidsinski *et al*., 2008). Secnidazole is a nitroimidazole antibiotic with a broad spectrum of activity against anaerobic microorganisms and has a longer half-life than metronidazol (De Backer *et al*., 2006; Christopher and Snehalata, 2000). Resistance to metronidazole and clindamycin in treating BV was demonstated by Belgi *et al.,* (2004) in their study on antimicrobial resistance associated with treatment of BV. Relapse is frequent after antibiotic treatment. The recurrence rate has been estimated at 58% 12 months after oral metronidazole therapy (Swidsinki *et al.,* 2007). *A. vaginae* have a variable susceptibility for metronidazole with some even showing a high-level resistance. Resistance to antimicrobial agent may be due to the survival of *A. vaginae* and *G. vaginalis* as a biofilm on the vaginal epithelium after therapy (Swidsinki *et al.,* 2007). Possible reinfection may explain the high recurrence rate as suggested by the highest cure rate observed among women who abstained from having sex or consistently used condoms during treatment.

2.6 BV intermediate score

Nugent's score below seven do not get treated for BV after diagnosis. Richard et al., (2009) in their study noted that upon follow up of the enrolled subjects with intermediate BV score (4-6) for up to 11 weeks, 32% acquired BV while 30% reverted to have normal flora. This current

study will follow up participants with intermediate score for 3 months from the time of diagnosis.

Nugent's (1991) criterion was used to score the organisms as follows:

Table 1: Nugent's score

Lactobacilli	**Score**	**Gardnerella, Bacteroides**	**Score**	**Curved gram –ve bacilli**	**Score**	**Nugent score**
30 or more	0	0	0	0	0	0
5-30	1	Less than 1	1	Less than 1	1	3
1-4	2	1-4	2	1-4	1	5
Less than 1	3	5-30	3	5-30	2	8
0	4	30 or more	4	30 or more	2	10

Nugent's score was interpreted as follows;

Normal (score of 0–3)

Intermediate flora (score of 4–6)

BV (score of 7–10) (Score 7-8 is Non-classic BV; while Score 9-10 is Classic BV)

2.7 Significance of the study

Determining the antimicrobial susceptibility pattern of the organisms on the locally available antibiotics will help select the appropriate drug for use to discourage resistance. This will help to reduce the burden of treating recurrent and persistent bacterial vaginosis in the population. Despite provision of treatment services for BV at the Sex Workers Operating points (SWOP) clinic, some antimicrobials have been associated with marked evidence of resistance among vaginal anaerobic bacteria (Belgi et al., 2004). Further more data on antimicrobial pattern of

isolates from bacterial vaginosis is scarce due to lack of appropriate laboratory methods for isolation of some of organisms involved yet the prevalence in women of reproductive age is shown to be high in Kenya. Female sex workers are a high risk group for BV and therefore if resistance to antimicrobials is not addressed, it may lead to increase in the vaginal reservoir of macrolide-resistant bacteria which may spread to other regions within the country. Bacterial vaginosis is documented as a common cause of malodorous vaginal discharge. It is also associated with sexually transmitted infections and adverse pregnancy outcomes. It accounts for 40% to 50% of all gynecologic conditions encountered by primary care physicians. Bacterial vaginosis prevalence is generally high among women who are sexually active than those who are not.

2.8 Hypotheses

This study hypothesized that:

1. There was no difference in response to the Metronidazole, Clindamycin, Tinidazole and Secnidazole used to treat BV in Female Sex Workers (FSW) in Nairobi.
2. There were no risk factors associated with BV in FSW in Nairobi.

2.9 Broad objective

To determine antimicrobial susceptibility of vaginal anaerobic bacterial isolates from FSW with bacterial vaginosis in Nairobi, Kenya.

2.9.1 Specific objectives

1. To determine the prevalence of bacterial vaginosis among FSW in Nairobi, Kenya.
2. To determine the antimicrobial susceptibility pattern of the isolates from FSW in Nairobi
3. To establish the risk factors associated with bacterial vaginosis in FSW in Nairobi.

3.0 STUDY DESIGN AND METHODOLOGY

3.1 Study Design

This was a Cross-sectional study.

3.2 Location of the study

The study was done in Nairobi SWOP clinics which included; Kariobangi, Majengo and SWOP City clinic. The samples were drawn from these clinics while the laboratory investigations were carried out at the University of Nairobi, Institute of Tropical medicine and Infectious diseases laboratory. Purposive sampling was used to select Nairobi SWOP clinics because that is where one could find the subjects when they seek treatment.

3.3 Target population and Recruitment Procedures

The target population was FSW attending SWOP clinics in Nairobi. The SWOP clinics serve a population of 7035 FSW patients from within Nairobi County. Among this 6572 are females while 463 are males. They provide services such as; free education and risk reduction counseling, condom use, HIV/STI testing and care (Kimani et al., 2013).

All FSW who presented to the SWOP clinic with vaginal discharge complain/signs and gave informed consent were eligible to participate in the study. Participants were purposively selected from the target population. Each patient was assigned a number as they were recruited until a target of 160 participants was achieved.

3.5 Inclusion criteria

All FSW attending SWOP clinics in Nairobi and with complains/signs of vaginal discharge with a pH greater than 4.5, aged 18 years and above and consented were included in the study.

3.6 Exclusion criteria

FSW attending SWOP clinics in Nairobi and were on antimicrobial therapy were excluded from the study.

3.7 Sample size

Sample size was calculated for single population proportion using the formula below;

$$n = \frac{(Z_{1-\alpha/2})^2 [p(1-p)]}{D^2}$$

Where; n = Sample size

$Z_{1-\alpha/2}$ = Critical value associated with significance level (1.96)

P = Estimate of proportion (18.4%)

D = Margin of error (6%)

$$n = \frac{(1.96)^2(0.184 \times 0.816)}{0.06^2} = 160$$

Convenience sampling technique was used. Any patient who presented with symptoms of BV and consented to the study was given enrolment number and registered in the enrolment record book using a given code. This was done from September to December 2014 where 160 participants were enrolled.

3.8 Sampling method

All female sex workers who presented at the SWOP clinic with vaginal discharge complain/signs were informed of the study. Those who understood the study after explanation and gave informed consent were enrolled to participate in the study. The questionnaires were administered to them to fill after which they were explained to that the endocervical swab was to be taken for analysis.

3.9 Consenting procedure

Female sex workers who presented with the signs and symptoms of BV were issued with the informed consent document (Appendix A and B) to read through. Those not capable of reading were assisted by an impartial witness who ensured that the document was read to the volunteer and that she understood the study. The volunteers were given chance to ask questions if any on the study. They were required to sign the consent form after comprehension and agreement to participate in the study voluntarily (Appendix B). Where the volunteer was assisted to comprehend, the witness was also required to sign the consent form as the volunteer used the thumb print. The personnel presenting the consent document was also required to append his/her signature. Those who consented were recruited as the study subjects.

3.10 Data collection procedures

Questionnaire was used to collect socio-demographic features in this study. It provided data in the same form from all participants. A two part questionnaire was developed (Appendix C). Section one was used to collect information on the demographic features of the participants. This included variables such as age, education level and their residence. Section two of the instrument included items that elicited participants' knowledge and attitude towards their reproductive health. The questionnaires were piloted in a few SWOP clinics before use. After determining their validity and reliability, they were administered to the participants who consented to fill and then taken back for coding, tabulation and analysis.

Vaginal secretion specimen was taken by the clinician using sterile vaginal swabs at the SWOP clinic, inoculated on Columbia blood agar and transported to the lab in a GasPak anaerobic chamber for anaerobic incubation at 37^{o}c for up to 48 to 72 hours in a carbon dioxide ($CO_{2)}$ incubator. Suspected colonies of *Mobiluncus species* appearing as gray-white or slightly

yellowish and pin-point with 1-2.5 mm diameter were confirmed by use of biochemical tests such as Oxidase, Catalase and hippurate hydrolysis. *V. mobiluncus* is oxidase and catalase negative, while it hydrolyses hyppurate. Colonies suspected to be *Gardnerella* species appearing as round, opaque and smooth measuring 0.4-0.5 mm in diameter were confirmed by performing starch hydrolysis, methyl red test and Voges proskeur test. Smears of the specimen on glass slide were also transported to the lab for gram staining.

Nugent's (1991) criterion was used to score the organisms as follows:

Table 1: Nugent's score

Lactobacilli	**Score**	**Gardnerella, Bacteroides**	**Score**	**Curved gram –ve bacilli**	**Score**	**Nugent score**
30 or more	0	0	0	0	0	0
5-30	1	Less than 1	1	Less than 1	1	3
1-4	2	1-4	2	1-4	1	5
Less than 1	3	5-30	3	5-30	2	8
0	4	30 or more	4	30 or more	2	10

Nugent score = the sum of the scores for each bacterial morphotype (no. of organisms seen/100x objective)

Nugent's score was interpreted as follows;

Normal (score of 0–3)

Intermediate flora (score of 4–6)

BV (score of 7–10) (Score 7-8 is Non-classic BV; while Score 9-10 is Classic BV)

The pH of the discharge was determined using pH indicators. Whiff test was performed by use of 10% KOH to determine the fishy odor of the discharge.

Susceptibility test for *Gardnerrella, Morbilluncus, Bacteroides* and *Streptococci* was done using disc diffusion technique. The organisms were inoculated on Mueller Hinton blood agar medium where discs impregnated with metronidazole (50µg/disc), clindamycin (10µg/disc), tinidazole (25µg/disc) and secnidazole (100µg/disc) were applied on to the inoculums and incubated at 37^{o}c for 48 hours in CO_2 incubator. Agar dilution technique was also used at the same time. The plates were then observed for susceptibility or resistance of the organisms to the drugs as per the National Committee for Clinical Laboratory Standards (NCCLS) or Clinical and Laboratory standards Institute (CLSI) guidelines.

3.11 Variables

3.11.1 Dependent variable – BV

BV was the predictor variable. The outcome was either susceptibility or resistance to the antimicrobials

3.11.2 Independent variable – Antibiotic susceptibility

This response variable depended on the type of antibiotic used. While some prevented development of BV, others did not.

3.12 Quality assurance procedure

3.12.1` Questionnaires

Validity of the questionnaires was determined using criterion-related technique. This established validity through a comparison standard by which the validity of the test was judged. If the scores of the measurement being validated related highly to the criterion, the measure was assumed to be valid. Reliability of the questionnaires was determined using a split-half technique. This

technique required only one testing session. The instrument was designed to contain two parts. The items were sampled from domains of indicator that measured the variable (BV). The scores from one part were correlated with scores from the second part after computing (Olive and Abel, 2003). According to Spearman-Brown prophecy formula, a coefficient of 0.80 to 1.0 implies that there is a high degree of reliability of the data (Olive and Abel, 2003). This was used to determine how reliable the instruments were.

3.12.2 Samples

Samples were inoculated immediately on culture media and put in an anaerobic jar before transportation to the laboratory. They were incubated in CO_2 incubator at a controlled temperature of 37^{o}c for 48 hours. Un inoculated culture plates were also incubated at the same time for quality control. The concentration of the susceptibility test organisms was compared to McFalands standard while the susceptibility patterns determined using National Committee for Clinical Laboratory Standards (NCCLS) guidelines. The susceptibility pattern of the organisms was compared with the pattern for *Bacteroides fragilis* using the same antimicrobials. The data collected was edited, coded and analysed using statistical packages.

3.13 Data collection instruments

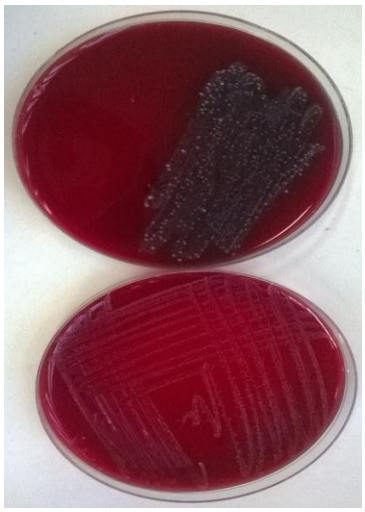
Questionnaire was used to collect socio-demographic features in this study. According to Sekran (1992), questionnaire is the main instrument it is used to obtain information on feelings, attitudes, beliefs, values, perception, personality and behavioural intentions. It provides data in the same form from all participants. A two part questionnaire was developed. Section one was used to collect information on the demographic features of the participants. This included items on age, education level and their residence. Section two of the instrument included items that

elicited participants' knowledge and attitude towards BV and STDs. The questionnaires were piloted in a few SWOP clinics before use. Vaginal specimens were collected and taken to the laboratory for bacterial analysis using conventional methods. Each participant was required to answer short questions about her background and reproductive health. Vaginal secretion specimen was taken by the clinician at the SWOP clinic, inoculated on Columbia blood agar and transported to the lab in a GasPak anaerobic chamber for anaerobic incubation at 37^{o}c for up to 48 hours. Smears of the specimen on glass slide were also transported to the lab for gram staining. The pH of the sample was determined by using pH indicator strip. Antibiotic discs and agar diluted with antibiotics were used to test for susceptibility.

Fig.1: Laboratory methods

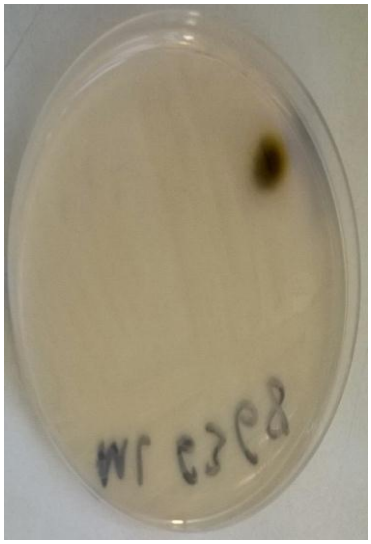
Anaerobic jar with culture plates

pH indicator

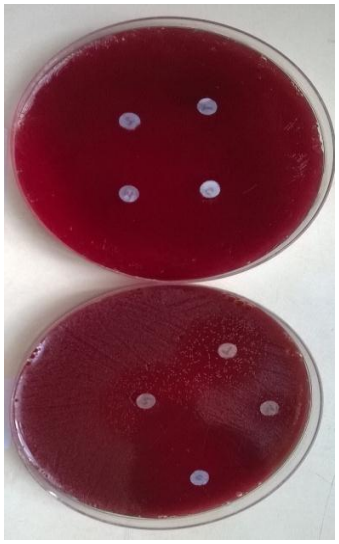


1^{o} and 2^{o} culture (*Bacteroides sp*)

Starch hydrolysis –ve



Starch hydrolysis +ve



Susceptibility to antimicrobials

3.14 Ethical consideration

The study proposal was presented to the University of Nairobi/Kenyatta National Hospital ethics and research committee for approval. The study was started after obtaining the approval from the ERC. Informed consent of the participants was sought and assurance of confidentiality of the information given was made to them. While there was no risk anticipated from this study, the participants benefited from the BV diagnosis services of the study in order to receive the appropriate drugs for treatment.

3.15 Data analysis

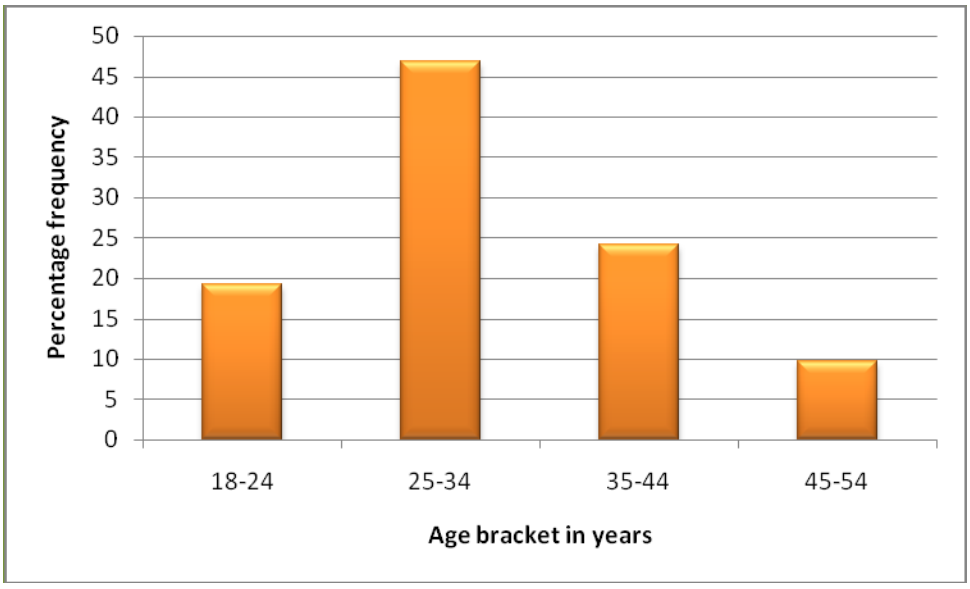
The data collected was first edited to identify and eliminate errors made by participants. Coding was done to translate responses into specific categories. The quantitative data (response to closed ended questions) was analysed using the descriptive statistics while multiple regression was used to determine the risk factors associated with the disease.

4.0: RESULTS

4.1 Demographic features of female sex workers in Nairobi

Out of 160 participants enrolled in to the study, 29 (18.1%) were confirmed to have Bacterial vaginosis. 130 (81%) of the study subjects were non-smokers while 30 (19%) smoked. 128 (80%) of the study participants had 1 to 3 children, 19 (12%) had 4 to 6 children while 8% had none. When asked about birth intention, 96 (60%) indicated that they had no intention of bearing children any more, 62 (39%) said yes while 2 (1%) were undecided. Out of 126 subjects who responded on their education level, 5 (3%) had tertiary education, 65 (41%) reached secondary level while only 2 (1%) had no formal education (table 2). 117 respondents stated their marital status and it was found that 65 (41%) were divorced, 7(4%) married 44 (27%) were single and 1(0.6%) widowed (table 3).

Fig. 2: The age of the participants



58 of the subjects were inclusively aged 25 to 34 years. The lowest age bracket was 18 to 24 years while the highest was 45 to 54 years. This formed 15% (24 participants) and 7.5% (12 participants) of the study subjects enrolled (fig. 2).

Table 2: Demographic features

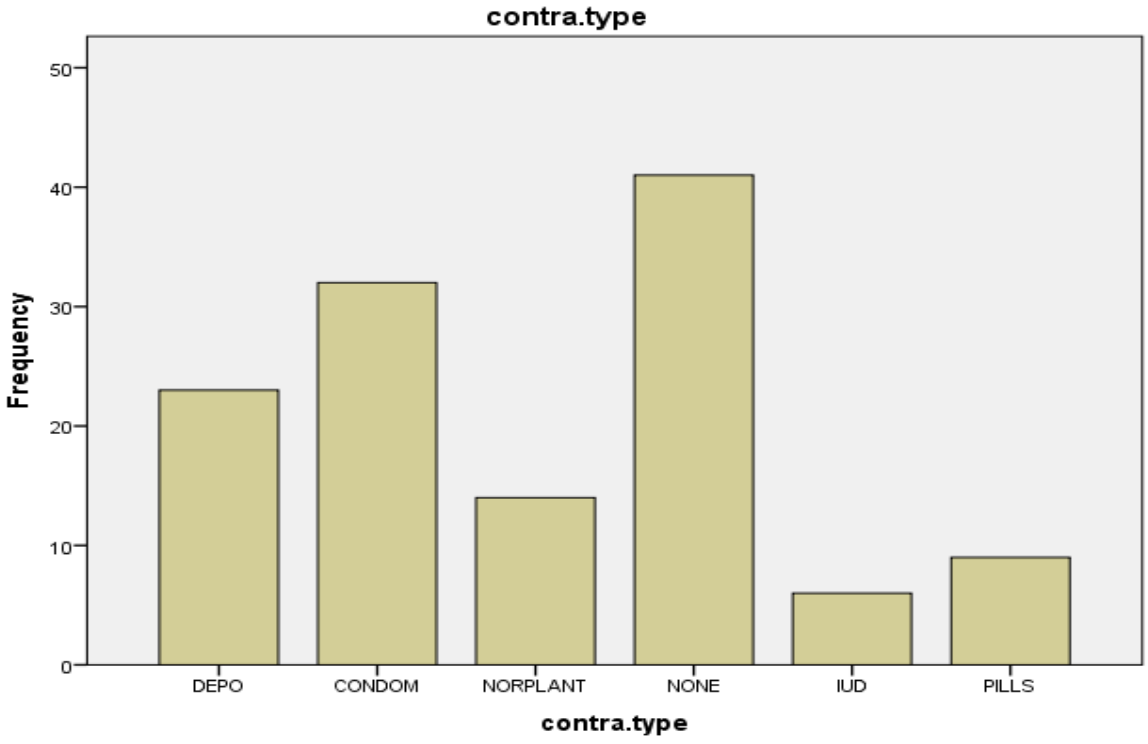
Category	Frequency	% Frequency	BV frequency	% BV Frequency
	Education level			
None	2	1.6	0	0
Primary	54	42.9	9	16.7
Secondary	65	51.6	13	20
Tertiary	5	4.0	0	0
Total	**126**	**100.0**	**22**	
	Marital status			
Married	7	6.0	1	14.3
Single	44	37.6	11	25
Divorced	65	55.6	10	15.4
Widowed	1	.9	0	0
Total	**117**	**100.0**	**22**	
	Age bracket			
18-24	24	19.4	7	29.2
25-34	58	46.8	7	12.1
35-44	30	24.2	5	16.7
45-54	12	9.7	3	25
Total	**124**	**100.0**	**22**	

Table 3: Reproductive health characteristics of female sex workers in Nairobi

Characteristic	Response		Total
	Yes	No	
Contraceptive use	85 (68.5%)	39 (31.5%)	**124**
Douching	64 (51.6%)	60 (48.4%)	**124**
Smoking	22 (18%)	100 (82%)	**122**
Previously treated for BV	74 (59.7%)	50 (40.3%)	**124**

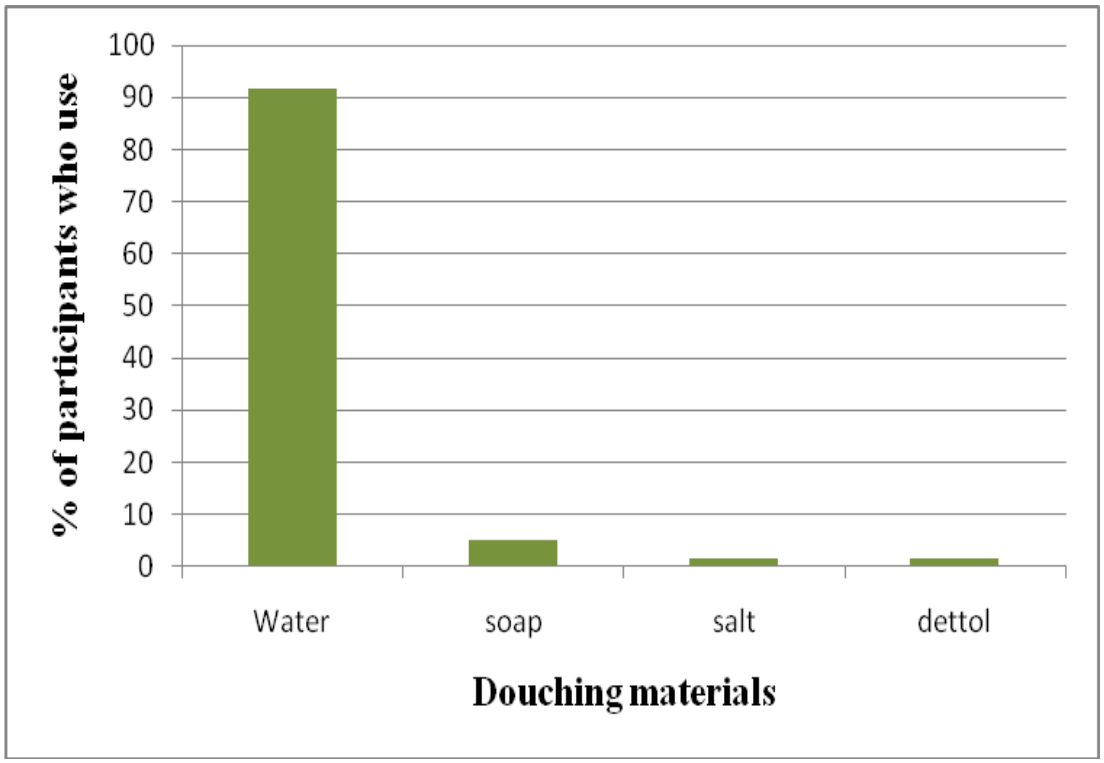
Contraceptive use was found to be common among the participants (68.5%), 51.6% practiced douching while only 18% indicated that they smoked. Seventy four subjects indicated that they had been treated for BV before while fifty had never had any BV treatment.

Fig. 3 Proportion of participants with respect to specific contraceptive use



Condom was the most common type of contraceptive used (32 participants) followed by Depo-Provera and Norplant (23 & 14 participants respectively) while pills and IUD were the least (9 & 6 participants respectively).

Fig. 4 Proportion of participants with respect to specific douching methods



Majority of the participants who douched preferred to use water (91.7%), 5% used soap while salt and dettol use was at 1.7% each (Fig.4).

Fig. 5: Regular sex partners per participant per week

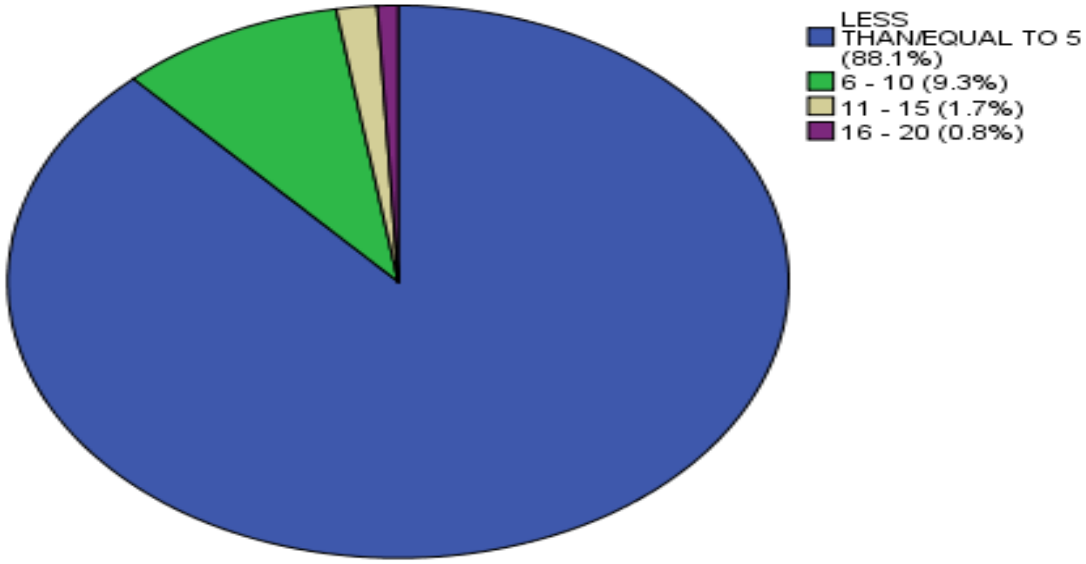
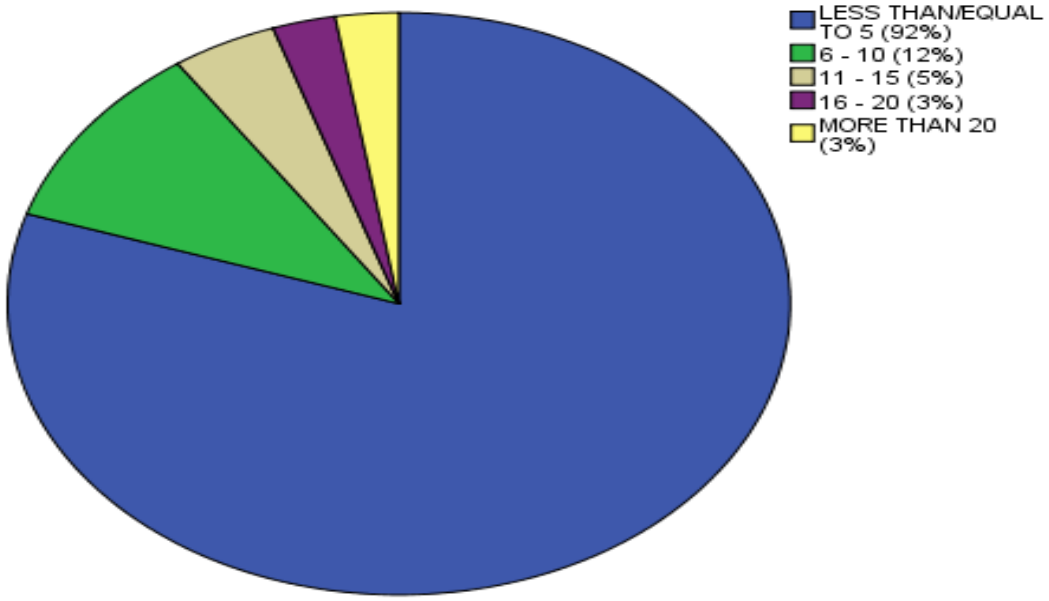
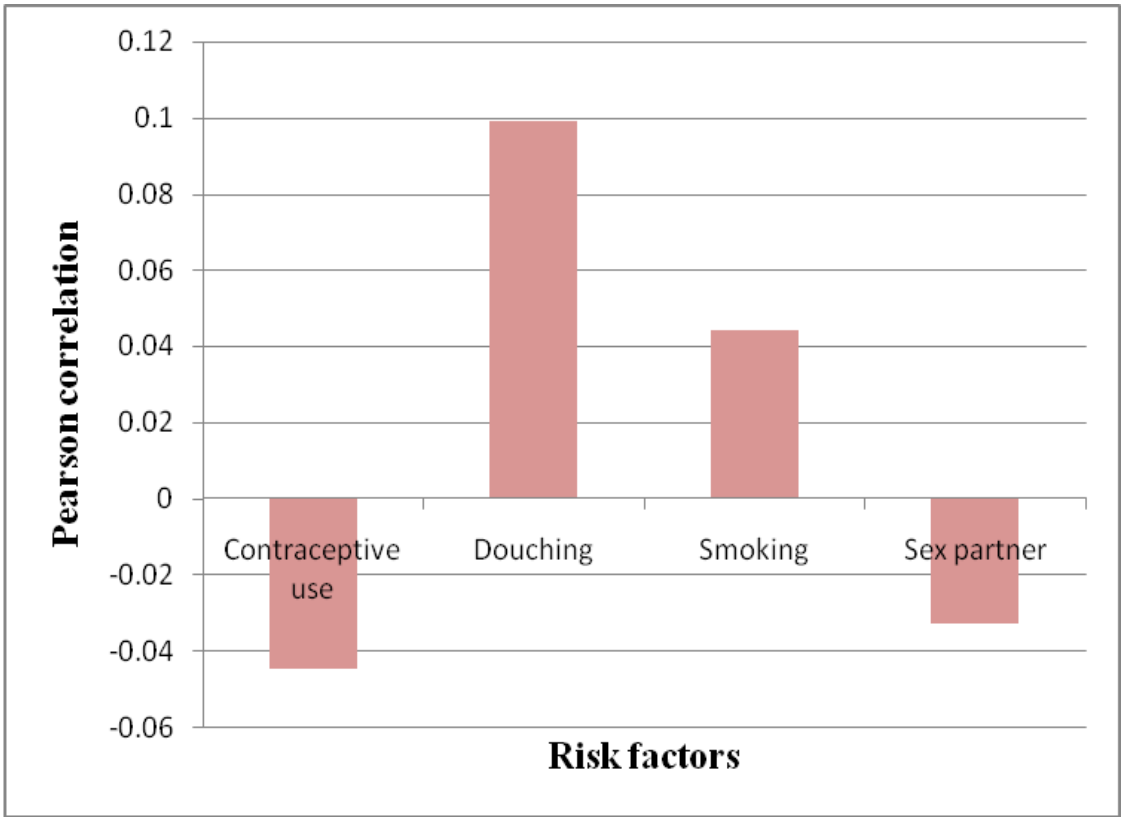


Fig. 6: Casual sex partner per participant per week



When participants were asked to state the number of regular sex partners they normally have per week. It was found that 88.1% of them had up to 5, 9.3% had 6 to 10, 1.7% had 11 to 15 and 0.8% had 16 to 20 regular sex partners per week (Fig. 5). It was found that 92% of the participants had up to 5 casual sex partners per week, 12% had 6 to 10, 5% had 11 to 15, 3% had 16 to 20 and only 3 had more than 20 casual sex partners per week (Fig. 6).

Fig. 7 Correlation between the risk factors and the bacterial vaginosis.



According to Pearson correlation results, BV was found to be inversely related to number of sexual partners and contraceptive use (Pearson correlation -0.033 and -0.045 respectively) while douching and smoking were directly related to BV condition (Pearson correlation 0.099 and 0.044 respectively) (fig. 4).

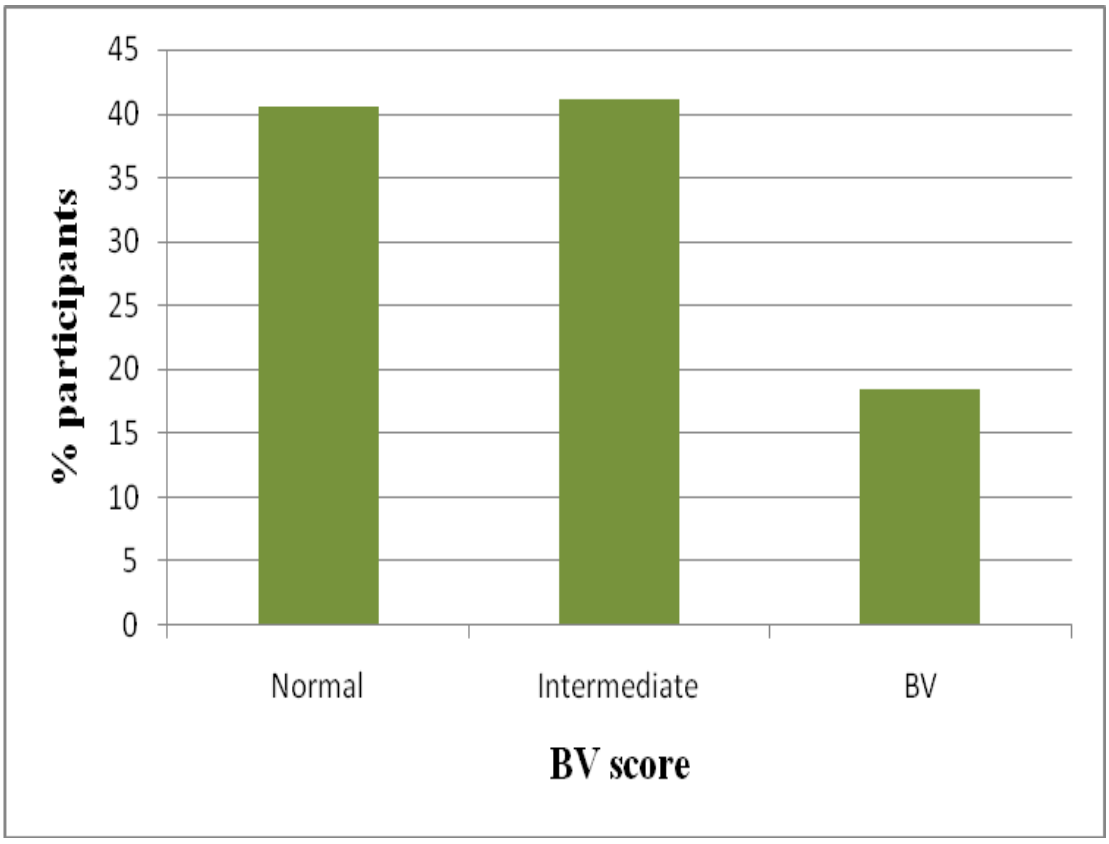
Table 4: Relationship between exposure factor and bacterial vaginosis

Exposure	Response	BV positive	BV negative	Total	Odds Ratio	95% CI
Contraceptive use	Yes	14	71	**85**	0.90	0.332, 2.448
	NO	7	32	**39**		
	Total	**21**	**103**	**124**		
Douching	Yes	9	55	**64**	0.65	0.258, 1.636
	NO	12	48	**60**		
	Total	**21**	**103**	**124**		
Smoking	Yes	3	19	22	0.72	0.193, 0.985
	NO	18	82	**100**		
	Total	**21**	**101**	**122**		

Odds ratio is a ratio of the odds of the outcome in exposed persons to the odds of the outcome in non-exposed. In this study it is the odds of having bacterial vaginosis in those who use contraceptives, douch or smoke to the odds of having the condition in those not exposed to the factors. Odds ratio of more than one indicates odds of exposure among those with the outcome. An odd ratio equals to one indicates no association between the exposed and the outcome. Odds ratio less than one indicates decreased odds or protective effects among those with the outcome. In this study, odds ratio for smokers is less than one and the confidence interval does not include

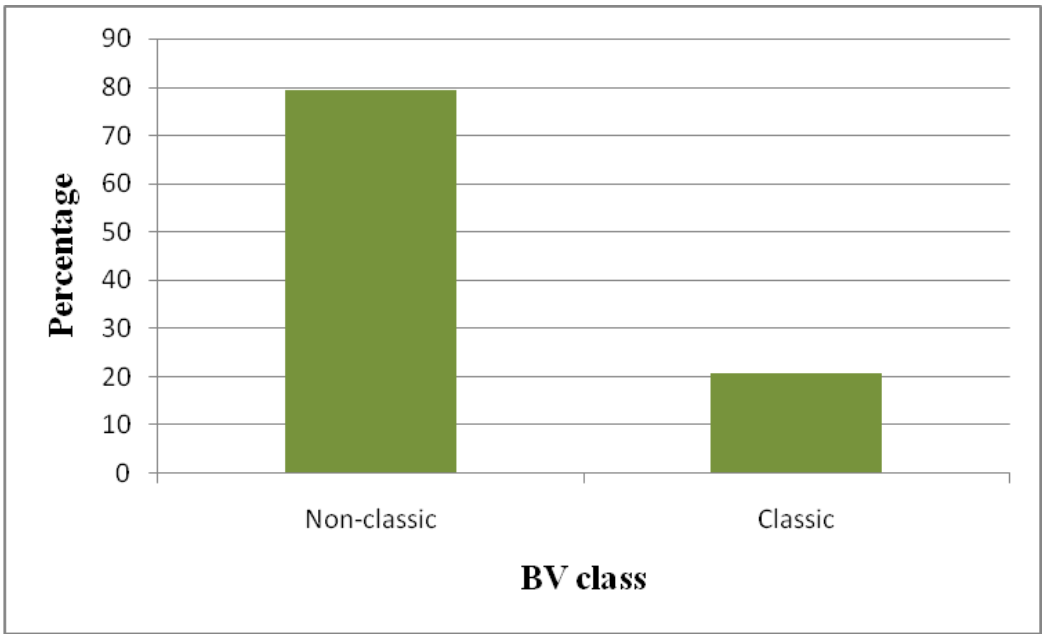
one. Therefore the exposure seem to have a protective effect to BV (OR= 0.72, CI= 0.193, 0.985) (table 4). Smoking also correlated to number of sexual partners (p=0.01) (fig 4).

Fig. 8: BV Score frequency



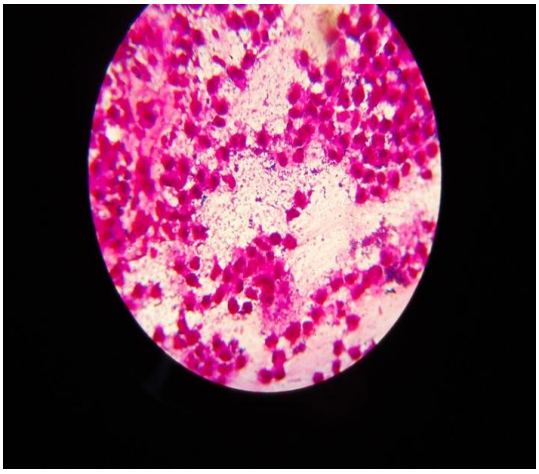
158 participants were tested for BV and the results identified 29 (18.4%) cases, 65 (41.1%) intermediate while 64 (40.5%) were normal.

Fig. 9: BV Class

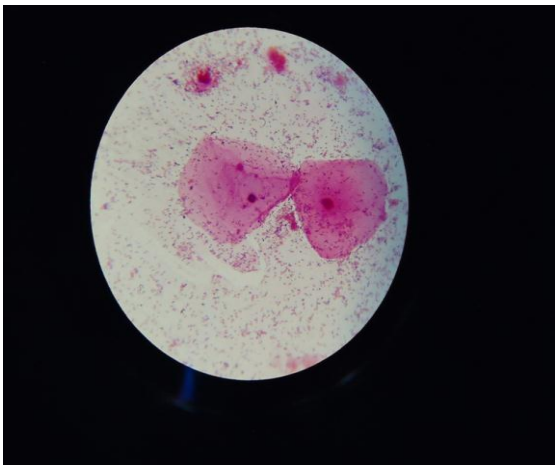


Out of the 29 BV cases, 23 (79.3%) were non-classic (BV score of 7 & 8) while 6 (20.7%) were classic (Score 9-10)

Fig. 10: Slide photos of BV categories

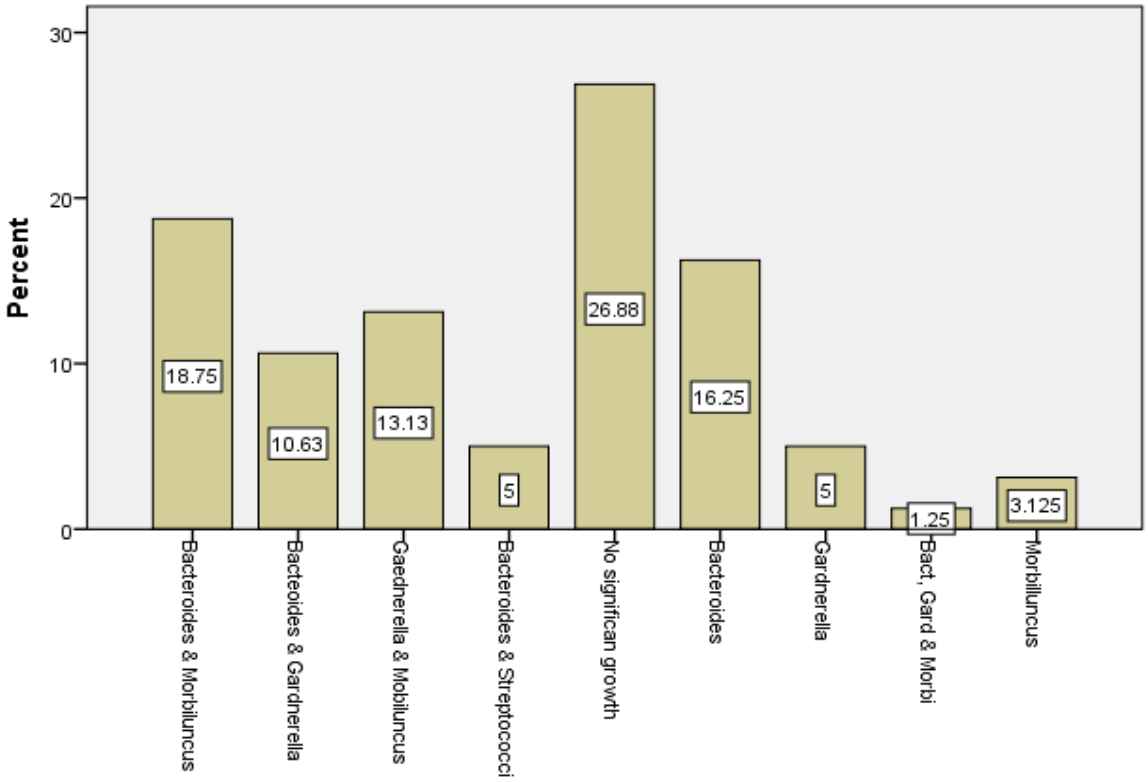


BV score of 8



BV score of 4

Fig. 11 Anaerobic organisms isolated from endocervical samples collected from FSW in Nairobi



Out of the 160 samples obtained from the participants, *Bacteroides* appearing together in a mixed culture with *Morbiluncus* were the most common organisms isolated (18.8%) while *Gardnerella* with *Morbiluncus* were the least (13.1%) (Fig.8). Single organisms isolated from the samples indicated that *Bacteroides* dominated the isolates at 16.3% while *Morbiluncus* was at 3.1%. Forty three samples out of one hundred and sixty had no significant growth (26.9%). The discharge could have been caused by other non-BV organisms which were not able to grow on the Columbia blood agar with antibiotics (Gentamycin sulphate, Nalidixic acid and Amphotericine B).

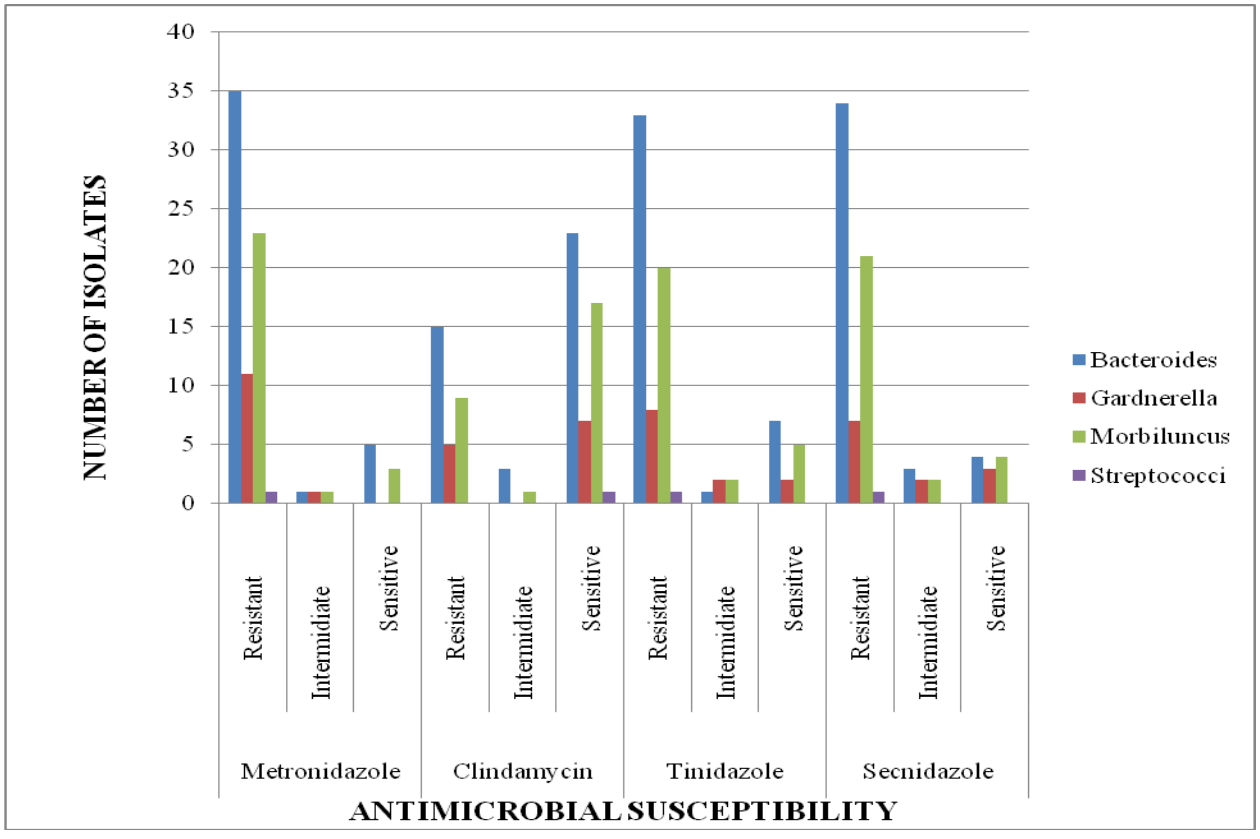
Table 5: Analysis of antimicrobials used in susceptibility testing

	Metronidazole	Clindamycin	Tinidazole	Secnidazole
Chi-Square	81.238[a]	34.588[a]	64.288[a]	58.513[a]
Df	2	2	2	2
P-value	.000	.000	.000	.000

a. 0 cells (0.0%) have expected frequencies less than 5. The minimum expected cell frequency is 53.3.

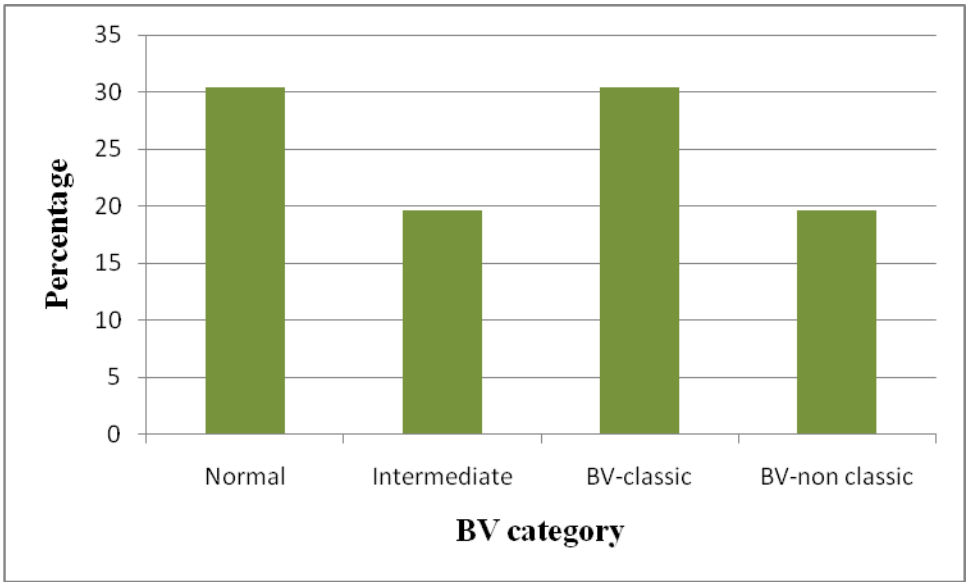
Chi-square results indicate that there is a significant difference in the antimicrobials used to treat BV (P<0.001) (Table 9).

Fig. 12: Antimicrobial susceptibility pattern



Most of the isolates were sensitive to clindamycin while majority were resistant to metronidazol.

Fig. 13: Follow up of participants 3 months after the first diagnosis (Intermediate BV score)



The second testing was carried out three months after the first BV diagnosis on participants who had intermediate score initially. 14 (30.4%) of them had a normal score, 9 (19.6%) had intermediate, 14 (30.4%) had classic BV while 9 (19.6%) had non classic BV.

5.0 DISCUSSION

The aim of this study was to investigate the antimicrobial susceptibility pattern of anaerobic bacteria isolated from female sex workers with BV. After collection of samples from participants, microscopy, culture and susceptibility tests were carried out. Its findings revealed that 18.4% of the FSW who participated in the study between October 2014 and December 2014 had BV. This is quite low as compared to a study conducted by Kouman et al (2007) which had a BV prevalence of 29.2% among women who participated in the National Health and Nutrition Examination Survey in United States. Nzomo (2010) in his study also got a prevalence of 43% in sexually active women attending family planning clinic in Thika, Kenya. While the two studies gave a high prevalence as compared to this study, the participants of this study were from a high risk group population as compared to the previous studies. The knowledge and awareness created among sex workers on sexually transmitted diseases could be a contributory factor to this difference.

This study found that BV was quite common among women who are single than those who are divorced, married or widowed. This could have been as a result of engagement in sex work more frequently than their counterparts, as they had more regular and casual sexual partners per week than the other groups. The mean number of regular sex partners per participant per week was found to be 3.39 (SE=0.32, CI=2.75, 4.02), while casual sex partners per participant per week was 4.48 (SE=0.44, CI=3.61, 5.36). The study also revealed that the mean age of participants was 31.80 (SE=0.77, CI=30.27, 33.33). Women among age group 18 – 24 years were the most affected by BV (29.2%), followed by 45-54 years age bracket at 25%. The former bracket had many sexual partners as compared to the latter group. While the former was a more sexually active group, the latter experiences hormonal change in menopause and this could have resulted

in the change of vaginal pH leading to alteration of the vaginal micro flora, hence the high frequency of BV.

Those with secondary education level had the highest BV prevalence (20%) while those with no formal education and tertiary education level were found to be BV negative. This finding contradicts that of Kouman (2007) who found that low educational attainment was positively associated with BV. The difference could be as a result of difference in number of sex partners. In the current study participants with secondary education level had the highest number of sexual partners which is a reverse in the previous study.

In this study 68.5% of the FSW indicated that they use contraceptives and out of this 24.7% were positive for BV. Contraceptive use was found to have an inverse relationship with BV (correlation coefficient = -0.45, table 5). The association was not statistically significant at 0.05 level (OR= 0.90, CI= 0.332, 2.448, table 6). This is supported by a study conducted by Bradshaw (2006) in Australia on recurrence of BV. He also found that hormonal contraceptive use had a negative association with recurrence of BV. Hutchinson et al (2007) in their study found that condom use was associated with a decrease in the risk for BV and associated vaginal microflora. Their study population was women at high risk for sexually transmitted diseases.

More than half (51.6%) of the women also indicated that they practiced douching and majority of them used water (91.7%). This was also found to have a direct relationship with BV (correlation coefficient = 0.099, table 5), the association was not statistically significant at 0.05 level (OR= 0.65, CI= 0.258, 1.636, table 6). Ness et al, (2002) in their study demonstrated that douching is associated with BV. It was a cross sectional study on the relationship between douching and bacterial vaginosis. Although they used a similar population on their study, douching materials

differed with the ones used in this study. The materials used in the previous study included water, vinegar, lemon juice and disinfectants. In the current study, participants used plain water, salty water and soapy water.

Several studies have associated smoking with BV. Koumans *et al.,* (2007) in their study on prevalence of BV in United States found that smoking and increasing life time sex partners had a significant positive association with BV. Their findings concur with this current study which revealed that 18% of the women smoked and among them 13.6% developed BV. In this study, odds ratio for smokers is less than one and the confidence interval does not include one. Therefore the exposure seem to have a protective effect to BV (OR= 0.72, CI= 0.193, 0.985, table 6). Even though smoking was directly correlated to BV (Pearson correlation=0.44, table 5), smokers had few numbers of sexual partners as compared to the non smokers and this could have contributed to their protection against BV. Despite this, number of sexual partners was still identified to be inversely related to BV. Since majority of the participants in this study used condoms to protect themselves and hormonal contraceptive use was also found to have a protective effect on BV, then this can explain the contradiction in the two studies on the effect of sex partners on BV. While Koumnans et al (2007) used the general population in their study, this current study used a high risk group population which had knowledge on their reproductive health according to the responses in the questionnaires.

Out of the 29 BV cases, 20.7% were identified as classic BV while *bacteroides* was the most common organism isolated and *Gardnerella* the least. Many studies have shown resistance of BV organisms to most of the antimicrobials used to treat the condition. Oduyebo et al (2009) looked at the effect of antimicrobial therapy on bacterial vaginosis in non pregnant women and found that clindamycin and metronidazole were effective for bacterial vaginosis. Lofmark et

al.,(2010) in their study indicated that *Garnerella vaginalis* responded well to metronidazole therapy and that resistance to the drug was low. De backer et al (2006) in their study on antibiotic susceptibility of *Atopobium vaginae,* found that clindamycin had a higher activity against *G.vaginalis* and *A.vaginae* than metronidazole. A study conducted in Australia (Bradshaw et al,. 2006) on high recurrence rates of bacterial vaginosis over the course of 12 months oral metronidazole therapy indicated that metronidazole therapy did not prevent recurrence of BV in majority of the women. This study revealed that that there was a difference between the antibiotics used to treat BV ($P<0.001$) while most organisms isolated were susceptible to clindamycin. Majority were resistant to metronidazole and secnidazole as compared to tinidazole. According to World Health Organization (2001) guideline, the recommended regimen for BV are metronidazole 500mg orally twice a day for 7 days or 2g orally as a single dose, Clindamycin 300mg twice daily for 7 days or tinidazole 2g orally once daily for 2 days. Among the WHO recommended drugs, clindamycin is the drug of choice for BV treatment in this population according to the findings of this study.

In this study, follow up was done three months later on participants who had intermediate score after the first diagnosis. BV classic was found to be 30.4% while 19.6% had non classic BV. This is in line with the results of a previous study on pregnant women who had intermediate score and were followed up for six to eleven weeks (Richard et al., 2009). The study revealed that 32% acquired BV while 30% turned to have normal flora. The current study used a high BV risk group population as compared to the previous one. This could have contributed to the difference in the percentage of BV score after the follow up.

6.0: CONCLUSION

According to the findings of this study, hormonal contraceptives were negatively associated with BV while smoking and douching were directly associated with the condition in FSW in Nairobi. *Bacteroides* sp was the most commonly isolated organism from samples collected from FSW in Nairobi. Clindamycin was the most effective drug for treating BV in FSW in Nairobi, Kenya and therefore should be the drug of choice for treating BV in this population.

7.0: RECOMMENDATIONS

Based on the findings of this study, it is recommended that upon diagnosis, an intermediate BV score should be treated using clindamycin. Antimicrobial susceptibility tests should be done to monitor the drugs that are occasionally used for treatment to detect resistance as early as possible.

8.0: LIMITATIONS OF THE STUDY

Not all the organisms were isolated and identified from the samples using the conventional methods available. The organisms being fastidious in their nutritional requirements and more biochemical techniques required for their identification, molecular techniques were necessary to capture and identify the organisms present in the samples including the ones that were not able to grow.

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9.0 APPENDICES

A: INFORMED CONSENT FORM

Introduction

Hello. My name is Millicent Ogutu from the University of Nairobi. I am conducting a study on "bacterial vaginosis in female sex workers in Nairobi". Bacterial vaginosis (BV) is a lower genital tract infection characterized by the presence of thin, white, homogeneous, fishy-smelling vaginal discharge. This discharge is present in the absence of signs of vaginal irritation. Its prevalence is reported to be generally higher among women who are sexually active than those who are not. Despite provision of treatment for BV, some antimicrobials have been associated with marked evidence of resistance among vaginal anaerobic bacteria. The prevalence in women of reproductive age is shown to be high in Kenya. Therefore the aim of this study is to investigate the antimicrobial susceptibility pattern of anaerobic bacteria isolated from BV in female sex workers in Nairobi, Kenya. You are not obligated to participate and can refuse to answer any question or stop the participation at anytime.

Purpose of the research

The purpose of this study is to find out the susceptibility pattern of the causative organisms to the locally used drugs. The results obtained will help us formulate targeted strategies aimed at addressing bacterial vaginosis in sexually active women.

Type of Research Intervention

This research will involve your participation by filling a questionnaire and giving specimen for diagnosis incase of bacterial vaginosis symptoms at the clinic.

Participant Selection

You are being invited to take part in this study because your views will help me know what the gaps are and see what interventions can be put in place to address the problem.

Voluntary Participation

Your participation in this study is entirely voluntary. It is your choice whether to participate or not. If you choose not to participate all the services you receive at this clinic will continue and nothing will change.

Procedures

I would like to invite you to participate because your opinion and views of other people like you will help in implementation of strategies to address this problem. This questionnaire should take about 15 minutes. Please be assured that the information you give me will not be linked to any names or other identifying information. Medical examination will be performed on the lower vaginal tract and the secretions will be collected by the clinician using speculum and sterile swab.

Risks/Discomforts

I am asking you to share with me some very personal and confidential information, and you may feel uncomfortable talking about some of the issues. You do not have to answer any question or take part in the discussion if you don't wish to do so. You do not have to give me any reason for not responding to any question in the questionnaire.

There is no risk or discomfort involved in the procedure as it does not involve traumatic/piercing of the vaginal wall but just swabbing the secretions which are present on the wall.

Benefits

Your participation is likely to help us formulate targeted strategies aimed at addressing bacterial vaginosis among female sex workers. If you are found to be suffering from BV, you will be given treatment at the swop clinic where you were first seen. If you have BV that is non

susceptible to the drugs available at the clinic, you will be referred to the City Council Clinic Casino within Nairobi.

Confidentiality

The information that we collect from this study will be kept private. Any information about you will be reported anonymously and no names will be used. You can ask me any questions about any part of the study, if you wish to.

Storage of samples

The samples will be stored for 5 years and after that they will be destroyed and disposed of. If you don't want your sample to be stored, you are given a period a period of 3 months to contact us. If you will not come, it will be assumed that you have agreed that your samples be stored for further analysis.

Whom Do I Call if I Have Questions or Problems?

For questions about the study, call or contact Millicent Ogutu, University of Nairobi, Mobile: 0723-532231 or Dr. Julias Oyugi University of Nairobi, Mobile: 0713-898564 or Ms. Susan Odera, University of Nairobi, Mobile: 0723-470211. For questions about your rights as a volunteer, contact Prof. M. l. Chindia, the Secretary of the Ethics Committee at Kenyatta National Hospital, Tel: 726300-9.

A: FOMU YA UFAHAMU WA UTAFITI KWA MSHIRIKI

Kielelezo

Jina langu ni Millicent Ogutu kutoka chuo kikuu cha Nairobi. Nafanya utafiti juu ya, “Viini vinavyo sababisha ugonjwa katika uke kwa wanawake wanaofanya kazi ya ngono”. Kuwa na viini hivi husababisha kutokwa na uchafu katika sehemu ya uke. Uchafu huo huwa na mnuko kama wa samaki. Ugonjwa huu husababisha asili mia arubaini hadi hamsini ya magonjua yote ya uzazi zinazo onekana na madaktari. Ugonjwa huu huripotiwa kwa wingi kwa wanawake wanao uzoefu wa shiriki sana ngono kuliko wasio nao. Pia uonekana kuwa juu sana kwa wanawake waliokatika umri wa uzazi nchini Kenya. Tafiti nyingi zimethibitisha kukosa kutibiwa kwa ugonjwa huu na madawa yanayotumika kuitibu kama,”metronidazole na clindamycin”. Unaulizwa kushiriki katika utafiti huu lakini pia una nafasi ya kujibu swali au kukataa kushiriki kabisa wakati wowote.

Madhumuni ya utafiti huu

Madhumuni ya utafiti huu ni kuchunguza madawa yanayotumika kutibu ugonjwa huu unaopatikana kwa wanawake wanao fanya kazi ya ngono. Maajibu yatakayo patikana yatatumika kusaidia kuweka mikakati maalum inayo tarajiwa kulenga ugonjwa huu kwa wanawake wanao uzoefu wa kushiriki ngono.

Njia ya kufanya utafiti

Huu utafiti utahusisha ushirikiano wako kwa kujaza maswali utakazo pewa na kwa kutoa uchafu unaotoka kwa uke wako kwenda kupimwa katika mahabara.

Uchaguzi wa washiriki

Unaalikwa kuwa mmoja wa washiriki wa utafiti huu kwasababu mawazo yako yatasaidia kujua pengo lililoko na ni njia gani itakayo tumoka kutatua shida hili.

Kushuriki kwa hiari

Kushiriki kwako katika utafiti huu ni kwa hiari yako nay a wengine. Ni chaguo lako kushiriki au kutoshiriki, na hata husiposhiriki, huduma zote unazostahili kupokea kwa kliniki hii bado utaendelea kupokea na hakuna litakalo badilika.

Utaratibu

Ninawaalika kushiriki kwakua maoni yako nay a wengine kama wewe itasaidia kuweka mikakati ya kutatua hili shida. Maswali yatachukua dakika 15 pekee. Nakuhakikishia kwamba mabo utakayo niambia hayata linganishwa na majina yako au chochote kinachoweza kukutambulisha.

Hatari

Ninakuuliza kuniambia mambo yako ya kindani na kisisri na unaweza kujisikia kuwa hauko tayari kuyazungumzia, silazima ujibu swali kama hujiskii kufanya hivyo. Pia si lazima uniambie kwanini ujajibu hilo swali. Hakuna uchungu wowote utahisi wakati uchafu wa uke utakapochukuliwa kwakua hudungui mwili.

Faida

Kushiriki kwako kutatusaidia kupata njia thabiti ya kusaidia kutatua hili shida kwa wanawake wanaofanya kazi ya ngono. Uchunguzi wa mahabara utasaidia kukupatia madawa zinazofaa kwa ugonjwa huu. Kwa wale ambao madawa hayo hayata wafaa, watatumwa kuenda kuhudumiwa katika kliniki ya “City Council- Casino” iliyo Nairobi kwa matibabu zaidi.

Siri

Mambo yatakayo kusanywa na utafiti huu yatawekwa kwa siri Chochote kitakachokuhusu kita ripotiwa bila kutumia majina. Unaweza kuniuliza swali lolote linalohusiana na utafiti huu.

Muda wa kuweka viini vya utafiti

Viini hivi vita wekwa kwa muda usiyo pungua miaka 5 kwa uchunguzi zaidi. Ikiwa hutaki viini hivi viwekwe kutumika kwa utafiti zaidi baadaye, unahimizwa kutujulisha katika muda wa miezi 3 baada ya kuchukuliwa la sivyo, itachukuliwa kuwa ulikubali iwekwe kwa matumizi zaidi.

Nikiwa na swali nita muuliza nani?

Kwa maswali kuhusu utafiti wapigie Millicent Ogutu wa chuo kikuu cha Nairobi, nambari ya simu: 0723-532231 au Dr. Julias Oyugi wa chuo kikuu cha Nairobi, nambari ya simu: 0713-898564 or Ms. Susan Odera, wa chuo kikuu cha Nairobi, nambari ya simu: 0723-470211. Kwa

maswali ya haki yako mpigie Prof. M. l. Chindia, mwandishi wa Kamitii ya sheria za kitafiti wa Hospitali kuu ya Kenyatta, nambari ya simu : 726300-9.

B: INFORMED CONSENT DOCUMENT

I, (name of the volunteer)_____

Of (address)_____

have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions I have asked have been answered to my satisfaction. I consent voluntarily to be a participant in this study.

Participant: Print Name: _____

Signature/mark or Thumb print _____

Date _____

Person Obtaining Consent:

I have explained the nature and demands of the above study to the volunteer and answered her questions:

Print Name: _____

Signature: _____

Date: _____

Impartial Witness: (only necessary if the volunteer was not able to read and understand the Consent Information sheet and informed Consent Document):

I affirm that the Informed Consent Document has been read to the volunteer and she understands the study, had her questions answered, and I have witnessed the volunteer's consent to study participation.

Print Name: _____

Signature/mark or Thumb print _____

Date _____

B: FOMU YA KUELEWA NA KUITIKIA KUSHIRIKI KATIKA UTAFITI

Mimi, (Jina la mshiriki)_____

Wa (anuani)_____

Nimesoma kuhusu utafiti huu au nimesomewa. Nime pewa nafasi ya kuuliza maswali kuihusu na maswali yote nilioulizia yamijibiwa nikaridhika. Najitolea kwa hiari yangu kushiriki katika utafiti huu.

Mshiriki: _____

Sahihi _____

Tarehe _____

Anaepeana fomu hii:

Nime mwelezea aina ya utafiti na matakwa ya utafiti kwa mshiriki na nikajibu maswali yake.

Jina: _____

Sahihi _____

Tarehe _____

Shahidi: (Inahitajika ikiwa mshiriki hawezi kusoma na kulielewa madhumuni ya utafiti):

Na Nimekusujudia madhumuni ya utafiti huu umesomewa na kuelezewa mshiriki na maswali yake yakajibiwa yote. Nilishujudia mshiriki akiikubali kushiriki kwa hiari yake.

Jina: _____

Sahihi _____

Tarehe _____

C: PARTICIPANT'S QUESTIONNAIRE

Questionnaire No……. Study No…….

Please answer the following questions

(*Tick the box provided against the choice of your answer where appropriate*)

SECTION I

SOCIO-DEMOGRAPHIC FACTORS

1. Year of birth ☐ Month ☐

2. Residence _____

3. Education level Primary ☐ Secondary ☐ Tertiary ☐ None ☐

4. Marital status Married ☐ Single ☐ Divorced ☐

5. Number of children if any _____

6. Do you still intend to have another child? Yes ☐ No ☐

7. What do you do for a living?

Business ☐ Employed ☐ Agriculture ☐ None ☐

SECTION II

REPRODUCTIVE HEALTH

8. What type of contraceptive are you currently on _____

9. Have you ever been treated for an abnormal vaginal discharge before? Yes ☐ No ☐

If yes, when was it? _____

10. Do you practice vaginal douching? Yes ☐ No ☐

If yes, go to 10

11. What do you use for douching? _____

12. Do you smoke? Yes ☐ No ☐

13. How many regular sexual partners do have?_____

14. How many casual sexual partners do have?_____

C: FOMU YA MASWALI KWA MSHIRIKI

Fomu ya maswali nambari……. Usomi numbari…….

Tafadhali jibu maswali yafuatayo

(*Jibu kwa sanduku zilizowekwa dhidi ya uchaguzi wa jibu lako inapohitajika*)

SEHEMU YA I

IDADI NA MASWALA YA KIJAMII

1. Mwaka wa kuzaliwa ☐ Mwezi ☐

2. Mtaa unapoishi _____

3. Kiwngo cha elimu Shule ya msingi ☐ Kidato cha pili ☐ Chuo Kikuu ☐ Hamna ☐

4. Hali ya ndoa Nimeolewa ☐ Sijaolewa ☐ Tulitalakiana ☐

5. Idadi ya watoto ulionawo kama wapo _____

6. Je bado unatarajia kumpata motto mwengine? Ndiyo ☐ Hapana ☐

7. Ni kazi gani unayo fanya maishani mwako kujimudu?

Biashara ☐ Nimeajiriwa ☐ Kilimo ☐ Hamna ☐

SEHEMU YA II

AFYA YA UZAZI

8. Kwa sasa unatumia mpango wa uzazi wa aina gani? _____

9. Je, umewai kutibiwa kwajili ya uchafu usiowakawaida unao toka kwa uke (sehemu ya siri) hapo awali? Yes ☐ No ☐

Kama ndiyo, ilikuwa lini? _____

10. Je, una uzoefu wa kuosha uke wako? Ndiyo ☐ Hapana ☐

Kama niyo, jubu namabari 10

11. Je, wewe hutumiiya nini kwa kuosha uke? _____

12. Je, wewe huvuta sigara? Ndiyo ☐ Hapana ☐

13. Una wateja wangapi wa mara kwa mara unao shiriki nao ngono?_____

14. Ni wateja wangapi ambao sio wa mara kwa mara unaowapata kishiriki nao ngono?_____

D: LABORATORY RESULT

Study No…….

(1). pH of the sample…….

(2). Odor of the sample…….

(3). **Gram stain microscopy (100x)**

Organism	*Lactobacilli*	*Gardnerella, Bacteroides*	Curved gram –ve bacilli	Nugent Score
Score				

Nugent score interpretation
Normal (score of 0–3)
Intermediate flora (score of 4–6)
BV (score of 7–10)

Culture result
Organism isolated

Susceptibility test result

Drug	**Susceptibility**
Metronidazole	
Clindamycin	
Tinidazole	
Secnidazole	

Investigator…… Sign……..
Date……..

E: LABORATORY PROCEDURES

PROCEDURE FOR RECRUITING PARTICIPANTS INTO THE STUDY

Identify subjects with discharge

Inform subjects of the BV study in detail

Let them consent to the study voluntarily after comprehension

Let them fill questionnaire

Collect endocervical specimen using three swabs

Using one swab, inoculate specimen on labeled Columbia blood agar plate

Place the plate in an anaerobic jar

Open an anaerobic sachet and place it in the jar

Close the jar tightly and transport it together with the remaining two swabs to the laboratory for analysis

PROCEDURE FOR HANDLING THE SAMPLE IN THE LABORATORY

Incubate the plates at 37^{o}c for 24-72hours in an anaerobic incubator

Use one swab to determine the pH and odor of the sample and record the results

Prepare a smear using the remaining swab, gram stain and examine microscopically

Record the BV score using Nugent's method

Examine cultures for growth

Perform gram stain technique on smears from cultures

Perform the biochemical identification tests

Perform susceptibility test

Examine results, record and give feed back to the respective clinics

PROCEDURE FOR GRAM STAINING

Heat–fix the smear

Flood the smear with crystal violet for 1 minute

Wash off with running water and flood with gram's iodine for 1 minute

Decolorize with acetone/alcohol

Rinse with running tap water

Flood with safranin for 30 seconds

Rinse with running tap water

Drain and air dry it in an upright position

PROCEDURES FOR BIOCHEMICAL TESTS

Oxidase test

Place a disc impregnated with oxidase reagent in a Petri dish

Pick a 48 hour growth colony with a wire loop and smear across the disc

A positive reaction is shown by development of a dark-purple color within 10 seconds

Starch hydrolysis test

Inoculate culture colonies into starch medium

Incubate at 37^{o}c for 3 days

Flood the plate with lugol's iodine solution

The presence of a clear zone indicate starch hydrolysis

Gelatin liquefaction

Inoculate gelatin agar with culture isolates

Incubate at 37^{o}c in anaerobic condition for 3 days

Flood the plates with mercuric chloride solution

The presence of opacity with clear zone around the colonies indicate gelatin liquefaction.

PREPARATION OF STARCH AGAR

Ingredients	**Grams/litre**
Peptic digest of animal tissue	5.0
Sodium chloride	5.0
Yeast extract	1.5
Beef extract	1.5
Starch, soluble	2.0
Agar	15.0

Suspend 30 grams of medium in 100mls distilled water. Boil to dissolve, sterilize by autoclaving at 121^{o}c for 15 minutes

PREPARATION OF GELATIN LIQUIFACTION AGAR

Ingredients	**Grams/litre**
Peptic digest of animal tissue	25.0
Sodium chloride	5.0
Meat extract	7.5
Gelatin	120.0
Agar	1.0

Suspend 158.5 grams of medium in 100mls distilled water. Boil to dissolve, sterilize by autoclaving at 121^{o}c for 15 minutes

PREPARATION OF COLUMBIA BLOOD AGAR

Ingredients	Grams/liter
Peptone, special	23.0
Sodium chloride	5.0
Corn starch	1.0
Agar	15.0

Suspend 44 grams of medium in 100mls distilled water.

Boil to dissolve

Sterilize by autoclaving at 121^{o}c for 15 minutes

Cool to 45-50^{o}c

Add 5% sterile defibrinated sheep blood

Add 2 vial of *G. vaginalis* supplement

Mix well and pour into petridishes

Let cool and solidify

Incubate one plate for 48 – 72 hours for quality control

Label the batch and store at 2-8^{o}c

PROCEDURE FOR PREPARATION OF G.VAGINALIS SUPPLEMENT

Ingredients	**mg/vial**
Gentamicin sulphate	2.00mg
Nalidixic acid	15.00
Amphotericine B	1.00

Rehydrate the contents of 1 vial aseptically with 2 mls of 0.2N Sodium hydroxide

Mix well and aseptically add it to 500ml sterile, molten Columbia blood agar base.